ROLE OF HOST RESPONSE TO HEPADNAVIRUS sAG IN IMMUNITY AND RECOVERY

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ANIMAL ETHICS

Animals were used during the studies described in this thesis.

Pekin-Ayelsbury crossbred ducklings were used in animal experiments. Ethical approval was obtained from the University of Sydney animal ethics committee. All ducks were handled with great care and respect, beyond that required by legislation. All animals were housed in designated animal care facilities at the University of Sydney.

The knowledge obtained by the sacrifice of these animals is appreciated.

DECLARATION

The study presented in this thesis contains original research performed by the author and has not been submitted previously for any other degree.

Robert Welschinger

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PUBLICATIONS

Work incorporated in this thesis has been accepted for refereed publication.

Welschinger, R., Pouliopoulos, J., Cossart, Y.E., and Vickery, K. (2003). The T-cell response of ducks to duck hepatitis B virus (DHBV) and the production of an associated DNA vaccine. *Proceedings 11th International Symposium on Viral Hepatitis and Liver Disease*. Sydney. Australia.

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PRESENTATIONS

Work incorporated in this thesis has been presented at several International, and National, and University conferences.

Welschinger, R., Vickery, K., and Cossart, Y.E. Patterns of duck hepatitis B virus infection. International Congress of Virology, IUMS. Sydney, Australia. August 1999.

Welschinger, R., Vickery, K., and Cossart, Y.E. Patterns of duck hepatitis B virus infection. Molecular Biology of Hepatitis B Viruses, University of California, Santa Cruz, USA. July, 1999.

Vickery, K., Welschinger, R., and Cossart, Y.E. Comparison of early patterns of duck hepatitis B virus (DHBV) infection with sequence changes. 10th International Symposium on Viral hepatitis and liver disease. Atlanta, USA. April, 2000.

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Welschinger, R., Pouliopoulos, J., Cossart, Y.E., and Vickery, K. The T-cell response of ducks to duck hepatitis B virus (DHBV) and the production of an associated DNA vaccine. MicroNZ 2003: A combined annual scientific meeting of the Australian and New Zealand Societies for Microbiology, Auckland, New Zealand. September, 2003.

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SUMMARY

Human Hepatitis B Virus (HBV) is a major global health problem affecting many millions of people. Individuals infected by perinatal transmission, become life long chronic carriers. They constitute a reservoir for the dissemination of infection, and many develop major health problems, such as cirrhosis, and hepatocellular carcinoma (HCC), later in life. Although new transmission can be limited by the use of a protein-based vaccine, the number of carriers continue to rise because the vaccine remains unavailable in many high prevalence, low-income areas. Treatment with nucleoside analogues and interferon is prolonged, expensive, and out of reach for most carriers. An inexpensive therapeutic vaccine which might be effective in established human carriers would have an immediate impact on a major global problem.

The first part of this study was undertaken to identify critical virus and host factors responsible for recovery from DHBV infection. The DHBV model has been pivotal in understanding the immunopathogenesis of hepadnaviral infections, and recent advances have opened the way to investigation of immunopathology.

Initially, the effect of age and dose on the kinetics, and outcome of infection was investigated, to define conditions where viral clearance could be studied. A biphasic pattern of infection was discovered, in which an initial peak of viraemia was cleared, only to be followed by rebound, and subsequent persistence. A mutation near the start of the surface open reading frame was identified in these cases, associated with attempted clearance of the infection. Transmission studies determined that the replication competency of the mutant genome was less than that of the wild type genome.

Because of earlier reports that immune response to DHBs predicted viral clearance, theoretical modelling of the surface gene was performed to determine the effect of the mutation on the genome, and associated polymerase protein. Immunogenic predictions for the S gene sequence were also undertaken and tested experimentally.

A lymphocyte proliferation assay was used to determine the CMI response of naïve, carrier, and protein vaccinated ducks to peptides spanning the surface protein. A DNA vaccine, was produced based on a polytope incorporating 7 peptides to which immune ducks selectively respond. This vaccine stimulated production of neutralising antibodies in naïve ducks, and also induced a 90% reduction in the average level of viraemia in chronically infected ducks. Such evidence suggests that co-operation of B- and T-cells occurs when these epitopes interact with the immune response.

A feature of the duck model system is that the cellular and humoral arms of the immune system can be modulated by surgical removal of the thymus, or bursa of Fabricius. The effect of reducing the total number of B- or T-cells on the outcome of DHBV infection was examined. Contrary to expectation, bursectomised ducks cleared the infection less efficiently than thymectomised ducks. While this indicates that antibodies play an essential role in clearance, such selective depletion of suppressor T-cells by thymectomy, may also promote removal of the virus.

The findings encourage further work into DNA vaccines with the expectation that incorporating a broader repertoire of peptides, in combination with cytokine sequences, will increase efficacy, to a level greater than current antiviral therapy.

CONTENTS

TABLE OF CONTENTS

TABLE OF FIGURES.

TABLE OF TABLES.

LIST OF ABBREVIATIONS

Abbreviation	Meaning
aa	amino acids
Ag	Antigen
cccDNA	Covalently Closed Circular DNA
CDx	Cell Differentiation marker x
CMI	Cell Mediated Immunity
conc	Concentration
DHBcAg	DHBV core Antigen
DHBsAg	DHBV surface Antigen
DHBV	Duck Hepatitis B Virus
ER	Endoplasmic Reticulum
FPV	FowlPoxVirus
HBV	Hepatitis B Virus
HCC	HepatoCellular Carcinoma
hr or hrs	Hour or Hours
id	intra-dermal
im	intra-muscular
ip	intra-peritoneal
IU	International Units
LB	Luria-Bertani media
MHC	Major Histocompatability Complex
min or mins	Minute or Minutes
o/n	Overnight
ORF	Open Reading Frame
PBMC	Peripheral Blood Mononuclear Cells
pi	post inoculation
RT	Room Temperature
s	Second
SMC	Spleen Mononuclear Cells
Th1 or Th2	T helper cell class 1 or 2
v/v	Volume per volume
vge	viral genome equivalents
vol	Volume
VV	Vaccinia Virus
w/v	Weight per volume

1. LITERATURE REVIEW

1.1. HUMAN HEPATITIS B VIRUS

1.1.1. Discovery and Historical Aspects

When acute icteric "serum hepatitis" was originally recognised as a complication of blood transfusion, it was attributed to transmission of a virus from the blood of healthy carriers (MacCallum and Bauer, 1944; MacCallum and Bradley, 1944). After more than 50 years of research there is still debate about basic mechanisms responsible for this dichotomy of clinical manifestations.

In 1963, a precipitating antibody from the blood of a haemophilia patient reacted with antigen in a serum sample from an Australian aborigine. It was named Australia antigen (Au), using the standard nomenclature for serum polymorphisms (Blumberg *et al.*, 1965). Almost simultaneously, SH antigen was described in acute phase fever from patients with post transfusion hepatitis (Prince, 1968a). When the identity of the relationship between SH antigen and Australia antigen was discovered they were renamed Hepatitis B surface Antigen (HBsAg) (Prince, 1968b).

Normal and diseased populations were then surveyed and it was shown that HBsAg was persistently present in serum of some healthy individuals and that infectivity survived for years in frozen or freeze dried serum samples. Other studies determined that the antigen is rare in normal populations from Northern America and Northern Europe, but common in tropical and Southeast Asian, the Pacific region and African populations (Prince, 1970b). These studies also noted that in Western countries the antigen was frequently to be found in patients who had been infused with various blood products for leukaemia and haemophilia (Blumberg *et al.*, 1967). Screening of blood donors was soon introduced (Prince, 1970a).

After observing laboratory transmission of Hepatitis B, Blumberg showed that HBsAg was present in serum from both acute hepatitis and various forms of chronic liver disease (Blumberg *et al.*, 1967). Fluorescently labelled antisera from HBsAg positive carriers was found to bind to the nucleus of hepatocytes of patients with HBsAg in their serum (Millman

et al., 1969), and this led to elucidation of the Hepatitis B core antigen/antibody system (Nowoslawski *et al.*, 1970).

Electron micrographs of Australia antigen were shown to have a 20nm virus particle-like appearance, with the additional presence of "sausage-shaped" and larger 40nm particles (Bayer *et al.*, 1968; Dane *et al.*, 1970).

In the late 1960s, isolated and partially purified particles from serum were used to transmit infection to non-human primates (marmosets, infant African green monkeys, and chimpanzees), and was subsequently passaged (Deinhardt *et al.*, 1967).

These studies opened the way to characterisation of the new agent and its disease associations. However, the virus remains uncultivatable in continuous systems, and the discovery of a related virus that infects ducks (DHBV) (Mason *et al.*, 1980; Wildner *et al.*, 1991), greatly facilitated studies of the molecular biology and pathogenic potential of the group. Hepadnaviruses have also been described in woodchucks, ground squirrels, herons, grey herons, snow geese, storks, cranes, and Ross Goose (Summers *et al.*, 1978; Marion *et al.*, 1980; Summers, 1981; Chang *et al.*, 1999; Pult *et al.*, 2001b; Prassolov *et al.*, 2003; Shi *et al.*, 2004).

1.1.2. Hepadnavirus Characteristics

1.1.2.1. Taxonomic Classification

HBV is the prototype of the *Hepadnaviridae* family of viruses characterised by a combination of morphological and genomic characteristics (ICTV, 2000) (Table 1, p.2), then subdivided into genus and species, on the basis of host range (Table 2, p.3).

Physical characteristics	Hepadnaviridae family
Nature of the genome	dsDNA-RT
Envelope	present
Morphology	Spherical
Genome configuration	circular
Genome size	3kb
Host	Vertebrates

Table 1. *Physical characteristics of the Hepadnaviridae family of viruses.*

Hepadnavirus genomes consist of a partially double-stranded circular DNA of approximately 3000-3200 bp, with a complete negative strand and approximately 55-90% of the positive strand. The 5' end of the negative strand is covalently bound to the terminal protein, which is produced by cleavage of the viral polymerase. The circular DNA encodes four overlapping Open Reading Frames (ORF): Surface (S), Core (C), Polymerase (P), and the X

gene (X), and three associated upstream regions (preC, preS1, and preS2), which are all located on the same (+) strand of DNA (Figure 1, p.4).

Numbering of the DNA sequence of the genomes begins at the unique EcoRI site. This form of numbering has lead to some confusion, because various subtypes do not have exactly the same number of nucleotides in various reading frames due to inserts or deletions.

Family	Genus	Virus	Host Species
Hepadnaviridae	Orthohepadnaviruses	HBV[1]	Human
		GSHV	Ground Squirrel
		WHV	Woodchuck
	Avihepadnaviruses	DHBV[2]	Duck
		HHV	Heron
		SGHV	Snow Goose
		SHBV	Stork
		CHBV	Crane

[1] Prototype of the Hepadnaviruses; [2] Prototype of the Avihepadnaviruses.

Table 2. *Taxonomic structure of the Hepadnavirus family.*

1.1.3. Virion Characteristics

The whole virion (Dane particle in HBV) is a 40–48nm sphere, which is composed of an envelope of lipid and viral proteins encasing a core, or nucleocapsid which encloses the DNA and the virus encoded RT-DNA polymerase. The physicochemical properties are listed (Table 3, p.3).

Physicochemical properties of Hepadnaviruses
Sedimentation constant 280.
Buoyant density in CsCl, 1.24-1.26 g cm^3.
Unstable at acid pH.
Ether soluble

Table 3. *Physicochemical properties of Hepadnaviruses.*

The icosahedral nucleocapsid is composed of 180 capsomers (mol. wt 22kD) arranged in a T=3 symmetry, which is surrounded by a 7 nm detergent-sensitive envelope composed mainly of two S molecules (24 and 27kD) derived from the host cell with virus enveloped insertions. The 27kD species has the same amino acid composition as the 24kD molecule but is glycosylated by N-linked glycans. In addition there are two preS2 proteins of mol. wts 33 and 36kD. These are composed of the 24kD protein with an additional 55 amino acids at the N terminus and two preS1 proteins of mol. wt 39 and 42kD, which have 120 extra amino acids. Host lipid is present in the virus and in the 22 nm surface antigen particles. The N terminus of the preS1 proteins is myristoylated. Other physicochemical properties have been described (Table 3, p.3).

The specific antigenic determinants of clinical and epidemiological importance include the '*a*' determinant, common to all HBsAg, and the *d, y, w,* and *r* determinants, located in the S region. The development of humoral immunity to HBsAg is protective, and recombinant HBsAg (S protein) provides the basis for the HBV vaccines currently available.

1.1.4. Characteristics of Genome

The hepadnaviruses have a characteristic genome (Figure 1, p.4), consisting of multiple overlapping reading frames encoding the Polymerase, Surface, and Core proteins, as well as the X protein in mammalian viruses.

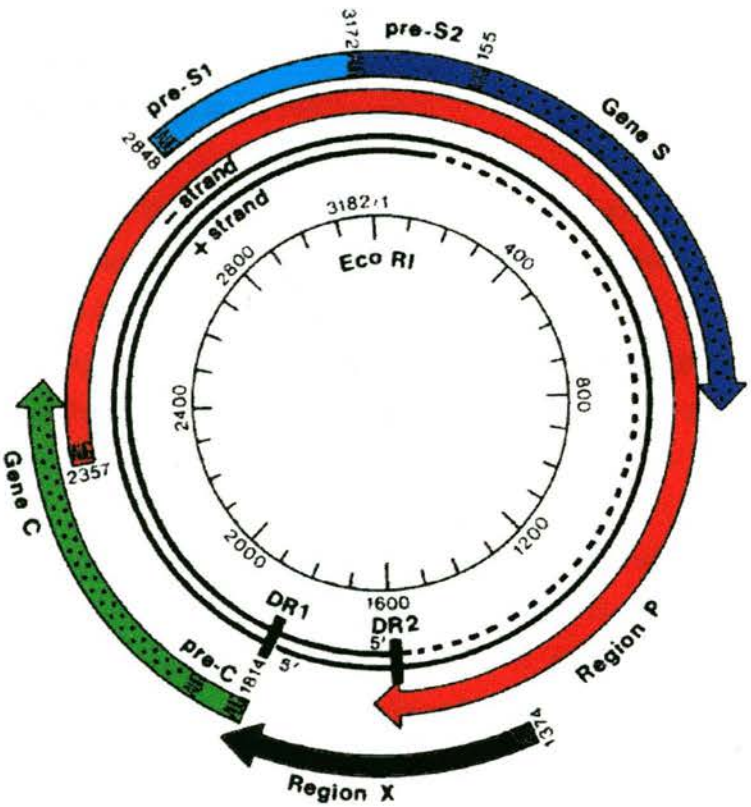


Figure 1. *Schematic diagram of the Hepadnavirus genome.*
Note the overlapping reading frames. Modified from published figures (Tiollais *et al.*, 1985; Bartenschlager and Schaller, 1993).

The long P gene encodes the DNA polymerase, which also serves a reverse-transcriptase function, since replication requires RNA intermediates. All three configurations of the HBV 'Surface' protein: large (preS1), middle (preS2), and major (S) proteins, are encoded by the Surface gene beginning transcription at nucleotide 2848, 3172, 155, respectively. Human HBV core gene contains two in-frame start codons. The shorter ORF produces the nucleocapsid (or Core protein, C) which form the basis of the core particle (HBcAg). The second ORF produces the preCore protein (preC), which contains an N-terminus addition to the core protein. This precore protein undergoes several cleavage steps to become HBeAg. The preC region product is required for the synthesis and secretion of hepatitis B e antigen

(HBeAg) (Ou *et al.*, 1986; Schlicht *et al.*, 1987b; Standring *et al.*, 1988). The X gene encodes two proteins that serve as transcriptional transactivators, aiding viral replication; these proteins may also play a part in the development of hepatocellular carcinoma. Several additional enhancer and promoter elements have also been identified within the genome.

1.1.4.1. Replication

Due to the strict species specificity and very restricted tissue tropism of HBV, conventional culture systems are not available for studies of replication. However, DHBV is more amenable, and the original description of hepadnavirus replication utilising reverse transcription of an RNA pregenome intermediate was made in this model (Figure 2, p.6) (Summers and Mason, 1982), followed by similar findings in HBV (Blum *et al.*, 1984; Miller and Robinson, 1984; Will *et al.*, 1987).

Following viral entry into the cell and uncoating, the viral DNA polymerase completes the plus strand of DNA leading to the formation of a covalently closed circular DNA that migrates to the cell nucleus. Cellular RNA polymerase transcribes the minus DNA strand, producing multiple copies of a 3.5kb RNA (pre-genome), and two subgenomic transcripts (2.1 and 2.4 kb). In the cytoplasm the core protein encapsulates the pre-genomic RNA, the viral DNA polymerase and a DNA-linked protein.

The pre-genome forms a template for reverse transcription and production of new HBV minus DNA strands as well as the synthesis of core, e antigens and polymerase proteins. As the minus DNA strand is synthesised the pre-genome is degraded except for a small fragment used to prime the synthesis of the plus strand using the minus strand as a template (Lien *et al.*, 1987; Will *et al.*, 1987). Envelope proteins are synthesised from the subgenomic transcripts and partially translocated across the endoplasmic reticulum membrane. HBcAg-derived peptides are expressed on the surface of hepatocytes by use of a signal sequence in the preC region which targets the protein for secretion (Standring *et al.*, 1988). The complete virion buds from the cell, receiving viral envelope proteins and host lipid simultaneously. DNA synthesis ceases when the virion is released from the cell containing the full length minus strand and a variable length plus strand. A small percentage of progeny viral cores containing relaxed circular DNA migrate to the nucleus to maintain the covalently closed circular (ccc) DNA pool (Miller and Robinson, 1984; Tuttleman *et al.*, 1986).

Hepadnavirus-cell interactions have a number of possible molecular outcomes, which include a) replicative infection with the production of many copies of single-stranded cytoplasmic viral DNA, cytoplasmic HBcAg and virion synthesis (Gowans *et al.*, 1985) and,

b) restricted infection of cells with limited viral genome expression, and, in mammalian hepadnaviruses c) integration of the viral genome into host cell DNA, with or without identifiable viral DNA replicative intermediates (Burrell *et al.*, 1984).

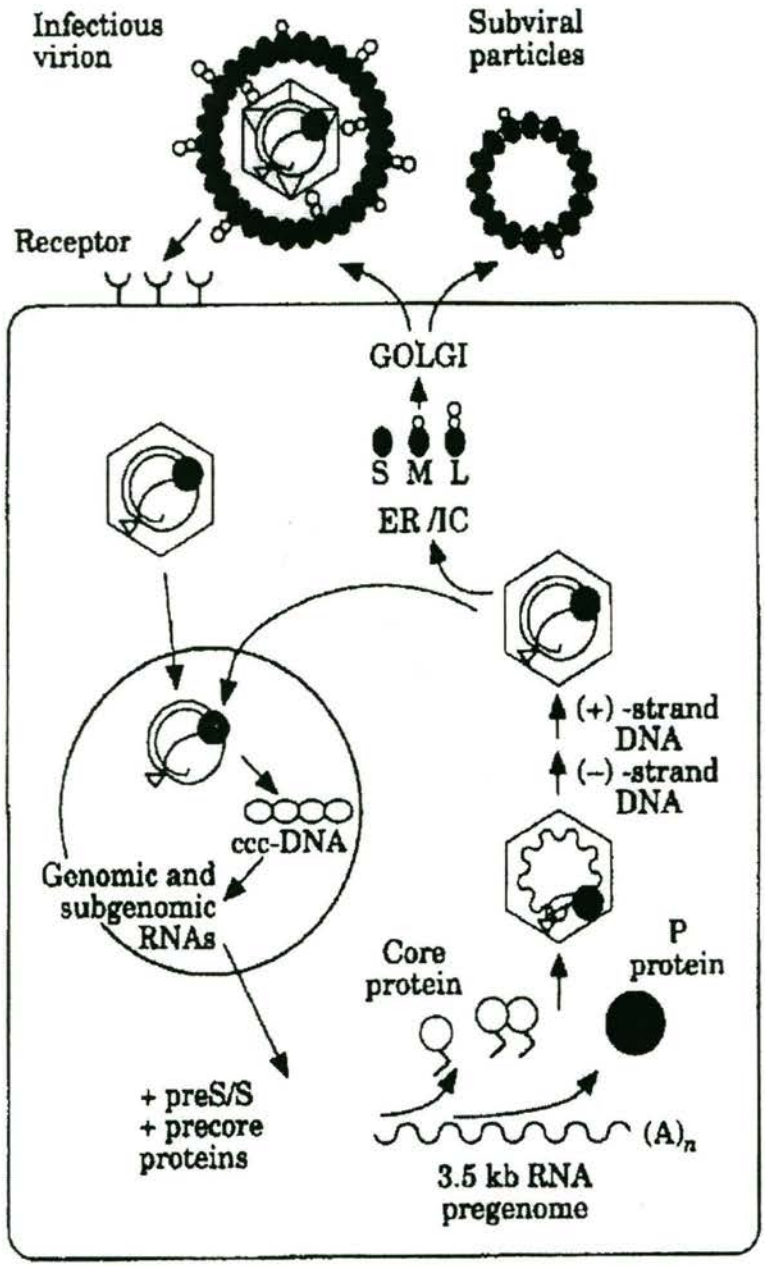


Figure 2. *Schematic view of the hepadnavirus life cycle.*

Infectious enveloped virions bind via the preS domain of the L protein to an uncharacterised receptor; capsids enter the cytoplasm, the DNA genome is transported to the nucleus, where the partially double stranded genome is completed becoming cccDNA. This serves as a template for transcription of genomic and subgenomic mRNAs which are translated in the cytoplasm. Core and Pol protein from the pregenome interact with the RNA forming new capsids. The RNA is reverse transcribed and the matured capsids either recycle the DNA back to the nucleus or are exported via interaction with the surface proteins at the membrane of the endoplasmic reticulum (ER), or intermediate compartment (IC). Empty envelopes (subviral particles) are secreted in excess over virions (Nassal and Schaller, 1996).

The presence and order of the genes for the principal viral components Core, Pol, preS-S (Gag, Pol, Env) are shared by hepadnaviruses and retroviruses. However, their replicative strategy is quite distinct. The extremely small size of the hepadnavirus genome has resulted in a largely overlapping arrangement of both coding regions and regulatory elements (Figure 1. p.4). In contrast to retroviruses, hepadnaviruses contain DNA rather than RNA; integration is not an obligatory step in replication; functional mRNAs are produced from several internal promoters on the circular DNA genome, and RNA splicing does not appear to play a critical role in the basic replication cycle.

1.1.4.2. Integration of Genome

Mammalian hepadnavirus integration in cellular DNA has been found in infected liver, as well as HCC. The possible role of integration in the development of HCC has been intensively investigated with much of the evidence of the structure of integration coming from investigation of HCC in humans (Nagaya *et al.*, 1987) and woodchucks (Ogston *et al.*, 1982), with less interest of viral integrations in non-tumourous liver (Ogata *et al.*, 1990). No apparent difference in the structure of viral integrations of HCC and non-tumourous liver have been identified. Hepadnavirus integration does not occur at a specific section or sections of the host genome, but tend to be randomly distributed. However, cis-activation of cellular oncogenes N-myc and c-myc by viral promoter insertion has been a common finding in woodchuck hepatitis virus associated HCC (Martinez *et al.*, 1994; Robinson, 1994). Some integrations consist of contiguous linear sections, while others are the result of complicated rearrangement and recombination (Matsubara and Tokino, 1990).

Complete viral genomes have not been found in any integrants, and deletions have been noticed in all integrants that have been sequenced, whether they arose from single or multiple genome integrations (Yaginuma *et al.*, 1987). The long terminally redundant HBV transcript that serves as a template for viral genomic DNA synthesis cannot be synthesised from such viral integrations and virtually all integrants are defective for virus replication. Thus hepadnavirus integrants are not involved in virus replication as is the integrated DNA provirus of retroviruses, but transcription and translation from integrated S sequences are observed in patients who have no evidence of ongoing productive infection (Yaginuma *et al.*, 1984; Mason *et al.*, 1998).

1.1.5. Infection Characteristics

Hepadnaviridae are capable of producing either acute self-limiting infection, or a persistent infection which may or may not be associated with liver disease (Robinson, 1977; Summers *et al.*, 1980; Ganem *et al.*, 1982; Marion *et al.*, 1983b).

Although the liver is the primary site of virus replication, hepadnaviruses have been found in pancreas, spleen, kidney, bile duct epithelial cells, and even skin (Shimoda *et al.*, 1981; Halpern *et al.*, 1983; Dejean *et al.*, 1984; Halpern *et al.*, 1986; Jilbert *et al.*, 1987b; Jilbert *et al.*, 1988; Nicoll *et al.*, 1997).

1.1.5.1. Acute Infection

Mammalian and avian hosts infected post-infancy develop acute hepadnavirus infection, which resolves in the face of a vigorous polyclonal and multi-specific host response. The appearance of surface antigen (and viral DNA in the serum), precedes the development of anti-core antibody. Elimination of infection is mediated by the immune response, through T-cell dependent activation of both antibody production and induction of immunomodulating factors such as Interferon (IFN). In acute infection, the disappearance of HBsAg is normally associated with the appearance of anti-HBs Antibody.

The absence of serum markers does not necessarily preclude virus persistence in the liver. This may be infectious as shown by reports of transmission of infection by transplantation of liver from a patient that has cleared their infection from the serum (Chazouilleres *et al.*, 1994), and reactivation of viraemia in anti-HBs positive patients who undergo immunosuppressive treatment (Nagington, 1977; Nagington *et al.*, 1984).

1.1.5.2. Persistent Infection

Persistence is conventionally defined as persistent viraemia of greater than six months duration whether or not it is associated with progressive liver damage. The mechanisms which determine persistence or clearance of hepadnavirus infection remain controversial, and the reason for the occasional spontaneous elimination of virus after many years of persistence is also unclear.

The importance of host immunity on the outcome of infection is best illustrated by the difference in the level of persistence between infection as a neonate, or as an adult. Infants, (possessing a naïve immune system), that are perinatally infected will develop a persistent infection in 95-100% of cases, while adults, (possessing a more mature immune system), develop persistence in only 5-10% of cases (Beasley *et al.*, 1982). The same age-related effects have been demonstrated with DHBV (Mason *et al.*, 1980; O'Connell *et al.*, 1983; Urban *et al.*, 1985; Jilbert *et al.*, 1992; Vickery and Cossart, 1996; Jilbert *et al.*, 1998), while self-limited acute infection has also been seen in woodchucks (Ponzetto *et al.*, 1984).

Another interesting observation, is that irrespective of the cause of the T-cell deficiency, (natural, such as tolerance or MHC restriction, or induced, such as immunosuppressive drugs

for transplant recipients etc.), the outcome of infection and the development of persistence is invariable (Planz *et al.*, 1996), while this is not the case for B-cell deficiencies.

1.1.6. Clinical Features and Outcome of HBV Infection

The clinical features and outcome of HBV infection differ according to the virus dose and the efficiency of the host response, both specific and non-specific. Early transmission studies in man revealed that the outcome and severity of hepatitis B is not dependent on the virus strain as some volunteers developed asymptomatic carriage while some developed severe hepatitis (MacCallum and Bauer, 1944; MacCallum and Bradley, 1944). Most adults infected with HBV develop an acute illness and recover within 6 months. A minority develop fulminant hepatitis and die, while up to 10% (mainly males), become chronic carriers. In contrast, chronic HBsAg carriage occurs in 90-100% of infected neonates (Beasley and Hwang, 1983), 20-30% of young children (Beasley *et al.*, 1982).

Natural clearance is frequently associated with changes in the hepatitis B core gene sequence. Core gene sequence is relatively stable and mutations are rarely detected in patients who are still in highly viraemic phase of infection but very high rates of changes were found during the immune clearance (Bozkaya *et al.*, 1996). After HBsAb seroconversion, a progressive and sufficient decrease of hepatitis B core antibody can predict the disappearance of hepatitis B virus DNA in Japanese patients with hepatitis B surface antigen clearance (Kobyashi *et al.*, 2000).

Serious sequelae can still develop in chronic HBV patients that clear sAg. A study in Taiwan of 1,355 chronic carriers from 1985 to 1997, found spontaneous HBsAg clearance in 55 patients. During a mean follow-up period of 23 months, 18 (all male) of the 55 developed serious complications, including 11 with HCC (9 underwent surgical resection), 6 with cirrhosis, and 1 with subfulminant liver failure (Huo *et al.*, 1998).

1.1.7. Immune Response to HBV

1.1.7.1. Non-specific responses

The incubation period from exposure to hepatitis is between 2 to 6 months (Howard, 1986). During the first few weeks of acute hepatitis there is an increase in the natural killer cell (NK) activity (Chemello *et al.*, 1986). Once viraemia occurs there is a transient increase in alpha-interferon (α-IFN) (Pignatelli *et al.*, 1986). IFN-α induces the hepatocytes to display major histocompatibility complex 1 (MHC 1) in conjunction with viral peptides on the cell surface permitting cytotoxic T-lymphocytes to clear infected hepatocytes (Grandits *et al.*, 1991), leading to elimination and recovery. IFN-α has been shown to decrease viral DNA levels in a few of the hepadnaviruses, such as woodchucks (Salucci *et al.*, 2002), and the

transgenic mouse models. Large antigens are broken down into smaller fragments prior to the macrophage presenting specific regions of the antigen to the lymphocytes (Unanue, 1980). It is known that HBV can itself alter the cellular response to interferon inducing low expression of HLA molecules (Onji *et al.*, 1989) and that the core protein of HBV can inhibit the production of interferon-beta (IFN-ß) (Whitten *et al.*, 1991).

1.1.7.2. The Humoral immune response

HBsAg is the first marker to appear in the serum and remains until recovery making it the most suitable and common serological marker for clinical diagnosis of HBV (Nordenfelt, 1975). The HBV DNA, HBeAg, DNA polymerase and anti-HBc then appear signalling the presence of mature virus and infectivity. The different immune responses for acute and persistent infection are shown diagrammatically (Figure 3, p.11).

Pre-S1 and pre-S2 antibodies also appear early in infection (Neurath *et al.*, 1985), and have a good correlation with HBV DNA detection. Pre-S1 binds the virus to the hepatocyte (Neurath *et al.*, 1986c), and so these antibodies may help prevent spread of the virus to other uninfected hepatocytes (Grandits *et al.*, 1991). The pre-S2 has a polymerised human serum albumin binding site (Michel *et al.*, 1984) which also has been postulated to be involved in viral binding to the hepatocyte (Machida *et al.*, 1984). Immunisation of chimpanzees with pre-S2 specific synthetic peptides or incubation of HBV with antibodies to these peptides was shown to be protective (Itoh *et al.*, 1986; Neurath *et al.*, 1986b; Emini *et al.*, 1989; Neurath *et al.*, 1989).

High titres of IgM anti-HBc are indicative of acute HBV infection in most patients, in turn developing into IgG anti-HBc, which can persist for many years (Hoofnagle *et al.*, 1973). IgM anti-HBc detected in chronic infection represents active viral replication (Sjogren and Hoofnagle, 1985) or induction by corticosteroid therapy for symptomatic flare in a chronic carrier (Alexander, 1990).

Development of anti-HBe correlates with the loss or a substantial reduction in viral replication, coincident with a rise in aminotransferase (ALT) levels, due to lysis of infected hepatocytes which is followed by a histologic improvement in liver disease. This recovery phase occurs weeks or months following anti-HBc production in acute hepatitis while it may never occur in chronic hepatitis (Realdi *et al.*, 1980). In most murine strains HBcAg and HBeAg are equivalently immunogenic and crossreactive at the level of T-cell activation (Milich *et al.*, 1988). HBcAg is both a T-cell dependent and independent antigen and as such can induce efficient antibody production in athymic mice (Milich and McLachlan, 1986) while HBeAg is strictly T-cell dependent, and thus less efficient at inducing an antibody response.

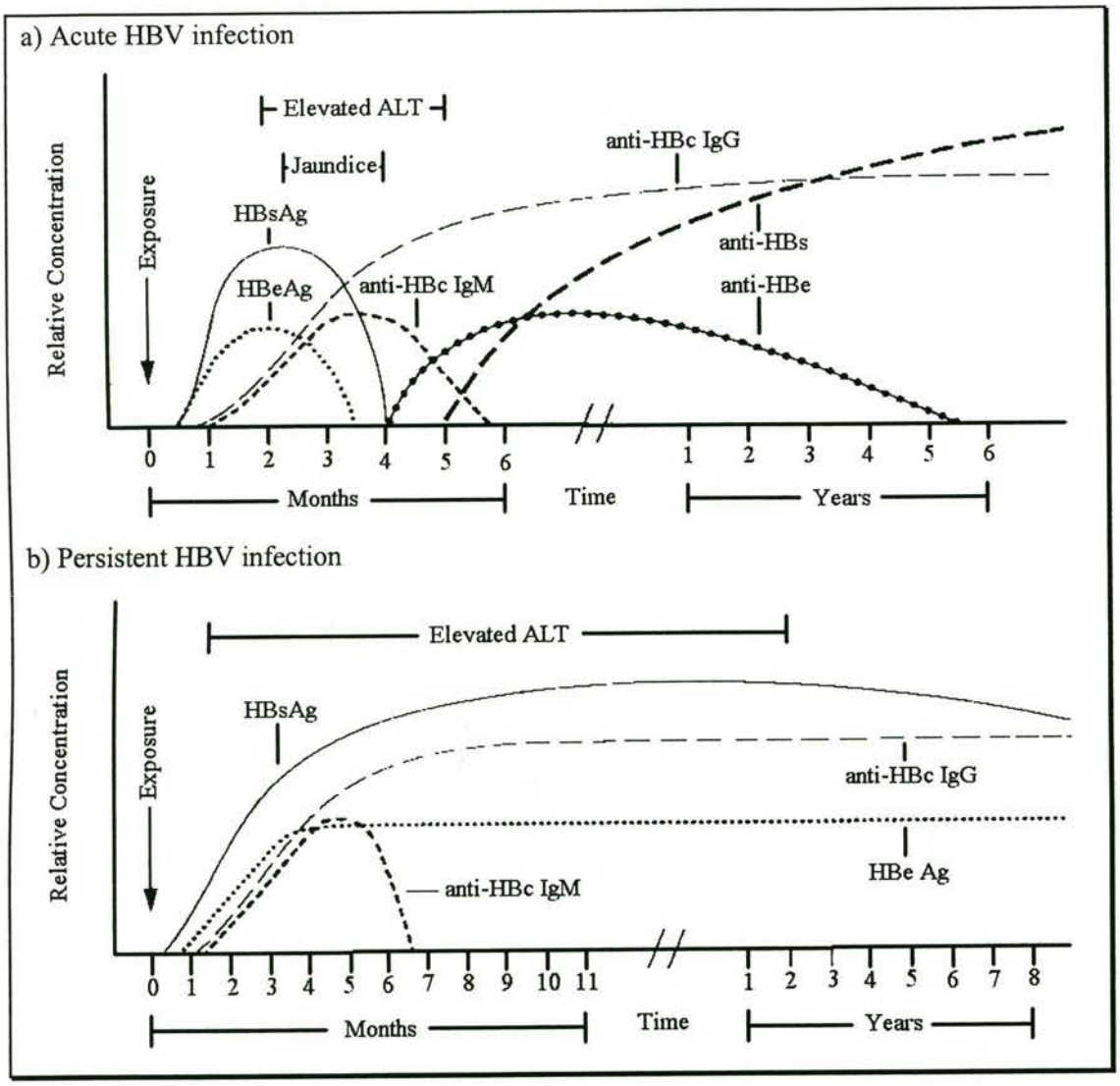


Figure 3. *Time course of HBV infection.*
(a) Acute infection, (b) Persistent infection. In chronic infection there is very little, if any, production of anti-HBeAg or anti-HBsAg.

The last antibody to appear is anti-HBs and its appearance usually indicates HBV recovery from infection and immunity. Anti-HBs antibodies are readily detectable in patients who clear the virus and recover from acute hepatitis, while they are usually undetectable in patients with chronic HBV infection, they are thought to play a critical role in viral clearance by complexing with free viral particles and removing them from circulation or possibly by preventing their attachment and uptake by susceptible cells. They also contribute to the pathogenesis of the extrahepatic syndromes associated with HBV infection (glomerulonephritis, cryoglobulinemia, polyarteritis nodosa) and to the prodromal syndromes of urticaria and arthralgias, by forming antigen-antibody complexes.

The role of the antibody response to the HBV nucleocapsid antigens (HBcAg and HBeAg) in HBV pathogenesis is not clear. It is generally accepted that they do not neutralise viral infectivity because they are present in high titres not only during acute hepatitis but also in

patients with chronic HBV infection. Interestingly, administration of anti-HBe antibodies, prolonged the incubation period of HBV in experimentally infected chimpanzees (Stephan *et al.*, 1984), suggesting that they may play some currently obscure role in HBV neutralisation. Because the T-cell response to HBc/eAg is strong during acute hepatitis and weak in chronically infected patients, the prevalence of a strong antibody response to HBcAg in chronically infected patients may be due in part to the fact that it can function as both a T-cell-independent and a T-cell dependent antigen (Milich and McLachlan, 1986).

Antibody responses to the polymerase and X proteins have been less well studied. The carboxy-terminus of polymerase, especially its RNAse H domain, appears to be immunodominant at the antibody level, and these antibodies may serve as early markers of infection and may reflect ongoing viral replication (Weimer *et al.*, 1990). While antibody response to the viral transactivator protein (pX), is principally associated with chronic hepatitis and HCC (Moriarty *et al.*, 1985; Stemler *et al.*, 1990; Vitvitski-Trepo *et al.*, 1990).

1.1.7.3. The Cell Mediated Immune Response

The CTL response to HBV is vigorous, polyclonal, and multispecific in patients with acute hepatitis who ultimately clear the virus, and it is weak or barely detectable in patients with chronic hepatitis (Bertoletti *et al.*, 1991; Missale *et al.*, 1993; Nayersina *et al.*, 1993; Rehermann *et al.*, 1995), except during acute exacerbations of chronic disease or after spontaneous or IFN-α induced viral clearance (Rehermann *et al.*, 1996b). Despite the vigour of the T-cell response to HBV during acute viral hepatitis, very low levels of virus persist in the circulation for several decades after complete clinical and serological resolution of disease (Rehermann *et al.*, 1996a). Long-term persistence of trace amounts of viral DNA is associated with equally long-term persistence of HBV-specific CTL that display recent activation markers. This suggests that transcriptionally active virions can apparently maintain the CTL response indefinitely after recovery, perhaps for life (Rehermann *et al.*, 1996a). Clinical reports that occult HBV may be responsible for transmission of virus to liver transplant recipients (Chazouilleres *et al.*, 1994), and after blood transfusions from HBV seronegative subjects (Thiers *et al.*, 1988), support the notion of incomplete viral clearance after recovery from acute viral hepatitis.

Studies with overlapping synthetic peptides have delineated some of the HLA restricted T-cell epitopes (eg. an HLA-A2 restricted epitope has been mapped to aa 18-27 of HBcAg), and aa 141-151 to both HLA-A31 and HLA-Aw 68 (Penna *et al.*, 1991). While study of T-helper cells (Th) has identified three HLA class II restricted immunodominant epitopes (Ferrari *et al.*, 1991), and one of these partly overlaps a HLA-A2 restricted CTL epitope (Penna *et al.*, 1991). These studies indicate that HBcAg can be a stimulus for both helper

and cytotoxic T-cells. Recent studies have suggested that treatment outcomes may depend on the development of type 1 T-helper responses, as activation of Th1 immunity accompanied by enhancement of CTL activity during therapy was a common immune mechanism associated with successful treatment not only of HBV, but also Hepatitis C Virus patients (Tsai *et al.*, 2003).

Development of transgenic mice provided more evidence of an association between liver disease and the CTL response during acute HBV infection, suggesting an important role for CTL in the pathogenesis of acute viral hepatitis. In mice that express and replicate HBV in their hepatocytes, it was found that they develop an acute necro-inflammatory liver disease after adoptive transfer of HBs antigen-specific CTL lines and clones (Moriyama *et al.*, 1990; Ando *et al.*, 1993). It has been shown that HBV gene expression and replication can be completely abolished in all of the transfected hepatocytes in the liver by a non-cytopathic antiviral process in which the viral nucleocapsids disappear from the cytoplasm and the viral RNAs are degraded in the nucleus of the hepatocytes under conditions in which <1% of the hepatocytes are destroyed (Guidotti *et al.*, 1996b). Thereafter, all of the viral gene products and virions disappear from the liver and the serum in the absence of serum transaminase elevations or histological evidence of liver disease (Guidotti *et al.*, 1996b). Viral clearance in this model is completely blocked when antibodies to IFN-γ and TNF-α are injected before the CTL, indicating that these cytokines are responsible for the antiviral effect.

A corollary of this observation would be that superinfection of the liver by other hepatotropic viruses might lead to the clearance of HBV if they induce the production of antiviral cytokines to which HBV is susceptible. These events have been shown to occur in the HBV transgenic mice during lymphocytic choriomeningitis virus infection (Guidotti *et al.*, 1996a). Isolated case reports have been published suggesting that superinfection by HAV is sometimes associated with clearance of HBV in chronically infected patients (Davis *et al.*, 1984). In contrast, co-infection of HBV and HCV has been associated with increased liver failure (Pouteil-Noble *et al.*, 1995), hepatocarcinogenesis (Koike, 1999), and chronic liver disease (Bukhtiari *et al.*, 2003).

These results suggest that a strong intra-hepatic CTL response to HBV during acute viral hepatitis can suppress HBV gene expression and replication and perhaps even "cure" infected hepatocytes of the virus in addition to killing them. Conversely, a weak immune response, such as that which occurs in chronically infected patients, could contribute to viral persistence and chronic liver disease by reducing the expression of viral antigens sufficiently for the infected cells to escape immune recognition but not enough for the virus to be eliminated. Therefore, the ability of CTL derived cytokines to inhibit HBV replication could

represent a survival strategy by the virus, contributing to persistence, or a tissue-sparing antiviral strategy by the host, contributing to viral elimination.

1.1.8. Mechanism of viral persistence

Elements of the innate, specific T-cell, and humoral responses are involved.

1.1.8.1. Specific T-cell response

Viral persistence is probably related to a specific failure of T-cells to recognise HBV antigens. This assumption is supported by the clinical observation that patients with a relative deficit in T-cell function (young, elderly, and immunosuppressed), are more prone to develop chronic HBV infection. *In vitro* peripheral blood T-cell activation is impaired in patients with chronic HBV infection but this is not associated with clinical evidence of immune deficiency, suggesting a redistribution of primed T-cells from the circulation to the liver.

1.1.8.2. Innate immunity

The finding of defective α-interferon production in patients with chronic HBV infection (Kato *et al.*, 1982; Abb *et al.*, 1985), and reduced capacity to produce α- and γ-interferon which is unrelated to the level of viral replication and the severity of liver disease (Ikeda *et al.*, 1986), has led to the hypothesis that this may be a primary defect which could be instrumental in the early stages of infection leading to persistence (Ikeda *et al.*, 1986). Alpha-interferon has immunomodulatory properties, and also stimulates the display of human leukocyte antigen class 1 (HLA-1) antigens on cell surfaces (Heron *et al.*, 1978), and should thereby enhance the presentation of viral antigens to cytotoxic T-cells. However, there is conflicting data regarding the levels and role of IFN-α. IFN-α was rarely detected in the circulation during chronic hepatitis B and virus-stimulated production of IFN-α was reduced in circulating mononuclear cells (Ikeda *et al.*, 1986) in one study. While in another study, IFN-α production was not significantly altered during HBV infection. IFN-α induces 2'5'-oligoadenylate synthetase, and levels of this enzyme in liver, and circulating mononuclear cells were found to be higher in patients with acute and chronic HBV infection, than in healthy controls, or interestingly, patients with HBV-related chronic active hepatitis, which have normal levels (Heathcote *et al.*, 1989). An alternative could be that the production of IFN-α by circulating cells may have been down regulated during its passage through the liver (Nouri-Aria *et al.*, 1991). Further complicating the issue, the HBV core gene has been found to suppresses the IFN-β gene in mouse fibroblasts (Twu *et al.*, 1988; Twu and Schloemer, 1989).

HBV has also been shown to reduce the cell's sensitivity to IFN-α, as when a HBV containing vector was transfected into an IFN-α sensitive cell line, the response to exogenous IFN-α was reduced (Onji *et al.*, 1989). Subsequently, it was found that the terminal protein of the HBV polymerase inhibited the response to not only IFN-α but also IFN-γ (Foster *et al.*, 1991). This may be one of the reasons that perinatally infected HBV carriers take many years to seroconvert from HBeAg positive to HBeAb positive, which occurs during the teens with transient hepatitis and appearance of mutant virus (Shimoyama and Sekiguchi, 1996).

The high incidence of chronic HBV carriage in babies born to HBeAg+ mothers suggests that circulating e antigen in the mother induces immunotolerance in the baby. In newborn transgenic mice that produce HBeAg, both HBeAg and HBcAg are tolerant at the T-cell level (Milich *et al.*, 1990), however these mice produce core, but not e antibody. The maintenance of T-cell tolerance was broken only when HBeAg had been withdrawn for more than 16 weeks. The close resemblance in the chronology of immunological events in HBeAg-expressing transgenic mice and in human HBV infection suggests that one function of e antigen may be to induce immuno-tolerance *in utero*, favouring the persistence of HBV in infancy and childhood. It has been demonstrated that patients with HBeAg negative HBV infections have a high rate of mutations present in the Core region (Thakur *et al.*, 2003), and the effect of these mutations on transmission rates is unknown. However transmission of preCore mutants does produce familial clustering of HBV infections (Santantonio *et al.*, 1997), similar to wild-type.

1.1.8.3. Tolerance

Tolerance is when the host does not mount an immune response to an antigen that is not 'self', and can be achieved during the negative selection phase of immune cell maturation. The specificity of a host's immune cells is tested before they are allowed into the circulation, in such a way that immune cells which are found to react to the host's normal cells are eliminated before they are released, thus stopping the host from producing an immune response which would destroy its own cells. Tolerance to an infection is achieved by this negative selection, which eliminates immune cells capable of reacting to the infection, thus leaving the host unable to mount an effective immune response to the infection.

Tolerance leading to persistence is normally obtained by parenteral transmission of the virus to the host during the early stages of life, when the negative clonal selection is most vulnerable, but can also be induced later in life. It is considered that persistence is established by a lack of the immune response to effectively eliminate infected hepatocytes. Although an alternative reason for persistence may be that the virus is able to change its

physical characteristics, such as developing a mutant genome, which is able to evade immune recognition, such as the truncated preCore mutants of HBV. This tolerance, be it natural or induced, as in individuals with impaired CMI response (eg. dialysis and transplant patients), is generally associated with persistent high titre viraemia, however there is usually very little acute liver disease (Alexander, 1990). This would indicate that the host's immune response to the infection could sometimes do more damage to cells than that caused by the virus.

Tolerance is not an eternally stable situation, and can be altered at any time, which is clearly demonstrated by some of the autoimmune diseases of humans, such as celiac disease, which is triggered by the ingestion of gluten (Bizzaro *et al.*, 2003), inducing an immune response that then targets the host's own antigens (Salaman, 2003). The onset of this intolerance is unknown, but can also be seen in HBV infection, when a chronic carrier spontaneously develops an immune response that is capable of clearing the virus (Hsu *et al.*, 2002), which is aided with successful treatment (Heathcote, 2003).

1.1.8.4. Humoral responses

In addition to the cellular immune reactions responsible for clearance of infected hepatocytes, neutralising antibodies are required to prevent spread of released virions to uninfected liver cells. A defect in viral clearance could be responsible for persistence of virus infection in chronic carriers.

Alberti *et al.,* first identified antibodies binding selectively to complete virions ('anti-Dane' antibodies) in sera early in acute hepatitis B (Alberti *et al.*, 1978). Observations that polymerised human serum albumin bound to the preS2 region (Machida *et al.*, 1984), lead to a hypothesis to explain the hepatotropism of HBV (Thung and Gerber, 1984), and anti-preS2 antibodies being neutralising. However, it is unclear whether such polymerised albumin exists *in vivo* in sufficient amounts to act as the proposed bridge between virions and hepatocytes (Yu *et al.*, 1985).

Whether anti-preS2 antibodies appear at the time of virion clearance, or later, is unknown (Alberti and Pontisso, 1987). Neurath *et al.*, using a model system to investigate hepatotropism of HBV, suggested that binding of virions to HepG2 cells is via sites predominantly in the pre-Sl region, and interestingly preS1 expression seems to be largely confined to envelope proteins of complete virions (Neurath *et al.*, 1986c).

1.1.9. Mechanisms of Liver Injury

A significant minority (up to 25%) of persistently infected HBV carriers develop severe pathologic consequences, including chronic hepatitis, cirrhosis, and hepatocellular carcinoma

(HCC) (Ryu, 2003). Despite many available treatment options, the prognosis of patients with HCC remains poor; surgical resection or liver transplantation still represents the only potentially curative treatments for HCC (Zhu, 2003).

There are major logistic and ethical problems in setting up studies of cytotoxic immune responses during early acute human HBV infection. Contact tracing was used to identifying five individuals in early stage acute HBV infection (Vento *et al.*, 1987). The first cellular immune response in these patients was to pre-S antigen, followed by HBcAg 10 days later, at which time IgM anti-HBc antibodies appeared in the serum, and then just prior to liver damage a cellular immune response to HBsAg was discovered. This HBsAg cellular immune response is absent during persistent infection (Vento *et al.*, 1985), and may be involved in not only the production of liver damage during acute HBV infection but also be of critical importance in determining recovery.

The search for an immune target in chronic HBV infection has centred on hepatocytes expressing HBcAg. Cytotoxic T-cells in the peripheral blood of chronic HBV carriers recognise nucleocapsid components of HBV on the surface of infected hepatocytes (Mondelli *et al.*, 1982; Pignatelli *et al.*, 1987; Bertoletti *et al.*, 1991), these findings have been corroborated in the woodchuck model (Shanmuganathan *et al.*, 1997). In cytotoxicity experiments, T-cells from patients with chronic liver disease lysed hepatocytes that expressed HBcAg, and this cytotoxicity could be blocked by antibody to HBcAg or by HLA class I molecules (Chu *et al.*, 1988). There is some evidence that anti-HBe can also block cytotoxicity, while no response to HBsAg was demonstrated. Immunohistochemical studies showed that cytoplasmic expression of core, but not surface, correlates with disease activity (Chu and Liaw, 1987).

Examination of the peripheral blood may give an imperfect view of the cells directly involved in hepatocyte damage, and most studies have concentrated on a phenotypic and functional analysis of lymphocytes in areas of liver necrosis. Direct immunofluorescence examination of liver biopsies has shown that T-lymphocytes of the CD8+ cytotoxic/ suppressor type predominate in areas of liver cell destruction in chronic hepatitis B (Eggink *et al.*, 1982).

Clonal expansion of cells from liver biopsies, has confirmed their cytotoxic potential. T-cells, incubated with IL-2 and a mitogen, were used to obtain clones which express cytotoxic effector function to heterologous rat hepatocytes and have suggested that secreted T-cell products may be responsible for hepatocyte injury whether or not the lymphocytes are recognising specific antigens on hepatocytes (Ramadori *et al.*, 1987). Similar T-cell lines

have been established from liver biopsies by stimulation with IL-2 and HBV antigens (Ferrari *et al.*, 1987). A mixture of CD4+ and CD8+ lines were obtained from which CD4+ HBcAg-specific T-cell clones have been derived. The full functional repertoire of these cells is still unknown.

The importance of T-cell responses to nucleocapsid antigens in the pathogenesis of liver damage in chronic HBV infection has overshadowed interest in the significance of T-cell responses to envelope antigens and their potential role in virus clearance.

Mediation of hepatocellular damage in chronic infection may also be attributed to the recruitment cells that form part of the non-specific immune response (Guidotti, 2002). Non-T lymphocytes cytotoxicity can be blocked by liver-specific membrane lipoprotein (LSP), aggregated IgG, or the $F(ab')_2$ fragment of anti-human IgG, suggesting that they may direct an antibody-dependent cell-mediated cytotoxicity against a component of LSP (Mieli-Vergani *et al.*, 1982). It has also been observed that when the HBV-specific CD8+ response is unable to control virus replication, it may contribute to liver pathology not only directly, but also by causing the recruitment of nonvirus-specific T-cells (Maini *et al.*, 2000).

Cytokines are also likely to be involved (Lau *et al.*, 1991; Schulte-Frohlinde *et al.*, 2002); in patients with HBV-related active liver disease, IFN-α is produced locally in the liver, and production of IL-1 and TNF-α by peripheral blood mononuclear cells are also increased. The possibility that these are non-specific consequences of inflammation remains to be excluded.

Abnormalities of lymphocyte proliferation in chronic HBV infection are well documented (Hanson *et al.*, 1984; Anastassakos *et al.*, 1987; Anastassakos *et al.*, 1988), but the underlying mechanisms are poorly understood, and it remains to be determined whether they are of primary importance in the failure of viral clearance or secondary to chronic liver damage. Lymphocyte proliferation to mitogens and antigens is defective, but although IL-2 production is decreased (Saxena *et al.*, 1985), exogenous IL-2 or IL-1 is unable to correct the low proliferative response (Anastassakos *et al.*, 1987; Anastassakos *et al.*, 1988).

Anastassakos *et al.*, also demonstrated that IL-1 production by monocytes is high, particularly in those with cirrhosis (Anastassakos *et al.*, 1987; Anastassakos *et al.*, 1988). One of the recognised biological properties of IL-1 is to stimulate fibroblasts to produce collagen (Dinarello, 1984), and it is of some interest that there was a rather close correlation in this study between IL-1 production and severity of fibrosis.

Although the evidence strongly suggests that HBV causes hepatocellular damage through an immune-mediated mechanism, other factors may be involved. Furthermore, high-level expression of HBsAg is associated with hepatocellular degeneration and necrosis in transgenic mice (Chisari *et al.*, 1987), and over-expression of HBcAg in a hepatoblastoma line induces cytopathic changes (Roingeard *et al.*, 1990). In patients transplanted for chronic hepatitis B, recurrence of infection is associated with a novel histological pattern (fibrosing cholestatic hepatitis) and fulminant clinical course, and in this situation HBV may be directly cytopathic (Lau *et al.*, 1992).

1.1.10. Diversity of HBV strains

The discovery of the Australia antigen by Blumberg, initiated systematic studies that eventually revealed that this antigen represented the surface protein of the hepatitis B virus (HBV) produced in excess as compared to the complete virions. Early on there were hints for the immunological heterogeneity of the antigen (Levene and Blumberg, 1969; Raunio *et al.*, 1970), although the fact that these variants represented genetically stable variants of the virus was not realised until later.

The occurrence of nine different subtypes of HBsAg reflecting genetic variability of HBV has been documented for a long time. The subtypes were *ayw1*, *ayw2*, *ayw3*, *ayw4*, *ayr*, *adw2*, *adw4*, *adrq*$^+$, and *adrq*$^-$ (Courouce *et al.*, 1976; Courouce-Pauty *et al.*, 1978). The entire nucleotide sequences of 18 human HBV genomes of various subtypes were classified into four genetic groups designated A-D based on an intergroup divergence of 8% or greater of the complete nucleotide sequence (Okamoto *et al.*, 1988). Two new genomic groups designated E, and F, were later identified on the basis of the variability in the Surface gene of genomes encoding the subtypes *ayw4*, and *adw4* (Norder *et al.*, 1992). The sequence divergence of a seventh genotype (G) was later determined (Kato *et al.*, 2001).

The identification of the two pairs of allelic variations, *d/y* (Le Bouvier, 1971), and *w/r* in the following year (Bancroft *et al.*, 1972), defined of the four major subtypes of hepatitis B surface antigen (HBsAg). These subtypes were *adw*, *adr*, *ayw* and *ayr*, where *a* was defined as the common determinant of all the subtypes (discussed in more detail in 1.1.10.2.1, p.21). It was observed early that the *ayw* subtype was the one found among i.v. drug users worldwide, with other subtypes related to specific geographic regions (Section 1.1.10.1, p.20).

With the description of four subdeterminants of *a*, later redefined as subdeterminants of *w* (*w1-w4*) at an international workshop in Paris in 1975 (Courouce, 1976; Courouce *et al.*, 1976), the issue of HBsAg subtypes acquired a considerable degree of complexity. These

subtypes were *ayw1*, *ayw2*, *ayw3*, *ayw4*, *ayr*, *adw2*, *adw4* and *adr*. With the identification of the *q* determinant (Magnius *et al.*, 1975) the number of subtypes increased from eight to nine, due to the subdivision of the *adr* subtype into a *q*-positive and a *q*-negative category (Courouce-Pauty *et al.*, 1978). Due to lack of reagents and the demand for experience in techniques such as immunodiffusion and IEOP, at that time mostly abandoned as routine diagnostic procedures for the demonstration of HBsAg, typing for nine different serotypes never became introduced outside the laboratory where it was once established. Indirect evidence such as from signature analysis of monoclonal antibody reactivities have, however, confirmed the existence of nine different subtypes (Wands *et al.*, 1984). Also the reactivity patterns obtained with sets of monoclonal antibodies provided an opportunity to identify some of them (Swenson *et al.*, 1991).

1.1.10.1. Geographic diversity

Geographic prevalence of HBV was investigated (Prince, 1970b), and it was soon determined that various subtypes of HBV are associated with various geographical regions. The *adw* subtype was found to be the dominant type among the carriers in North-Western Europe (Schmidt *et al.*, 1972; Magnius *et al.*, 1973; Mazzur *et al.*, 1974), while the *r* determinant subtypes were exclusively confined to populations of the Far East (Courouce and Soulier, 1974; Mazzur *et al.*, 1974). A more precise study to define the worldwide distribution of HBV subtypes was undertaken in a large study during the early 1980s (Courouce-Pauty *et al.*, 1983), while more recent data has been reviewed (Robertson and Margolis, 2002) (Table 4, p.20, and Figure 4, p.21).

Genotypic group	Subtype	Areas of high prevalence
A	adw2	North-Western Europe
	ayw1	Central Africa
B	adw2	China, Indonesia
	ayw1	Vietnam
C	adw2	East Asia
	adrq+	Korea, China, Japan
	adrq-	Polynesia
	ayr	Vietnam
D	ayw2	Mediterranean area
	ayw3	India
E	ayw4	West Africa
F	adw4	American Natives, Polynesia

Table 4. *Geographic Distribution of HBV genotypes and subtypes.*

1.1.10.2. Molecular Basis for the Major Subtypic Variations

Sequencing of complete genomes encoding *adw2* and *ayw3* subtypes revealed numerous substitutions throughout the genome (Galibert *et al.*, 1979; Valenzuela *et al.*, 1980; Ono *et*

al., 1983; Okamoto *et al.*, 1988). A number of these substitutions in the S-gene were claimed to be associated with the expression of *d* and *y* specificity (Prince *et al.*, 1982; Gerin *et al.*, 1983; Ionescu-Matiu *et al.*, 1983; Okamoto *et al.*, 1986).

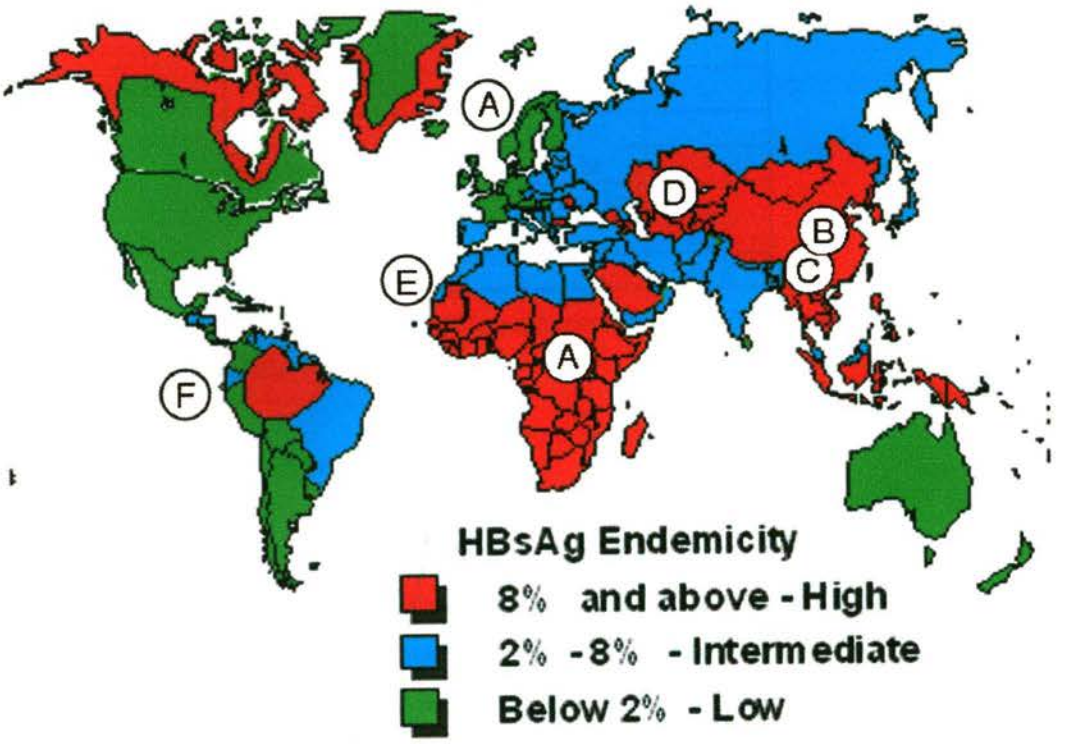


Figure 4. *Geographic Distribution and Endemicity of HBV genotypes.*
Endemicity based on WHO data (WHO), while genotype based on Robertson and Margolis (Robertson and Margolis, 2002).

From studies of HBV subtype infections of chimpanzees there is little difference in the infectivity of the various HBV subtypes (Barker *et al.*, 1975).

1.1.10.2.1. Monoclonal Antibody Mapping

Analysis of reactivity patterns with monoclonal antibodies after chemical modification of HBsAg revealed the importance of Lys (K) 122 for the expression of the *d* determinant (Peterson *et al.*, 1984). Later studies on two blood donors carrying surface antigens of compound subtypes, *adyr* and *adr* respectively, showed that amino acid substitutions at positions 122 and 160 alone explained the expression of *d/y* and *w/r* specificity, respectively (Okamoto *et al.*, 1987). Both the *d* to *y* and *w* to *r* changes were mediated by a shift from Lys to Arg at the corresponding positions (Okamoto *et al.*, 1987). The dependence of the *w* specificity on a Lys 160 was later also supported by site-directed mutagenesis (Okamoto *et al.*, 1989). Previous failures to unambiguously identify the *d/y* site by synthetic peptides, may be partially explained by the reagents used to identify the subtypes not being entirely mono-specific, since they were obtained with antisera that were absorbed with antigens

differing at several positions outside residue 122 as compared with the immunogen. A summary of the molecular basis for the major subtypic variations is given in Table 5 (p.22).

Specificity	aa 122	aa 127	aa 160
d	Lys	-	-
y	Arg	-	-
w1*	Arg	Pro	-
w2	-	Pro	-
w3	-	Thr	-
w4	-	Leu/Ile	-
w	-	-	Lys
r	-	-	Arg

Table 5. *Amino acid residues specifying determinants of HBsAg subtypes.*
*w1 reactivity also requires Phe 134, and/or Ala 159.

1.1.10.3. Definition of HBV Genotypes

Once complete sequences for a number of HBV genomes became available, four genomic groups of HBV were defined based on a divergence of 8% or more of the complete genome (Okamoto *et al.*, 1988). Genotyping parallels subtyping (Table 4, p.20): genomes encoding *ayw* were found in group D, those encoding both the *adr* and *ayr* subtype occurred in group C alongside with *adw*, which was also found in groups A-C. In a later study from Indonesia, genomes encoding *ayw* were also encountered in group B (Sastrosoewignjo *et al.*, 1991). The genomic groups E, and F, were identified as subtypes *ayw4*, and *adw4* (Norder *et al.*, 1992). At present it seems that the genotype designation has gained wider usage as compared to subtype group (Li *et al.*, 1993; Naumann *et al.*, 1993).

1.1.10.4. PreC Mutants

Recognition of a subgroup of patients with HBV that were HBeAg negative, and anti-HBeAg positive, indicative of clearance, but were HBV DNA positive, and suffering from liver disease. This led researchers to ponder whether HBV genomes were capable of variation or deletions of a protein, and began a search for mutations or variations in the core gene. Three types of variant have yielded a viable HBe negative phenotype: inactivation of the start of transcription sequence (ATG) (Okamoto *et al.*, 1990), insertions or deletions causing frame shifts (Okamoto *et al.*, 1990), and mutations producing stop codons (Carman *et al.*, 1989; Okamoto *et al.*, 1990; Ulrich *et al.*, 1990). The most common are the stop codons, of which a G to A point mutation at nucleotide 1896 (M1896) creates a novel translation stop codon that prevents HBeAg production.

All three types of mutations prevent effective production of HBeAg, but do not affect HBcAg production.

The most common preC mutation is a G⇒A at nt 1896, which produces a stop codon (TGG⇒TAG). This mutation prevents synthesis of preC protein, but produces a short preC peptide, which has been demonstrated in the cytoplasm of infected hepatocytes. A similar mutant has been produced for WHV (Delaney *et al.*, 1990). Hypermutation of G⇒A is thought to be responsible for the high rate of mutations found at nt 1896.

Initially this mutation was found in individuals that were persistently infected with severe hepatitis, and it was thought that this mutation was the cause of their excessive hepatitis. It was considered that a random mutation, which was inevitable in a chronic carrier, or possibly by positive antibody selection during attempted clearance by the host, was selected for and eventually increased the severity of disease. This was corroborated by such observations that dual (B and C) and triple (B, C, and D) chronic hepatitis infections, which often present minimal hepatitis, did not appear to have preC mutant genomes in circulation. A preC mutant was also associated with post transfusion fulminant hepatitis (Kojima *et al.*, 1991; Shimizu *et al.*, 1995), and was found in HCC (Clementi *et al.*, 1993; Ni *et al.*, 2003). However, the incidence of preC mutations was determined to be relatively high in persistently infected individuals without associated risk of increased disease (Bozkaya *et al.*, 1996).

Although technically defective, it appears that HBeAg is not essential for *in vivo* or *in vitro* replication in humans (Ulrich *et al.*, 1990), Woodchucks (Chen *et al.*, 1992), and DHBV (preCore) (Chang *et al.*, 1987). The duck studies have indicated that an artificially constructed preC mutant (which has a lower replication rate than that of the wild type, and thus possibly different from that found naturally in humans), when injected as a mixed infection with wild type produces several outcomes. Either the mutant or the wild type slowly began to dominate, or there was a fluctuation in the ratio of variants. PreC mutant domination was not associated with a faster replicating variant of itself but retained its original replication rate, it was however associated with elevated anti-Core Ab, which could be analogous to selection of HBeAg negative mutants in humans.

The consequences of preC mutants on the competency and effect of infection is uncertain, with some studies indicating enhanced RNA encaspidation (Hasegawa *et al.*, 1994; Baumert *et al.*, 1998) and/or replication following cytotoxic treatment (Yoshiba *et al.*, 1992), while others have shown no effect (Sterneck *et al.*, 1998). One of the reasons for the uncertainty is that most studies have just looked at a short length of sequence, usually only a fraction of the preC/C. This leaves the vast majority of the genome as an unknown quantity, in which other factors affecting transcription rates are certainly located, and those studies that have used entire genome sequence data, suggest that there are many areas of variation.

1.1.10.5. Surface Mutants

Hepatitis B surface antigen (HBsAg) is not only critical to the biology of HBV, but is also the basis of current vaccines, detected in serum for diagnosis, and antibodies against it are used clinically to suppress infection of transplanted livers. All of these rely on antigenic interactions between HBsAg and HBsAb.

PreS1 and PreS2: Amino acids 21-47 of preS1 are involved in *in vitro* hepatocyte attachment (Petit *et al.*, 1991), as such it is considered a conserved region in which no significant variants have been described either before or after liver transplant (Trautwein *et al.*, 1996). Point mutations and deletions have been described downstream (Trautwein *et al.*, 1996), and have been associated with severe disease. *In vitro* studies have shown that preS2 is not required for virus production (Santantonio *et al.*, 1992; Fernholz *et al.*, 1993a), and most *in vivo* cases have lost the preS2 ATG (start of translation), thus producing only small and large surface proteins. These mutations indicate an escape from antibody pressure, as the preS2 sequence is part of the large surface protein and still presentable to the cellular immune system. These variants are frequently seen in anti-HBeAg positive carriers, also often with preC mutants.

Small Envelope Surface Protein: The major protective epitope of HBV is highly conserved and found within 23 amino acids of the surface antigen (HBsAg). This 'a' determinant, believed to form two loops on the outside of the virus (Figure 5, p.24), is found in all known subtypes of HBV, and binds most of the anti-HBs found in hyperimmune globulin.

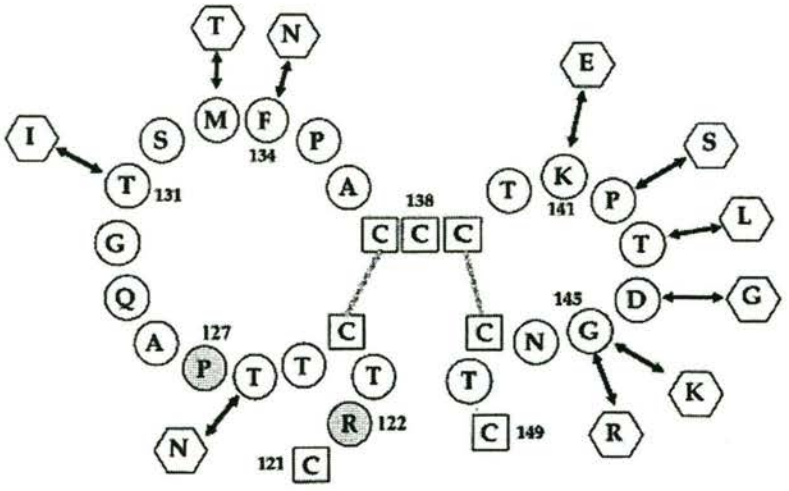


Figure 5. *Two loop structure of the 'a' determinant.*

The double arrows point to common point mutations that have been found in the 'a' determinant (Torre and Naoumov, 1998). The shaded proteins are involved in HBV sub-typing (Table 5, p.22).

Adequate levels of anti-HBs produced by HBV vaccines do not prevent infection in all cases, but infection is normally transient, and rarely associated with disease.

Mutants of this epitope have appeared under pressure generated by antibodies, both vaccine induced (Wilson *et al.*, 1999) and therapeutic (Carman *et al.*, 1996; Shields *et al.*, 1999). Most 'a' determinant mutations are a substitution of G>A, at aa 145 of HBsAg. This mutation has been shown to inhibit most of the anti-HBsAg binding (Fujii *et al.*, 1992; Chakravarty *et al.*, 2002). Other 'a' determinant mutations have been found but seem less clinically important (Carman, 1997).

However one of the most important aspects of these 'a' determinant mutations is that the majority of HBV diagnostic tests are based on serology which may have altered sensitivity in the detection of these mutations.

1.1.10.6. Polymerase Mutants

Several naturally occurring mutations alter the expression, structure, or function of the P protein. Deletions in the C gene may change the structure and expression of the P protein; deletions in the preS1, or preS2 regions remove sequences from the dispensable spacer region; and 'a' determinant mutations lead to changes in the RT domain. There is little evidence that these mutations interfere with the usual functions of P. A single patient was found to have a mutation which prevented encapsidation of the pregenomic RNA (Blum *et al.*, 1991).

The use of nucleoside analogues however, has been associated with functionally important mutations. Resistance to lamivudine therapy is associated with amino acid substitutions in the YMDD motif (located in the catalytic site of the RT) (Bain *et al.*, 1996; Ling *et al.*, 1996; Fischer *et al.*, 2001a; Germer *et al.*, 2003; Yu and Keeffe, 2003). In immunocompetent patients the cumulative incidence of mutations in the YMDD motif during lamivudine therapy was estimated to be as high as 39% after 1 year of treatment (Honkoop *et al.*, 1997). Changes in the YMDD motif strongly decreased the polymerase activity in transfection assays (Fu and Cheng, 1998), viraemia rebounds to a lower level than that originally associated with the wild-type, and wild-type rapidly emerges again after cessation of antiviral treatment (Niesters *et al.*, 1998). Resistance to famciclovir (FCV) has been documented, in which reduced sensitivity to FCV was associated with mutations upstream (in the template binding region of the RT) from the conserved YMDD motif in the HBV polymerase gene (Bartholomeusz *et al.*, 1997).

1.1.10.7. Quasi-Species

Hepadnaviruses replicate by means of a reverse transcription step which is similar to that seen in RNA viral replication. The proof reading ability of these reverse transcription polymerases is poor, resulting in a high substitution rate, which leads to a heterogeneous

mixture of related genomes (quasispecies) within the one individual (Domingo *et al.*, 1985). The quasispecies virus population share a consensus sequence but differ from each other and the consensus sequence by one, several, or many mutations. Le Bouvier first suggested the heterogeneity of HBV subtypes (Le Bouvier, 1971). Since then evidence for quasispecies has been mounting from individuals with heterogeneous subtype populations (Burda *et al.*, 2001; Cacciola *et al.*, 2002; Dong *et al.*, 2002; Jeantet *et al.*, 2002).

Mutant viruses have been associated with unusual hepatitis B virus serology: one patient, HBsAg and HBeAg positive, was also anti-HBc negative by radioimmunoassay (Zoulim *et al.*, 1996). Hepatitis B virus genotype was determined by size polymorphism of the core gene and the pre-S region was found to be D/E and consistent with the results of serological subtyping (HBV ayw2-4). DNA sequence analysis of the pre-C/C region showed the presence of significant nucleotide changes: in association with a wild type hepatitis B virus strain, they detected at least four hepatitis B virus variants with nucleotide deletions leading to a frame shift in the core gene. According to the position of the mutations, these hepatitis B virus core variants were expected to be defective for B-cell epitopes and Th-cell epitopes (Zoulim *et al.*, 1996).

Single strand conformational polymorphism analysis performed on PCR fragments of a conserved core region and a surface antigen region of HBV DNA from sera of 27 Korean chronic hepatitis B patients, was followed by DNA sequence analysis. The results showed that heterogeneous HBV mutants in both regions were present in a single as well as in various hepatitis B patients. Sequence analysis revealed a defective interfering particle with missense mutation in the core region. They also found that two subtypes of *adr* and *adw* coexisted in a single patient, as well as a point mutation causing a stop codon in the surface antigen region (Keum *et al.*, 1998).

Mutation of the preS2 gene sequence of HBV was investigated to clarify the significance of HBV quasispecies groups in Chinese patients with chronic HBV infection. Quasispecies were displayed in the PCR products from 52.9% (27/51) of patients. The phenomena of multiple bands in PAGE was detected in both HBeAg (36.1%) and anti-HBe (93.3%) positive patients. A deletion in the preS2 gene sequence may influence the recognition by neutralising antibodies (Huangfu *et al.*, 2002). Pre-transplantation pre-S2 and S protein heterogeneity has been shown to predispose HBV recurrence after liver transplantation (Grottola *et al.*, 2002).

1.1.11. Models of HBV

Although the clinical literature regarding human hepatitis B infection is now vast, critical data about the pathogenesis of infection has been very difficult to obtain for ethical and practical reasons. Animal models permit prospective studies using defined doses and timing of infection and have been used in pivotal molecular studies of viral replication as well as in prospective studies of the virus and host response at different stages of infection.

There are currently several well-characterised animal models that provide useful information for human HBV infection. Those most studied are the woodchuck, ground squirrel, and duck hepadnaviruses, each of which exhibit different advantages and limitations as an experimental model (Summers *et al.*, 1978; Marion *et al.*, 1980; Mason *et al.*, 1980; Summers, 1981). Mice transgenic for HBV and individual HBV genes have also provided critical data about the mechanisms of regulation of hepadnavirus replication *in vivo* (Chisari *et al.*, 1985; Uprichard *et al.*, 2003; Wieland *et al.*, 2003).

1.1.11.1. Woodchuck Hepatitis B Virus

The discovery of a naturally occurring hepadnavirus in woodchucks (Summers *et al.*, 1978), and its association with acute and chronic liver disease and HCC (Summers *et al.*, 1978; Popper *et al.*, 1981), laid the groundwork for much of our current understanding of hepadnavirus biology and pathogenesis. As with HBV, neonatal infection by WHV invariably leads to persistent infection while adult onset infection leads to acute self-limited hepatitis and viral clearance (Korba *et al.*, 1989b). HCC is an almost invariable outcome. Discovery of the extrahepatic replication of WHV (Korba *et al.*, 1990), especially its ability to replicate efficiently in lymphomononuclear cells (Robertson *et al.*, 1981; Korba *et al.*, 1986; Korba *et al.*, 1987; Korba *et al.*, 1989a; Chemin *et al.*, 1992), reinforced the concept that HBV is not strictly hepatotropic and that extrahepatic reservoirs of virus may exist that can contribute to viral persistence and serve as a continuing source of virus and viral antigens to maintain the immune response long after seroconversion and recovery from acute viral hepatitis.

The WHV model has also greatly strengthened the concept that the antiviral T-cell response plays a critical role in viral clearance and disease pathogenesis, since cyclosporine A treated woodchucks with suppressed T-cell function fail to terminate WHV infection when infected as adults (Cote *et al.*, 1991). This model also documented the dependence of the hepatitis delta virus (HDV) on coincident or preceding HBV infection (Negro *et al.*, 1989). Furthermore, due to the ability to infect the woodchuck liver by direct intrahepatic injection of cloned WHV genomes, it has been shown that the precore protein is dispensable for viral

replication *in vivo* (Chen *et al.*, 1992) but that the X protein is not (Chen *et al.*, 1993; Seeger and Zoulim, 1994).

The woodchuck model has also been used to examine the physiological basis for viral clearance during acute WHV infection (Kajino *et al.*, 1994). The results of these studies are compatible with a hypothesis from a transgenic mouse model of viral hepatitis (Guidotti *et al.*, 1994), that, in addition to destroying infected hepatocytes, the immune response can also deliver a noncytolytic signal that eliminates the virus from the hepatocyte without killing it.

Perhaps the most important contribution of the woodchuck model was in the area of hepatocarcinogenesis. Not only was it shown that virtually 100% of neonatally woodchucks develop persistent WHV infection and chronic hepatitis that progresses to HCC, but the insertional or transcriptional activation of the *myc* family of oncogenes was established as a critical early element in hepatocarcinogenesis in these animals (Martinez *et al.*, 1994).

Woodchucks, however, have not been imported into Australia, because of quarantine restrictions, which make the model unavailable to us.

1.1.11.2. Ground Squirrel Hepatitis Virus

During a search for a HBV-like virus in Californian relatives of the woodchucks, the Ground Squirrel Hepatitis Virus (GSHV) was discovered in Beechey ground squirrels (Marion *et al.*, 1980). The GSHV shares many characteristics of the Orthohepadnaviruses including virus morphology, viral DNA size and structure, a virion DNA polymerase that repairs a single-stranded region in the viral DNA, crossreacting viral antigens, and persistent infection with viral antigen continuously in the blood. Although similar, GSHV and HBV are not identical. The ground squirrel virion has a slightly greater diameter, there are many unusually long filaments, the viral surface antigens crossreact only partially, and GSHV DNA has two restriction endonuclease EcoRI cleavage sites in contrast to the single site in HBV DNA (Marion *et al.*, 1983b).

GSHV has been used to demonstrate many of the characteristics found in other hepadnaviruses such as: acute infection (Ganem *et al.*, 1982), genomic organisation (Seeger *et al.*, 1984b), replication by reverse transcription (Seeger *et al.*, 1986), S gene products (Feitelson *et al.*, 1981), preS gene products (Schaeffer *et al.*, 1986), pregenomic mRNA (Enders *et al.*, 1987), infectious cloned DNA (Seeger *et al.*, 1984a), and genetic recombination (Seeger *et al.*, 1987).

1.1.11.3. Transgenic Mice

Another useful model is the transgenic mouse system, in which DNA of various forms of HBV, from the whole genome of the virus to single proteins is transgenically introduced into a strain of mouse by embryonic microinjection. The transgenic mouse then has the viral DNA as part of its own genome and may also occasionally expresses some of the viral proteins to various degrees, which would allow direct study of some aspects of HBV immunobiology and pathogenesis. The expression of viral proteins by the mouse induces tolerance for the proteins, and the mouse then is a model for a chronically infected host. The mouse model is useful in determining the various effects of the CMI on hepatocytes, as allograph transfer of specific cells can be easily achieved.

Using constructs containing HBV derived regulatory sequences, several laboratories (Chisari *et al.*, 1985; Farza *et al.*, 1988; Araki *et al.*, 1989) have produced transgenic mice that preferentially express all of the viral gene products, and even replicate the virus in the hepatocyte. These mice also express the viral gene products in kidney tubular epithelial cells, sometimes preferentially, and they also display sporadic and unpredictable expression in miscellaneous other tissues that are unique to each transgenic lineage, presumably reflecting integration site influences. It has also been demonstrated that most of the HBV gene products, and even the process of viral replication, are not directly cytopathic. Most importantly, the supercoiled form of HBV DNA (cccDNA) has not been detected in any of these lineages, and naïve hepatocytes cannot be infected.

Adoptive transfer of HBV specific CTL into such mice induced hepatocytes expressing HBV antigens to undergo apoptosis, representing a critical initiating event in the elimination of HBV particles (Ando *et al.*, 1994). However; the direct cytopathic effect of the CTL was limited to very few hepatocytes; possibly because the Effector:Target (E:T) cell ratio in the liver was low and the free-ranging CTL movement was severely limited by the architectural constraints of solid tissue. There are several strains of transgenic HBV mice that reproduce various aspects of HBV infection, and some of these strains produce transient and relatively mild disease (like most cases of acute viral hepatitis in humans), which destroy no more than 5% of the hepatocytes. In acute necroinflammatory liver disease transgenic mice, injury can be completely prevented by the prior administration of neutralising antibodies to IFN-γ, it was assumed that most of the liver cell injury was mediated by non-specific inflammatory cells that the CTL recruited, most probably by IFN-γ mediated release of chemotactic and inflammatory cytokines (Ando *et al.*, 1993; Guidotti, 2002).

Direct evidence for non-cytolytic clearance of hepadnavirus infection came from a series of experiments done by transferring HBsAg specific CTL into allogeneic HBV transgenic mice

(Guidotti *et al.*, 1996b). Secretion of IFN-γ and TNF-α by CTLs were able to almost completely suppress the expression of HBsAg in hepatocytes by a noncytolytic mechanism. These findings confirmed earlier studies which revealed that IFN-γ and TNF-α suppress the liver specific expression of hepatitis B virus mRNA in transgenic mice (Maggi *et al.*, 1992; Seder *et al.*, 1992; Lenschow *et al.*, 1996).

However, because of the intrinsic limitation of HBV transgenic mice as a non-infectious model; the further investigation of the role of cytokines in the clearance and pathogenesis of HBV infection has been greatly hampered and elucidation of the effect of these molecules on the outcome of hepadnavirus infection will require studies in model systems such as DHBV infected ducks.

Determinants of HBV Host Range and Tissue Specificity: Murine studies have demonstrated that HBV has the potential to be expressed and to replicate in many cells besides the hepatocyte. Together with evidence of extrahepatic viral DNA and virus expression in infected patients and the various hepadnaviruses, such data strongly suggests that the relative liver specificity of HBV must reflect multiple constraints at the levels of viral entry, replication and gene expression, and that none of these constraints individually, is absolutely specific for the human hepatocyte.

Assembly, Transport and Secretion of HBV Structural Proteins: An important by-product of the murine studies was the demonstration that most of the HBV gene products, and the process of viral replication itself, is not directly cytopathic for the hepatocyte, at least at the levels attained in animals containing the complete viral genome (Farza *et al.*, 1988; Araki *et al.*, 1989). This was further examined, by production of an assortment of transgenic lineages that express each of the HBV gene products under the control of the native viral regulatory elements or liver specific cellular promoters.

Transgenic mice have been produced in which the envelope coding region was controlled either by the native HBV regulatory elements, the inducible liver-specific mouse metallothionein promoter, or the constitutively active mouse albumin promoter. In these studies, it was shown that the middle and major envelope proteins assemble into small 22 nm spherical particles that bud into the endoplasmic reticulum (ER) and are rapidly secreted by the cell (Chisari *et al.*, 1986; Chisari *et al.*, 1987). In contrast, the HBV large envelope protein assembles into long; branching, filamentous HBsAg particles that become trapped in the ER and are not secreted (Chisari *et al.*, 1986; Chisari *et al.*, 1987). It was subsequently shown that the progressive accumulation of these subviral filamentous particles leads to a dramatic expansion of the ER in the hepatocyte, eventually causing ultrastructural and

histologic changes that are characteristic of the ground glass hepatocytes found in the liver of chronically infected patients with integrated HBV DNA (Gerber *et al.*, 1974b; Gerber *et al.*, 1974a).

To examine factors that influence the intracellular localisation of nucleocapsid proteins and particles in the primary hepatocyte *in vivo*, transgenic mice that express the HBV core and precore proteins under the transcriptional control of the liver specific mouse major urinary protein (MUP) promoter were produced. In these studies it was learned that the pre-core protein is strictly secreted into the blood as HBeAg and that it is not detectable within any compartment in the hepatocyte by immuno-histochemical techniques.

There are many difficulties involved with the study of native human HBV proteins in transgenic animals, not least of which are the theoretical problems of having highly host specific viral proteins in a foreign environment, but the mouse model also suffers from the lack of cccDNA presenting a practical problem when investigating viral clearance; as it is the cccDNA that is most resist to antiviral treatments. In, addition untransfected naïve mouse hepatocytes cannot be infected by HBV, so the spread by cell-to-cell transmission is not mimicked, and cannot be investigated. As such, animal models of HBV have been found to be highly effective and relatively simple to use, with one of the most valuable being DHBV.

1.1.11.4. Ducks

Experimental transmission of DHBV has provided an excellent system for *in vivo* studies of virus transmission, organ tropism, and dissemination in ducks (Mason *et al.*, 1983; Omata *et al.*, 1984; Freiman *et al.*, 1988a). The cultivation of the virus in primary duck hepatocytes has been a very useful tool for studying replication and the effect of antiviral agents.

1.2. Duck Hepatitis B Virus

1.2.1. Discovery and Historical Aspects

Studies in both Chinese Pekin ducks, and American Pekin ducks (which were originally imported into America from China in the early 19th Century) demonstrated the presence of a virus with similar morphology, genetic organisation and hepatotropism to human HBV (Mason *et al.*, 1980).

1.2.2. Duck Breeds

The host range for DHBV is relatively restricted. DHBV was initially detected in the serum of Pekin ducks (*Anas domesticus*) from mainland China (Zhou *et al.*, 1980), followed by

commercial flocks of Pekin crossbred ducks in the USA, Australia, and Europe, as well as, other duck breeds (Indian Runner, and Khaki Campbell) (Mason *et al.*, 1980; Cova *et al.*, 1985; Freiman and Cossart, 1986). The Pekin duck originated in China, and was introduced into other parts of the world towards the late 19th century. DHBV has also been isolated from domestic geese (*Anser domesticus*), wild mallards (*Anas platyrynchos*), maned ducks (*Chenonetta jubata*), and other species of wild duck (Cova *et al.*, 1986; Dixon *et al.*, 1989). However there are distinct genotypes associated with the different duck species.

1.2.3. DHBV Infection in Nature

DHBV appears to be highly endemic in non-captive ducks from many parts of the world such as China, France, and Australia (Dixon *et al.*, 1989). Very high levels are also found in some commercial flocks in the USA (~60%) (Cova *et al.*, 1985; Marion *et al.*, 1991), and Australia (up to 70%) (Freiman and Cossart, 1986).

Observations of duck HCC from Qidong, appeared to be more prevalent in domestic brown ducks, than Pekin ducks, so it was suspected that the brown duck was more susceptible to liver disease (Yokosuka *et al.*, 1 985). Further comparison of duck HCC in Qidong, and Shanghai, (which have similar carrier rates) showed that they had high and low rates of HCC respectively. The HCC rates correlate with the level of human liver cancer in the two areas, which indicates some form of environmental factors (Gu, 1992) possibly toxin ingestion (Carnaghan, 1965).

The X gene in orthohepadnaviruses, encodes a multifunctional protein that can regulate cellular signalling pathways, interact with cellular transcription factors, and induce hepatocellular oncogenesis (Lee *et al.*, 2002; Shamay *et al.*, 2002; Kim and Seong, 2003). The effect of these diverse activities on HBV life cycle remains unclear, and while the X protein is not absolutely essential for HBV replication or maturation in transgenic mice, it can enhance viral replication by activating viral gene expression (Xu *et al.*, 2002). Interestingly, variations in the production of antibodies to X have been associated with various outcomes (Stemler *et al.*, 1990; Vitvitski-Trepo *et al.*, 1990).

The avihepadnaviruses differ from the orthohepadnaviruses in the lack of an obvious X gene, lack of stable integration, and low levels of HCC. There has long been speculation on the existence of an incomplete ORF in DHBV that may be an analogue of the mammalian X gene (Kay *et al.*, 1985; Feitelson, 1986). Until recently it was thought that DHBV was unable to express such a protein, but it is apparently able to do so from a hidden ORF (Chang *et al.*, 2001), and has similar activities to the mammalian X protein (Schuster *et al.*, 2002). The lack of integration into the host genome, may be another important factor in the low

HCC rate, as metastasis is often associated with the viral genome being incorporated into an oncogenic gene which is then either improperly regulated or increases its oncogenic potential. However, this property is more of an advantage for the study of persistence and clearance since it avoids consequences of viral DNA incorporated in the hepatocyte genome. For instance, the lack of integration has been used to determine the half life of cccDNA (Civitico and Locarnini, 1994), and whether the cccDNA infects the stem cells of the liver or if it is diluted when the hepatocytes divide.

1.2.4. Virion Structure of DHBV

The whole infectious virion is a 40nm sphere, which is composed of an envelope of lipid and viral proteins surrounding an 27nm inner core structure which appears to be covered in spike-like projections (Marion *et al.*, 1983a; Marion and Robinson, 1983). Similar to human HBV, the serum of infected ducks contains non-infectious, pleomorphic, roughly spherical particles, which vary from 35-60nm in diameter (Mason *et al.*, 1980). However, in contrast to human HBV infection, no filamentous forms have been described for DHBV.

1.2.5. Replication of DHBV

The 3021-7bp DHBV genome, is composed of similar characteristics, arranged in the same manner as for other hepadnaviruses (Mason *et al.*, 1980). It, however, differs from mammalian hepadnaviruses by containing only S, C and P ORFs, ie. it lacks an obvious X gene (Mandart *et al.*, 1984), although, recently an analogue to the X protein has been found expressed from a hidden ORF (Chang *et al.*, 2001). Despite this possible difference the replication cycle of Hepadnaviruses was first elucidated by use of the DHBV model (Summers and Mason, 1982).

As described in section 1.1.4.1 (p.5), the main features of the replication cycle are repair of the single stranded region producing the double stranded, cccDNA which serves as a template for the synthesis of the RNA pregenome. The RNA pregenome is reverse transcribed to produce the DNA minus strand which is copied to produce the DNA positive strand.

The complete minus strand of DNA is covalently bound to a protein at the 5' end (Molnar-Kimber *et al.*, 1983). Reverse transcription in hepadnaviruses is primed by the viral reverse transcriptase (protein priming) and requires the specific interaction between the RT and a viral RNA signal termed epsilon, which bears the specific template sequence for protein priming (Bartenschlager and Schaller, 1992). The product of protein priming is a short oligodeoxynucleotide, which represents the 5' end of the viral minus-strand DNA and is covalently attached to the RT (Lien *et al.*, 1986). The protein and the oligonucleotide are

fundamental to the protein-primed initiation of reverse transcription in hepadnaviruses (Wang and Hu, 2002).

The number of copies of cccDNA in each infected hepatocyte appears to vary in relation to the type of infection. It has been found that in congenitally infected ducks, each hepatocyte was estimated to contain 20 copies of cccDNA from six weeks to 2 years of age (Jilbert *et al.*, 1992). While, in ducks experimentally infected at one-day of age it was found that hepatocytes contained at least 2000vge/cell during acute infection, and 550vge/cell in hepatocytes from a chronic infection (Freiman *et al.*, 1988b).

1.2.5.1. Surface protein

The pre-S reading frame (position 693-1283) contains up to 6 in frame AUGs (start codons) (Mandart *et al.*, 1984). Just as in HBV, the S reading frame (position 1284-1785) encodes the major envelope protein of 167 amino acids, with a molecular weight of approximately 17 kDa (Marion *et al.*, 1983a).

Although only one major DHBV pre-S mRNA has been described, which according to ATG mutants (Schodel *et al.*, 1991), initiates at the second AUG (nt 801), and translates into a 36 kDa preS protein (Buscher *et al.*, 1985), several other minor species of preS protein ranging from 28-37kDa have been detected in serum, as well as livers, of infected ducks. Various workers have described two Pre-S1 proteins of 34 and 36 kDa (Feitelson *et al.*, 1983; Marion *et al.*, 1983a; Pugh *et al.*, 1987) or 35 and 37 kDa (Schlicht *et al.*, 1987a). Additional bands ranging in size from 23 to 35 kDa with predominant bands at 30 and 35 kDa have been reported (Wen *et al.*, 1990). Similarly, additional bands have been found and referred to as Pre-S1 (37kDa) and Pre-S2 (28 kDa) (Yokosuka *et al.*, 1988). In some liver extracts the 28kDa appears to be the major preS (Lambert *et al.*, 1990; Chassot *et al.*, 1993). Mutational analysis suggests that the 28kDa protein may be generated by proteolysis of the 36kDa protein, and not initiated from an internal start codon of the preS/S open reading frame (Fernholz *et al.*, 1993b).

The confusion that arises from the all of these multiple bands may arise from our incomplete knowledge of how and where the DHBV proteins are translated into proteins. DHBV does not translate its proteins in the standard eukaryotic manner as it does not contain the well established Kozak sequences at the start of any of the ORF (Kozak, 1981; Kozak, 1987). Although the first AUG codon is not immediately preceded by a TATA box, which is normally associated with the start of translation, it does not however exclude, the full ORF from being translated.

The preS/S protein is myristilated at its N-terminus (Macrae *et al.*, 1991), at a conserved sequence for all hepadnaviruses (Persing *et al.*, 1987).

As with HBV, DHBV envelope proteins function as the entry receptor and contain neutralising epitopes, as such DHBV infected ducks permit the study of neutralisation mechanisms both *in vitro* (Pugh *et al.*, 1987; Cheung *et al.*, 1989; Lambert *et al.*, 1990), and *in vivo* (Lambert *et al.*, 1991a; Chassot *et al.*, 1993).. Adult ducks repeatedly inoculated with DHBV remained non-viraemic, but developed neutralising antibodies to envelope proteins (Vickery *et al.*, 1989). Similar experiments demonstrated that there may be a more frequent and extensive response to the L, than the S protein, during convalescence of infected ducks (Cheung *et al.*, 1990). Other experiments in which rabbits were immunised with undenatured S particles (consisting of both S and preS antigen) the major immune response was directed against the preS determinants (Schlicht *et al.*, 1987a). This data fits well with a computer prediction in which the preS region is hydrophilic, while the S region contains two hydrophobic regions (Lambert *et al.*, 1990). It has been shown that polyclonal antiserum raised against the first 131aa of bacterially expressed preS protein abolished infectivity of DHBV *in vivo* (Lambert *et al.*, 1991a). Thus it can be seen that the preS region of DHBV is very important in the infectivity and neutralisation of infection, because antibodies induce protection to DHBV infection. Similarly it has been shown for HBV that antibodies to preS1 or preS2 protect chimpanzees against infection (Itoh *et al.*, 1986; Emini *et al.*, 1989; Neurath *et al.*, 1989).

The sequence of HBs and DHBs are described and compared in more detail in the Theoretical Modelling chapter (Chapter 6, p.150).

1.2.5.2. Polymerase protein

The polymerase ORF (position 170-2528) encodes the viral polymerase (Sprengel *et al.*, 1985), which consists of several regions with specific functions (Fourel *et al.*, 1987). The Terminal protein is a primer for initiation of transcription of the RNA pregenome (Bartenschlager and Schaller, 1988; Bosch *et al.*, 1988), the Spacer, the Reverse Transcriptase is an enzyme that transcribes the first DNA strand from the terminal protein-primed RNA pregenome, and the RNase H which is an enzyme that degrades the RNA pregenome as the DNA is produced (Summers and Mason, 1982; Radziwill *et al.*, 1990).

The polymerase gene participates in several steps in the viral life cycle: packaging of viral RNA, providing the primer for synthesis of minus-strand DNA, synthesising minus-strand DNA from an RNA template and plus-strand DNA from a DNA template, and degrading viral RNA in RNA-DNA hybrids. Experimental evidence demonstrated that the RNA

packaging function could be uncoupled from DNA synthesis, however RT could not be separated from RNase H activities, as has been done with human hepatitis B virus .(Chang *et al.*, 1990). The viability of a mutant with a large insertion (123 amino acids) upstream of the RT and RNase H domain indicates that the spacer region may act as a hinge separating parts of the polymerase protein implicated in priming and polymerisation (Chang *et al.*, 1990).

1.2.5.3. Other DHBV proteins

The C reading frame (position 2518-412) codes for the core protein (Sprengel *et al.*, 1985) with a molecular weight of approximately 35 kDa (Halpern *et al.*, 1984; Yokosuka *et al.*, 1988). C terminally truncated core proteins (30 and 33 kDa) similar to HBeAg have been detected in the sera of DHBV infected ducks (Schlicht *et al.*, 1987a). The Pre-C region does not appear to be essential for genomic replication, core particle morphogenesis, intrahepatic virus spread (Chang *et al.*, 1987; Schlicht *et al.*, 1987a) or viraemia (Schlicht *et al.*, 1987a). A DHBV X protein has been found to be expressed from a hidden ORF (Chang *et al.*, 2001).

1.2.6. DHBV Infection

Day old hatchlings infected with high doses of DHBV (intravenously or intraperitoneally), have detectable antigen and DNA in scattered single hepatocytes within 24 hours of inoculation (Vickery and Cossart, 1996), while slightly lower doses progressively increase this period to several days (Jilbert *et al.*, 1987a; Jilbert *et al.*, 1988; Vickery and Cossart, 1996). In humans, the incubation period appears to be longer, with Human Hepatitis B Surface Antigen (HBsAg) only being detected 21-77 days after subcutaneous inoculation, with clinical symptoms 21-66 days later (Hoofnagle *et al.*, 1978). Virus dose was found to be inversely related to the incubation period for both antigenaemia and clinical illness (Barker and Murray, 1972).

Histological inspection of persistent DHBV infection of ducks reveals milder hepatic inflammation than woodchucks, or ground squirrels. In ducks it ranges from no lesions (in congenitally infected ducks) to portal inflammation and necrosis (in experimentally infected ducks) (Omata *et al.*, 1983; Marion *et al.*, 1984; Omata *et al.*, 1984; Uchida *et al.*, 1988; Lambert *et al.*, 1991b).

Suggestions that duck HCC may take longer to develop were possible considering the initial data which came from ducks 2-4 years old, while the lifespan of a duck may be considered 10 years. However, after 10 years of investigation, no HCC was reported outside of China (Marion *et al.*, 1991), while in China, HCC has been reported in ducks which were no more than 3 years (Yokosuka *et al.*, 1985), suggesting a role for carcinogenesis, duck genetic variability, or environmental factors.

The route of administration also has a large effect on the dose of hepadnaviruses required to initiate infection; *intraperitoneal* inoculation requires a much higher dose of virus than *intravenous* inoculation. For the *intravenous* route the number of genomes in an infectious dose has been reported as low as a single genome (Jilbert *et al.*, 1996; Anderson *et al.*, 1997), while for *intraperitoneal* inoculation, the virion must negotiate added biological barriers to reach and infect hepatocytes.

One of the main contributing factors of the decreased susceptibility may be the genetic adaptation of the wild DHBV strains to their natural host. It is well known that *hepadnaviruses* have a narrow host specificity (Ganem *et al.*, 1982; Davis and Woolcock, 1986), which is attributed to the PreS receptor sequences of the various *hepadnaviruses*. These are distinctive between the hepadnaviruses and approximately cover the PreS portion of the Surface gene (1-180 aa). Because the PreS sequence is considered to contain the virus attachment factor, the variation may well cause this specificity.

Inoculating a range of avian species with DHBV from domestic duck species shows reduced susceptibility in parallel with phylogenetic relationships. A standard inoculum of DHBV was able to produce viraemia in all of 107 2-5 day old Pekin ducklings, while no evidence of viral infection was detectable in 2-5 day old chicks, or Muscovy ducklings, while two domestic geese breeds were infectable with delayed viraemia (Marion *et al.*, 1987). Snow goose HBV was found to infect not only Pekin duck hepatocytes but also chicken hepatoma cells (Chang *et al.*, 1999). Stork HBV infected primary Pekin duck hepatocytes very inefficiently which suggests a restricted host range, similar to other hepadnaviruses (Pult *et al.*, 2001b).

Crane HBV is closely related to DHBV, even though phylogenetically, cranes are very distant from geese and ducks and are most closely related to herons and storks. Naturally occurring hepadnaviruses in the last two species are highly divergent in sequence from DHBV and do not infect ducks or do so only marginally. In contrast, CHBV from crane sera and recombinant CHBV produced from LMH cells infected primary duck hepatocytes almost as efficiently as DHBV did. This experimental data implies either the use of at least similar, if not the same entry pathways and receptors by DHBV and CHBV, unusual host/virus adaptation mechanisms, or divergent evolution of the host genomes and cellular components required for virus propagation (Prassolov *et al.*, 2003).

There is an absence of a detectable viraemia in Muscovy ducklings experimentally infected with DHBV; one of the reasons for this may be that the Muscovy hepatocytes have decreased susceptibility to infection with DHBV *in vitro*. As it has been shown *in vitro* that

DHBV is initially able to infect approximately 1% of Muscovy duck hepatocytes in culture, and that virus spread does occur so that by 3 weeks approximately 5-10% of hepatocytes are infected (Pugh and Simmons, 1994). An interesting feature to be observed from the Muscovy duck hepatocyte experiment was that although the cells had decreased susceptibility, their rate of DHBV replication was similar (Pugh and Simmons, 1994).

The ID_{50} of different DHBV isolates is relatively consistent in a particular duck variety. For instance, Japanese ducks can be infected with a Chinese strain of DHBV (Omata *et al.*, 1984). Ducks from one hatchery can be infected by different strains of DHBV with similar outcomes (Lenhoff *et al.*, 1998).

Hepadnaviruses originally isolated from species of wild ducks (geese, mallard, maned duck) generally have reduced infectivity in domestic ducks routinely used for experimentation, but many are still susceptible. A Duck Hepatitis B Virus isolated from wild mallards in France was able to produce a persistent infection in not only mallards, but also Pekin ducklings (Cova *et al.*, 1986). Grey heron virus was found to be able to infect Pekin ducks when injected as a cloned genome (Wildner *et al.*, 1991). It has also bee shown that a particular strain of a hepadnavirus obtained from Mallards produces higher serum titres than a normal strain in Mallards, then it also produced higher serum titres in Pekin ducks (Lambert *et al.*, 1991b).

Human (Will *et al.*, 1982), ground squirrel (Seeger *et al.*, 1984b), and duck (Sprengel *et al.*, 1984) hepadnavirus infections have been produced from the direct injection of DNA into the liver of susceptible hosts. For Hepadnavirus infection a full length genome has been either ligated to itself to form a covalently closed circular genome (similar to the bacterial plasmid) (Will *et al.*, 1985), or has been ligated to another full length genome to produce a dimer (Will *et al.*, 1983), of which a head to tail dimer will contain at least one complete copy of every gene. Both methods have produced patent infections with complete viral particles and the same pathogenesis as natural infection.

HBV infection from direct DNA injection has been achieved in chimpanzees (Will *et al.*, 1982; Will *et al.*, 1983). Both dimerised and closed circular DNA of three different serotypes was injected intravenously, directly into the liver, and intramuscularly. Seven weeks after inoculation, the chimpanzee developed a typical, mild self-limited, acute hepatitis. HBsAg (subtype ay) appeared a week before an increase in aspartate aminotransferase (AST) and alanine aminotransferase (ALT), followed by the first signs of the typical histology of a mild, acute hepatitis in liver biopsies. Resolving hepatitis was eventually seen with no further pathological changes. HBeAg appeared two weeks after

HBsAg, with both disappearing three weeks later. Development of HBsAg, HBeAg, and HBcAg antibodies was detected with usual kinetics. The HBV DNA detected in both liver and serum during the acute phase of infection, differed significantly (by Southern blot), from the material injected, which indicates selective replication.

Direct DNA injection has not only been shown to produce DHBV infection *in vitro* (Yang and Summers, 1998), but also *in vivo* recombination (Sprengel *et al.*, 1987). Again both dimerised and closed circular DNA were used, and both produced an infection. In the DHBV experiment, three different sequences were all separately injected into ducklings, to determine if their sequence variation would affect the infection produced by the different types. After 3-5 weeks most of the injected ducklings showed, low-titre and transient viraemia, by dot blot. Restriction analysis showed that the produced virus had the same pattern as the injected cloned material, and as the naturally occurring DHBV on which the cloned material was produced. The infectivity of the virus was tested by injection of the serum into new ducklings, which also became infected, proving that the clone produced virus was replication competent. Dot and southern blot were used to analyse the liver and showed that cloned DHBV DNA had initiated a normal replicative cycle, with the morphology of the natural and cloned viruses indistinguishable.

Further analysis of the early stages of DHBV infection have shown that the conversion of relaxed circular (RC) DNA into covalently closed circular (ccc) DNA does not require the viral polymerase. Primary duck heptocytes from embryonated eggs, were infected with DHBV and at the same time treated with a potent inhibitor of the viral polymerase. It was determined, by selective PCR, that cccDNA was produced in the absence of an effective viral polymerase, indicating that the genome repair of the viral DNA is or can at least be undertaken by the hosts natural polymerases (Kock and Schlicht, 1993). This has also been correlated with cell cycle progression (Borel *et al.*, 2001) and cccDNA is reduced when a cell cycle blocker is used (Turin *et al.*, 1996). This would allow the production of an infection simply by somehow inserting into cells the complete DHBV viral genome, as has been done (Sprengel *et al.*, 1984; Sprengel *et al.*, 1987).

1.2.7. Humoral Immune Responses to DHBV

Humoral responses to DHBV infection were initially performed by testing sera of naturally, or experimentally infected ducks for anti-core and anti-surface antibodies. Anti-core antibodies were present in the sera of experimentally infected ducks as detected in the serum by immunohistochemical assays to detect DHBV antigens in infected duck tissues (Halpern *et al.*, 1987). Anti-surface antibodies were detected by indirect radioimmunoassay (RIA), using polyclonal anti-sera from rabbits, which were immunised with purified DHBsAg

particles, and by *in vivo* neutralisation assays (Vickery *et al.*, 1989). These studies demonstrated that 6 week old ducks (which were inoculated 4 times) produced detectable anti-surface antibodies by RIA at 17 days post-inoculation (*pi*). Further investigations determined that serum, which had been collected 40 days *pi* (after 3 doses of DHBV), was able to neutralise DHBV infection in 1 day old ducklings. RIA assays have also been used to study serological responses to DHBV infection in ducks of different ages (Qiao *et al.*, 1990) where variable levels of anti-surface antibodies were detected in 20-40% of ducks inoculated with DHBV from 3 to 8 weeks of age. Further studies employed *in vitro* DHBV neutralisation assays in primary duck hepatocytes that detected neutralising activity in the serum of adult ducks inoculated with DHBV from as early as 7 days *pi* (Jilbert *et al.*, 1992).

ELISAs have been developed for detection of anti-surface and anti-core antibodies (Jilbert *et al.*, 1996; Vickery and Cossart, 1996; Jilbert *et al.*, 1998; Triyatni *et al.*, 1998) using anti-DHBV PreS/S monoclonal antibodies (Pugh *et al.*, 1995) and recombinant DHBcAg (Jilbert *et al.*, 1992). However these assays do not distinguish between IgM, IgY, and IgY(ΔFc) responses, because only anti-duck Ig is detected. IgY and IgY(ΔFc) were previously referred to as IgG (Zimmerman *et al.*, 1971).

In congenitally infected ducks anti-core antibodies can be detected in the serum from ~80 days posthatch, while experimentally infected ducks with persistent DHBV infection, anti-core antibodies are detected from as early as 7-10 days *pi* and persist throughout the course of infection (Vickery and Cossart, 1996; Jilbert *et al.*, 1998). These ducks do not resolve their DHBV infection and do not develop anti-DHBs antibodies.

Humoral immune responses to DHBV infection have been investigated in adolescent ducks; increasing the virus inoculum, decreased the time required for antibodies to become detectable (Vickery and Cossart, 1996; Jilbert *et al.*, 1998). The increased inoculum also saw an increase in anti-core Ab titre, which reflected by a more extensive infection of the liver. Ducks receiving lower doses of DHBV had lower levels of anti-core Ab, and no detectable replication in liver tissue collected between days 7-12 *pi*. Two of three, 4 month old ducks, which received the larger dose of DHBV ($2x10^{11}$ vge), were able to resolve their infection, and developed anti-surface, and anti-core Ab, despite extensive viral replication in the liver, histological evidence of moderate to severe acute hepatitis on days 9-12 *pi*., and detectable viraemia early after infection (Jilbert *et al.*, 1998).

In humans with persistent HBV infection, liver damage is associated with HBeAg in serum (Niederau *et al.*, 1996), as such it is disappointing that assays for the DHBeAg and antiDHBe antibodies are not currently available.

Several studies have defined neutralising and non-neutralising epitopes within the DHBV preS/S and S proteins. These have been generally mapped within the preS domain (Cheung *et al.*, 1989; Cheung *et al.*, 1990; Lambert *et al.*, 1990; Yuasa *et al.*, 1991), with only a single epitope mapped to the S domain (Cheung *et al.*, 1990; Pugh *et al.*, 1995).

1.2.7.1. Antibody Mapping of Neutralising Epitopes

The preS epitopes involved in DHBV neutralisation have been investigated by the use of murine monoclonal antibodies (Marion *et al.*, 1983a; Chassot *et al.*, 1993). Work based on *in vitro* competitive binding assays, identified three non-overlapping preS epitopes (Cheung *et al.*, 1989). Using peptide mapping and a preS/S fusion protein, three epitopes on the DHBV preS sequence were localised to aa 58-66, 91-99, and 139-145 (Yuasa *et al.*, 1991). Although the third epitope was recognised by a neutralising MAb, it does not appear to be directly involved in viral neutralisation. It has since been demonstrated that antibodies against a preS peptide lacking this epitope were able to completely neutralise DHBV infectivity (Lambert *et al.*, 1991a), and that mutants carrying deletions (aa 138-141, and 143-147) within this epitope were still infectious (Li *et al.*, 1989). Other preS epitopes have been recognised by MAb 900 and SD20, which reduce infectivity *in vivo* by 90% and 75% respectively (Lambert *et al.*, 1990; Chassot *et al.*, 1993). Subsequently, it was found that Mab900 mapped to residues 82-95, which is the same section that protective polyclonal serum recognised (Lambert *et al.*, 1991a), and MAb SD20 mapped slightly downstream (aa100-107). One of epitopes previously described aa91-99 is located between MAb 900 and SD20 (Yuasa *et al.*, 1991). Using single amino acid replacement, it has been demonstrated that W88 is a key reside for binding MAb 900, since it could not be replaced by any other naturally occurring amino acids in Pepscan analysis (Chassot *et al.*, 1993). This is in accordance with other studies that have described the importance of aromatic residues in the antigenic determinants of peptides (Appel *et al.*, 1990).

The preS domain containing the three neutralisation epitopes has been shown to be highly conserved among all cloned DHBV isolates (Lambert *et al.*, 1990), and to be immunodominant in infected ducks (Cheung *et al.*, 1990). This area is located within the main antigenic and hydrophilic site (aa75-100) of DHBV, as computer model predicted (Lambert *et al.*, 1990).

The identification of preS epitopes had not demonstrated that these epitopes were involved in the viral attachment to hepatocyte receptors. However, in other studies it has been demonstrated that the preS sequence aa81-120 was important for the *in vitro* binding of DHBV to hepatocyte membranes. This would suggest that some of the previously described neutralisation epitopes (Cheung *et al.*, 1989; Lambert *et al.*, 1990; Yuasa *et al.*, 1991;

Chassot *et al.*, 1993) could be part of the cell receptor binding site on DHBV since they appear to be the same region.

1.2.8. CMI Responses to DHBV Infection

Neutralising antibodies play an important role in recovery from infection with lytic viruses by containing the spread of infection in the infected host, facilitating the removal and destruction of viral particles, and prevent re-infection by blocking the ability of virus particles to bind to receptors on target cells. While the cell mediated immune (CMI) responses are most important in the elimination of viruses that do not have a lytic cycle in the host and for any tissue damage seen during either transient and/or persistent infection.

The demonstration that HBV specific CTLs were present in HBV infected patients was consistent with this view. As such, it has been assumed that viral clearance was mediated chiefly by destruction of infected cells by viral antigen specific CTLs (Chisari *et al.*, 1989) and that pathogenesis of persistent hepadnavirus infection is also mediated by these cells (Chisari and Ferrari, 1995). Recent studies in HBV transgenic mice provided some experimental evidence for this view, but it was evident that a non-cytolytic mechanism was more important in clearance of hepadnavirus infection from the liver, and several *in vivo* studies of transient DHBV and WHV infections (Jilbert *et al.*, 1992; Kajino *et al.*, 1994; Jilbert *et al.*, 1998) have also suggested a non-cytolytic mechanism. At the peak of infection, > 95% of hepatocytes were shown to support viral replication, but infections were rapidly cleared from the liver, anti-surface antibodies became detectable in serum, and although viral replication was accompanied by mild to moderate mononuclear cell infiltration of the liver and increases in levels of liver enzymes in the serum, histological evidence of significant cell regeneration was not observed.

Although there have been several studies on humoral immunity to DHBV, there are very few studies examining cellular immunity. The development of an antigen specific blastogenesis assay for DHBV (Vickery *et al.*, 1997), opened an opportunity to observe the natural CMI response in the various outcomes of infection. This lymphoblastogenesis assay has been successfully utilised to examine the group cellular immune responses to native DHBV surface (DHBsAg) and core (DHBcAg) antigens in uninfected, acute or chronically infected, and immune ducks (Vickery *et al.*, 1999a), as well as the kinetics of CMI response in ducks that have differing outcomes to DHBV infection (Vickery *et al.*, 1999b).

The CMI response correlates well with the outcome of infection (Table 6, p.43) (Vickery *et al.*, 1999a). The study indicated that the CMI response in immune animals differs from

acute, and chronically infected ducks, and that the response of peripheral cells is different to that of splenic cells (Vickery *et al.*, 1999a).

Antigen	Cells	Controls	Immune	Acute	Chronic
DHBsAg	PBMC	-	+++	++	+
	SMC	-	+++	+	+
DHBcAg	PBMC	-	++	+	+
	SMC	-	++	--	+

Table 6. *Relative lymphoblastic CMI response related to outcome of DHBV infection.*

The kinetics of the PBMC CMI response to DHBsAg and DHBcAg was determined using the lymphoblastogenesis assay for both infected and immune ducks. Acutely infected ducks that failed to clear the infection also failed to develop a significant cellular immune response to both antigens, while ducks with chronic infection acquired as neonates or as the result of the failure to clear acute infection had an increasing cellular immune response over time. Immune ducks demonstrated significant cellular responses following challenge with DHBV irrespective of the level of their responses prior to challenge. There was however, a reduction in the response of their PBMC over a 4-week-period postchallenge (Vickery *et al.*, 1999b).

The results of the above investigations into the CMI response of ducks to DHBV by Vickery *et. al*, (Vickery *et al.*, 1999a; Vickery *et al.*, 1999b) have been reproduced and confirmed by (Tang *et al.*, 2001).

1.2.9. Cytokine Response

IFN-γ is one of the most important mediators in the immune system. It is known to exert inhibitory effects on viral replication (Farrar and Schreiber, 1993; Boehm *et al.*, 1997). Recently, duck interferon gamma (DuIFN-γ) cDNA was cloned from a phytohaemaglutinin-stimulated duck spleen cDNA library screened using a chicken IFN-γ (ChIFN-γ) cDNA probe (Kaiser *et al.*, 1998; Schultz and Chisari, 1999; Huang *et al.*, 2001). Curiously, duck IFN-α (DuIFN-α) was initially found to have little cross-reactivity when tested on chicken cells, although it shows 50% identity to its chicken homologue at the amino acid level (Ziegler and Joklik, 1981a; Schultz *et al.*, 1995; Huang *et al.*, 2001). Later, functional homology between chicken and duck lymphokines produced by PHA stimulated lymphocytes was observed in an *in vitro* proliferation assay system (Bertram *et al.*, 1997), and pre-treatment of chicken cells with COS-derived DuIFN-γ 15h prior to challenge with VSV induced a significant degree of antiviral activity (Schultz and Chisari, 1999). Experimental investigations have revealed that IFN-γ inhibits the synthesis of progeny DHBV cccDNA *in vitro* (Schultz and Chisari, 1999), while in combination with TNF-α

suppresses the liver-specific expression of HBV mRNA in transgenic mice (Guidotti *et al.*, 1994).

1.3. THE AVIAN IMMUNE SYSTEM

1.3.1. Introduction

Despite the importance of the duck as an economic species, and its ability to act as a reservoir for several important agents, such as Influenza A virus (Shortridge, 1982), information on the duck immune system is relatively simplistic. In comparison, the chicken is well studied. However, more recently, the intricacies of the duck immune system are starting to be unravelled.

1.3.2. Duck Lymphoid Organs and Ontogeny

The avian and the mammalian lymphoid systems developed from a common reptilian past with approximately 160 million years of evolutionary dichotomy (Welty and Baptista, 1988). Similar to mammals, the avian immune system is divided into the humoral and cellular arms.

The bursa of Fabricius (bursa) is a primary lymphoid organ that is associated with the humoral immune response, and was crucial to the discovery of the two arms of the immune system; the humoral and cellular (Cooper *et al.*, 1966a). Mammals lack a comparable anatomical structure but maintain a similar division of humoral and cellular components.

In the chicken the bursa is a spherical lymphoepithelial organ that is formed by a dorsal diverticulum of the cloacal proctoderm at day 4 of incubation and attains a maximum size 10 weeks post hatch (Kollias, 1986). In the duck it is long and cylindrical in shape and attached to the cloaca by a narrow stalk. The bursa contains 10,000 follicles that are colonised by 2-3 stem cells which proliferate until 2-4 weeks post-hatching (Lydyard *et al.*, 1976; Olah and Glick, 1978). By day 12 of incubation, the B-cells are capable of secreting antibodies (initially IgM). By the 20th day of incubation a more specific and diversified immunoglobulin, IgG is produced (Kollias, 1986). In the chicken, the bursa provides the proper environment for immunoglobulin gene rearrangement and diversification (McCormack *et al.*, 1991). The post bursal stem cells do not require the bursal environment for differentiation and are responsible for the maintenance of the B-cell pool following bursal involution (Toivanen *et al.*, 1974). The resulting postbursal stem cells leave the bursa for secondary lymphoid tissues from 3 weeks post hatching and are responsible for the maintenance of the B-cell repertoire following bursal involution at 5-6 months of age (Toivanen and Toivanen, 1987).

The importance of the bursa in humoral immunity has been shown by manipulation. Early surgical bursectomy (Huang and Dreyer, 1978) or chemical ablation by testosterone treatment (Meyer *et al.*, 1959), results in B lymphocytes with a very restricted diversity. Post-hatch cyclophosphamide treatment of ducks lead to lymphoid follicle loss, and a lack of specific antibody to *Salmonella pullorum* (Hashimoto and Sugimura, 1976a).

Bone marrow develops between days 8-9 of incubation and may also be a derivative of cells from the yolk sack membrane (Kollias, 1986). Post bursal stem cells migrate to the bone marrow and form a life long source of B-cells.

In the duck the thymus consists of multiple lobes (3-5) on either side of the neck, close to the jugular vein, extending from the pharyngeal region to the thoracic inlet and occasionally into the thoracic cavity. In both the duck and the chicken, the thymus consists of an outer cortex containing a large number of thymic lymphoblasts, an inner cortex containing smaller lymphocytes and a pale medulla with fewer lymphocytes. The thymus is essential for the maturation of T lymphocytes, the principal cells of cellular immunity (Sharma, 1991).

Development of the thymus in birds begins at day 5 of incubation as an outgrowth of the pharyngeal pouches (Kendall, 1980). Precursor cells originating from blood-borne lymphoblasts within the yolk sac, enter the thymus from 7 days of incubation (Jotereau *et al.*, 1980), differentiate into T-lymphocytes within the special microenvironment of the thymus. The T-lymphocytes that are incapable of recognising self-antigen undergo extensive proliferation within the thymus independently of antigenic stimulation. Successive waves of thymocyte precursors enter the thymus and undergo both positive and negative clonal selection. The T-cells then populate the lymphoid organs. The thymus reaches its maximum size by 4 months of age, it then involutes with most of the thymic parenchyma bring replaced by adipose tissue (Kollias, 1986). However, the lymphoid tissue that remains retains the same function.

The important role of the avian thymus is readily shown by neonatal thymectomy, which results in loss of cell-mediated responses such as delayed hypersensitivity reactions and skin allograft rejection (Cooper *et al.*, 1966a).

Although lymphoid stem cells develop in the cloacal bursa and the thymus, none of these organs contain pure populations of T- and B-cells (Kollias, 1986). During embryonic development the spleen is involved in granulopoiesis and erythropoiesis. The principal role of the spleen is blood filtration and antibody production post hatch. Active proliferation of immunologically competent B-cells occurs in the germinal centres where there is close

contact between B-cells and dendritic reticular cells (Toivanen and Toivanen, 1987). Germinal centres appear approximately 10 days post hatch and contain dendritic reticulum cells, macrophages and B and T lymphocytes (Vainio *et al.*, 1987). Plasma cell are found adjacent to the germinal centres.

Shortly after hatching these immature post-bursal precursor cells from the bursa infiltrate the spleen and thereafter settle in bone marrow and thymus (Toivanen *et al.*, 1974). Although stem or precursor lymphoid cells infiltrate the spleen, they do not mature sufficiently enough to reconstitute the B-cell lineage in cyclophosphamide bursectomised embryonic or day old recipients (Toivanen *et al.*, 1972; Toivanen *et al.*, 1976).

Maturation of the immune system to competently mount sufficient cell-mediated immune responses in chickens occurs at one to three weeks after hatching. One day old chicks are capable of antibody production to certain antigens, however a complete adult level response with immunoglobulin production is usually not observed until six weeks of age (Kollias, 1986).

Histocompatibility genes control the diversity of immune function. In ducks, limited knowledge of this gene, combined with the unavailability of inbred duck strains, has limited research into DHBV immunology. In avian species the B-histocompatibility locus is responsible for controlling such functions as skin graft rejection, graft versus host reactions, complement production, leukocyte antigen production, resistance to certain viral diseases, tumour regression of lymphoid leukosis and regulation of autoimmune reactions (Kollias, 1986).

1.3.3. Lymphocytes

Lymphocytes are the most frequently occurring leukocyte in avian blood (approximately 60-66%) (Soliman *et al.*, 1966). Of the lymphocytes in the chicken spleen, approximately 55% are T-cells, which are located in the red pulp, while B-cells are located in the germinal centres (Boyd and Ward, 1978; Ellsworth and Ellsworth, 1981). The B-cells are principally located in the Haderian gland, the bursa, and the caecal tonsil, while the T-cells predominantly located in the thymus (Albini and Wick, 1974).

Monoclonal antibodies have differentiated chicken T lymphocytes into functionally diverse subpopulations. Remarkable similarity has been revealed between the surface antigens of T lymphocytes of chickens and mammals (Sharma, 1991). The chicken T-cell markers include CD2, CD1, CD5, CD4 and CD8 (Cooper *et al.*, 1991). As in mammals, thymic T-cells express both CD4 and CD8 molecules, while more mature cells in the peripheral lymphoid

tissues express either CD4 or CD8 molecules. CD4 cells have helper cell functions and CD8 cells have cytotoxic activity (Chen *et al.*, 1988).

The normal location of the two cell types (CD4, and CD8) is tabulated from various investigators (Table 7, p.47) (Lillehoj, 1991; Hala *et al.*, 1992). At one month post hatch approximately 80% of thymocytes are CD4+ (Lillehoj, 1991).

Chicken	CD4+	CD8+
Blood	40-45%	15%
Spleen	10-20%	50%

Table 7. *Normal location of CD4, and CD8 cells in the Chicken.*

Surface membrane antigen receptors on chicken cells appear as CD3/TcR (antigen-specific T-cell receptor) complex. Three types of CD3 positive cells have been recognised, two correspond to their mammalian counterparts: TcR-1 (mammalian TcR-gamma/delta), and TcR-2, (mammalian TcR-alpha/beta) (Chen *et al.*, 1988; Cihak *et al.*, 1988; Sowder *et al.*, 1988), while the third sublinage is unique to birds (Chen *et al.*, 1989), and may be a subfamily with TcR-2 (Char *et al.*, 1990).

The T-cell occupies a central role in antigen-dependent immunoregulation in mammals, and appears to have a similar function in the chicken. The major functional T-cells are helper or inducer T-cells, suppressor T-cells, cytotoxic T-cells and delayed type hypersensitivity T-cells. The recognition of antigen by avian T-cells is restricted to the MHC-II for cells of delayed hypersensitivity (Ewert *et al.*, 1984; Vainio and Lassila, 1989), graft rejection (Cooper *et al.*, 1966a), and B-cell help (Ratcliffe *et al.*, 1987). In reticuloendotheliosis virus, cytotoxic T-cells recognise MHC-I antigens (Maccubbin and Schierman, 1986).

1.3.3.1. Other Avian Leukocytes

Other cells important to the cellular immune response include macrophages, dendritic cells, natural killer cells and effector cells of antibody dependent cellular cytotoxicity (Qureshi *et al.*, 2000). Important mediators of non-specific immunity include thrombocytes and heterophils.

Avian macrophages are derived from bone marrow stem cells, which differentiate into monoblasts, promonocytes and monocytes. The monocytes are continually released from the bone marrow into the blood stream where they remain for 3 to 5 days before migrating into the tissues to become macrophages. Macrophage phagocytic function appears as early as day 12 (in liver) or 16 (in spleens) of chicken embryonic development (Jeurissen and Janse,

1989). The tissue macrophage has a limited capacity to divide during its lifetime of around 5 weeks (Powell, 1987).

The natural killer cell system is well developed in birds (Fleischer, 1980) and its role against some poultry diseases is very important (Lillehoj, 1991). The NK cell activity increases in activity with age (Sharma, 1981). Chicken NK cells are thermolabile, non-phagocytic, and non-adherent to the plastic normally utilised for tissue culture (Sharma and Coulson, 1979). They lack immunological memory and are not MHC restricted (Petit *et al.*, 1985; Carman *et al.*, 1986; Ernst *et al.*, 1986).

1.3.4. The Immune Response

Development of an immune response requires interactions between T- and B-lymphocytes in which the macrophage cooperates as an initiator and a moderator. Interactions between B and T lymphocytes and macrophages are essential for development of humoral immunity to thymus-dependent antigens that involve both physical contact and interleukins (Powell, 1987).

In mammals activated macrophages present antigen in conjunction with MHC determinants to antigen specific T-cells and secrete IL-1, which serves as a signal to activate T helper cells. The activated T-cells then secrete IL-2 and other factors eg gamma interferon which mediates a variety of functions critical to the progression of the immune response.

In chickens, adherent spleen cells, peritoneal macrophages, blood monocytes and cells of macrophage lineage may be stimulated *in vitro* to secrete IL-1 by mitogens (Vainio and Ratcliffe, 1984), and bacterial endotoxins in the presence of suboptimal doses of mitogens (Sharma, 1991). In the chicken, the binding of IL-1 to the receptor on T-cells initiates production of IL-2, IL-3 and the IL-2 receptor (Hagiwara *et al.*, 1987) and results in clonal expansion.

Chicken macrophages were also shown to be required for *in vitro* IgM antibody production by chicken B-cells (Evans and Ivanyi, 1975), and mitogen presentation (Vainio and Ratcliffe, 1984) and subsequent *in vitro* transformation of peripheral duck lymphocytes to mitogens (Higgins, 1992). Induction of cell mediated immunity in avian T-cells requires MHC-II antigen presentation by macrophages (Ewert *et al.*, 1984; Vainio and Lassila, 1989).

Antibody dependent cell mediated cytotoxicity requires antibody (IgG) to attach to antigen displayed on cell surfaces via its Fab portion and to an effector cell (macrophage) by its Fc

portion (Powell, 1987). This type of cytotoxicity has been reported in the chicken (Fleischer, 1980), and in the duck (Bubenik *et al.*, 1970).

Lymphokines are important in the regulation and differentiation of cells responding to antigens as well as in inflammatory and physiological interactions between immune and non-immune cells (Lillehoj, 1991; Lowenthal *et al.*, 2000).

1.3.5. Immunoglobulins

Ducks have three types of serum immunoglobulins, IgM, IgG, and IgY, plus an immunoglobulin of bile and intestinal secretions, IgA (Zimmerman *et al.*, 1971; Higgins and Warr, 1993; Magor *et al.*, 1998).

Immunoglobulins are composed of Constant (C) and Variable (V) regions. Birds are the most primitive extant species to have recognisable orthologues of three mammalian C region genes. Three C region genes (μ-, ν-, and α-chain) are in translocus arrangement (Du Pasquier, 1993), with the μ-chain gene located adjacent to, and downstream of, the J_H region (Kitao *et al.*, 1996). Studies at the cDNA level indicate that the α-chain gene of birds, despite having four exons, is homologous to the α-chain gene of mammals (Mansikka, 1992; Magor *et al.*, 1998). The ν-chain gene of birds shares structural features of γ- and ε-chain gene of mammals, and was probably the evolutionary precursor of both these genes (Parvari *et al.*, 1988; Warr *et al.*, 1995).

IgY antigenically resembles an $F(ab)_2$ fragment of IgG. Lacking an Fc portion IgY is unable to fix complement or bind to Fc receptors (Zimmerman *et al.*, 1971). Originally described as IgX (Ng and Higgins, 1986; Higgins *et al.*, 1987), and more accurately defined as IgA (Magor *et al.*, 1998), and studies revealed physical and antigenic similarities between duck bile immunoglobulin (IgX) and serum IgM. Differential screening was used to clone, from a duck spleen library, the cDNA encoding the heavy (H) chains of IgM and the IgX, which was identified as IgA, occurring in duck secretions (Magor *et al.*, 1998). Several chains of the C region were related closest to chicken regions. The previously noted antigenic overlap of duck IgM and IgA, was found to be in the C4 domains. IgA was first detected in ducks 26 days of age, and its appearance was unrelated to serum levels of IgG or IgM (Ng and Higgins, 1986). It has since been determined that messenger RNA for IgA is most abundant in the respiratory, alimentary and reproductive tracts, and first appears around 14 days of age and reaches adult levels of expression only at 35-50 days (Magor *et al.*, 1998). As such, the duck has a mucosal immune system, which utilises IgA; however, the delayed expression and secretion of IgA explains the susceptibility of ducklings to mucosal pathogens.

1.3.6. Effects of Bursectomy

Bursectomy is the removal of the bursa of Fabricius, which has several important implications for the duck. In birds, B-lymphocytes undergo maturation in the bursa, and its role in B-cell differentiation makes it essential for expansion and creation of the antibody repertoire (Jalkanen *et al.*, 1984). Dipping of eggs in testosterone (Glick, 1970), or injection of embryos with 19-Nortestosterone (Meyer *et al.*, 1959), by day 5 of incubation prevented development of the bursa. In the murine system depletion of the B-cells can be achieved by γ-irradiation, and reconstitution by allograph transplant of T-cells, or destruction of B-cells by injection of anti-B-cell antibodies. In the duck, surgical removal of the bursa at embryonic day 18 (three days prior to hatch) completely abrogates B-cells, while bursectomy at hatch may not completely remove all traces of B-cells, it does significantly reduce the B-cell population.

Splenic lymphoid tissue has been shown to be bursa dependent in chickens that have been neonatally surgically or chemically bursectomised with colchicine or cyclophosphamide. Chemical bursectomy (cyclophosphamide treatment) of ducks post hatch severely decreased the immune response to *Salmonella pullorum* (Hashimoto and Sugimura, 1976a). The reduced antibody titre was related to the reduction in the number of bursal follicles (Sato and Glick, 1970). Similar *in ovo* surgically bursectomised birds lacked specific responses to nine different antigens (Jalkanen *et al.*, 1984) despite the production of IgM, Ig G and IgA. Prebursal stem cells enter the bursa between 8 and 12 days of embryonic development but have also been found in the spleen by day 14 and the bone marrow by day 16 (Back *et al.*, 1973), suggesting these sites might function to produce Ig, but as they failed to undergo maturation in the bursa they lack Ab specificity. This phenomenon also resembles human patients, which suffer antibody deficiencies but have a normal level of serum immunoglobulin (Rothbach *et al.*, 1979).

After bursectomy, germinal centre formation in the spleen and caecal tonsils are significantly decreased (Jalkanen *et al.*, 1984), the amount of white pulp tissue and its compartments, periellipsoidal lymphoid tissue and periarteriolar lymphoid tissue were also decreased (Romppanen and Sorvari, 1981). The periellipsoid lymphoid tissue contains splenic dendritic cells which trap and process antigen and then migrate to the periarteriolar lymphatic sheath where they associate with T and B-cells. Bursectomy at hatch produced extensive necrosis of the periellipsoid tissue and the dendritic cells failed to act as splenic messengers (Olah *et al.*, 1985), perhaps explaining the reduction in plasma cells after antigen injection reported by others (White and Timbury, 1973). However, no difference was found in body weight, weight of the thymus or spleen in ducks hormonally bursectomised by testosterone at day 5 of incubation (Sugimura *et al.*, 1975).

Immunoglobulin switching from IgM to IgG (Andersson *et al.*, 1978), and the amount of immunoglobulin secreting precursors and B-lymphocytes are thought to be bursa dependant (Lawton *et al.*, 1975). Surgical bursectomy of chickens at 60 hours of incubation has a marked negative effect on the frequency of cytoplasmic IgA positive cells (c-IgA$^+$) with minimal changes to the frequency of c-IgG$^+$ and c-IgM$^+$ cells (Veromaa *et al.*, 1987). In contrast, interaction with T-cell systems are needed (Romppanen and Sorvari, 1981), showing that heavy chain class switching is not bursa dependant (Jalkanen *et al.*, 1984). However, bursectomised birds can reject skin grafts and develop normal cell mediated immunity.

In ducks, surgical bursectomy at 1 day post hatch resulted in a significant decrease in antibody responses to viral antigens (Di *et al.*, 1987). Successful bursectomies were verified by immunising ducks with bovine serum albumin (BSA) or Newcastle disease virus (NDV), which resulted in lower antibody titres. A summary of the effect of bursectomy on cell numbers is tabulated (Table 8, p.51) (Wick *et al.*, 1975).

Immunomodulation	Peripheral blood		Spleen	
	B-cell	T-cell	B-cell	T-cell
untreated	22	58	36	55
Bursectomised	1	89	18	81
untreated	22	58	36	55
Thymectomy 2 lobes left	38	57	65	31
Thymectomy 1 lobe left	76	1	71	15
Complete thymectomy	-	-	84	6

Table 8. *Effect of Bursectomy or Thymectomy on immune cell composition in the Chicken.*

Values given are percentage. Top: In ova bursectomised chickens (day 18). Bottom: Neonatal thymectomised chickens and sublethal radiation at 7 days.

1.3.7. Effects of Thymectomy

Both birds and mammals have developed dual immune systems however only birds have separate organs for B and T-cell maturation which are the bursa of Fabricius and thymus respectively. While the association between the bursa and the humoral response in chickens was crucial to the discovery of the duality of the immune response, the early experiments in mice were pivotal to determining the role of T lymphocytes in the cell mediated immune response.

In these experiments thymectomy in mice resulted in diminished CMI specific responses including graft rejection. Due to loss of T- and B-cell collaboration, the mice were also limited in their capacity to generate primary antibody responses to certain antigens, such as sheep erythrocytes. The peripheral lymphoid tissues became depleted. The cortex of lymph

nodes, including the germinal centres and medulla with its foci of plasma cells remained unaffected yet a significant depopulation of the deep cortex or tertiary nodules occurred. Within the spleen, the white pulp around the central arterials became deficient of lymphocytes (White and Timbury, 1973).

Similarly the important role of the avian thymus is shown by the loss of the CMI responses following thymectomy. Thymectomised chickens fail to reject skin allografts (Warner and Szenberg, 1962; Aspinall *et al.*, 1963; Cooper *et al.*, 1965; Cooper *et al.*, 1966a). Furthermore, there was a rough correlation between graft rejection time and the number of circulating lymphocytes (Warner and Szenberg, 1962). Chickens lost their ability to mount a delayed hypersensitivity reaction (Jankovic and Isakovic, 1963; Cooper *et al.*, 1966a). The development of the chicken peripheral lymphatic organs, such as the spleen and caecal tonsil were shown to be dependent on the thymus (Cooper *et al.*, 1965; Cooper *et al.*, 1966a) and neonatal thymectomy plus irradiation significantly depleted numbers of lymphocytes in the white pulp of the spleen. One group of researchers (Hoshi and Mori, 1973) found that X-radiation of chicken thymuses resulted in loss of germinal centres while another found no significant difference (Cooper *et al.*, 1966a). A summary of the effect of thymectomy on cell numbers is tabulated (Table 8, p.51) (Wick *et al.*, 1975).

The effect of thymectomy on the antibody response is more variable. In chickens neonatal thymectomy may result in loss of antibody production to thymus-dependent antigens (Bhogal *et al.*, 1984), without any change in serum antibody levels (Baba *et al.*, 1978). Thymectomy with irradiation resulted in significantly decreased total leukocyte counts (Cooper *et al.*, 1966a). Thymectomy significantly reduced the white blood cell count (Warner and Szenberg, 1962; Sugimura *et al.*, 1975).

In ducks no significant change in body weight (Sugimura *et al.*, 1975), weight of bursa or spleen was detected between control ducks and ducks surgically thymectomised at hatch (with or without X radiation) (Sugimura *et al.*, 1975; Hashimoto and Sugimura, 1976b; Hashimoto and Sugimura, 1976a). However, 1/5 ducks thymectomised without radiation showed a decrease in the size of bursal lymphoid follicles (Sugimura *et al.*, 1975). Similar to the chicken, thymectomy in ducks results in prolonged survival of skin grafts (Vojtiskova *et al.*, 1963).

Impairment of T-cell responses in individuals with DiGeorge's syndrome (congenital athymic aplasia), acquired immunodeficiency syndrome, leukaemia, or immunosuppressive therapy, enhances the frequency and severity of viral infections (White and Timbury, 1973). In most instances, some impairment of antibody response is observed (White and Timbury,

1973). Even with adoptive transfer of hyperimmune immunoglobulin, viral infection can be moderated but not cleared.

1.4. PREVENTION AND TREATMENT OF HEPADNAVIRAL INFECTIONS

Evidence from contacts of HBV infected individuals led to the recognition that antibodies to HBsAg were protective, and that HBsAg possibly could be used as a vaccine (Almeida and Waterson, 1975). This concept was investigated in both people and chimpanzees, using both heat inactivated and formalin fixed sAg preparations (Soulier *et al.*, 1972; Krugman, 1975; Prince *et al.*, 1975).

The original vaccines were derived from purified proteins that had been extracted from the plasma of chronic carriers of HBV and inactivated with formalin (McAuliffe *et al.*, 1980). Eventually, HBsAg purified from transformed bacteria became available (Charnay *et al.*, 1980). Several vaccine trials were undertaken (Bergamini *et al.*, 1983; Coutinho *et al.*, 1983; Desmyter *et al.*, 1983), and finally a subunit protein vaccine incorporating the 'a' determinant became widely available.

The protective properties of specific anti-HBs immunoglobulin were tested for prevention of HBV transmission (Courouce-Pauty *et al.*, 1975), and would become the basis of Hyperimmune Hepatitis B Immune Globulin (HBIG) therapy. HBIG was originally derived from human serum of patients that contained anti-HBsAg antibodies. HBIG was, and still is, used for prophylactic treatment of HBV. If administered soon after exposure, either perinatally, or by needlestick injury, the HBsAg antibodies effectively neutralise the virions, preventing establishment of infection. Original trials in Taiwan demonstrated its efficacy in preventing perinatal transmission of HBV infection (Beasley *et al.*, 1983).

Finally combination therapy of protein based vaccine and simultaneous HBIG administration was shown to be effective at providing immediate followed by longer term protection, which was useful for immunocompromised patients (Goudeau *et al.*, 1983), and prevention of mother to baby transmission.

Several years after the commercial HBV protein vaccine became available, escape mutants were discovered. Escape mutants are not neutralised by the antibodies produced to the normal 'a' determinant. A Japanese child born to an HBeAg-positive carrier mother received both HBIG and protein vaccine, but developed chronic hepatitis by 12 months of age. Unusual serology was noticed: HBsAg, anti-HBs and HBeAg were all positive. The

nucleotide sequences of the S region of HBV DNA obtained from the patient, the mother and a HBeAg-positive brother were completely identical except for one nucleotide at position 587, giving an amino acid change: Gly to Arg at position 145 of the major HBs protein (Fujii *et al.*, 1992). Several other studies produced similar findings (Okamoto *et al.*, 1992; Waters *et al.*, 1992; Yamamoto *et al.*, 1994; Carman, 1997; Chakravarty *et al.*, 2002; Shizuma *et al.*, 2003). The findings that such escape mutants are infectious (Okamoto *et al.*, 1992), is evidence that although the 'a' determinant is immunodominant, it is not absolutely required for infection.

The discovery of vaccine escape mutants lead to the consideration of inducing an immune response to the viral cell receptor, considered to be contained within the preS region. Escape mutations would then be very much restricted, as the virus would need to mutate away from the immune response but still be able to bind the cell. Experiments using rabbit antisera to the preS protein were shown to protect chimpanzees (Neurath *et al.*, 1986b; Neurath *et al.*, 1989), similar results were obtained with preS2 region Ab (Emini *et al.*, 1989).

The use of protein vaccines has generally been considered unsuccessful in the treatment of already chronic infections; a form of tolerance prevents a successful immune response form being generated. However, there is some evidence that after protein vaccination of chronic patients without cirrhosis, they may eliminate DNA from the serum (~20%, 3/14 patients), or significantly decrease replication (~28%, 4/14 patients), within 3 months of the final inoculation (Pol *et al.*, 1993), but no long term data has been produced. This has lead to the use of both antiviral and immune boosting treatments.

Nucleoside analogues originally developed for use with retroviral infections were tested because hepadnaviruses also utilise an RT step in replication. Several drugs (eg. Lamivudine, adefovir, and entecavir), all with varying degrees of cytotoxicity, have been trialled with various degrees of success (Bain *et al.*, 1996; Foster *et al.*, 2003; Le Guerhier *et al.*, 2003; Okamoto *et al.*, 2003; Yu and Keeffe, 2003). The drawback of antiviral therapy is quick development of resistance (Fischer *et al.*, 2001a), and combination therapy is now being evaluated (Soemohardjo, 2003).

IFN is now being successfully used to treat HBV (Bahar *et al.*, 2003; Yalcin *et al.*, 2003). It was shown to upregulate expression of viral peptides in conjunction with MHC-1, which leads to elimination and recovery from infection (Grandits *et al.*, 1991).

Most of the currently available treatments were originally investigated in the animal models of HBV (Zoulim *et al.*, 2002). DHBV has been used for the testing of most of the antivirals

(Sherker *et al.*, 1986; Tsiquaye *et al.*, 1986; Zuckerman, 1987; Wang *et al.*, 1995), as well as combination therapy (Chen *et al.*, 2001), and immune modulating therapies are starting to be tested as well (Huang *et al.*, 2001).

The major drawbacks of current HBV therapy are the relatively low effectiveness, the high cost, and toxicity of the treatments used. Successful treatment of persistent infection is measured not by complete eradication of the virus from the liver of the individual, but rather seroconversion and removal of virus from the bloodstream. Even so, current treatments can be 12 months, or longer, followed by rebound soon after cessation of treatment. Even in combination therapy utilising IFN and an antiviral for twelve months, only 45% (15/33) had decreased DNA levels, while IFN monotherapy had an even lower effect with only 19% (3/16) of patients responding with lower DNA levels (Yalcin *et al.*, 2003). Even so, there was no significant difference in rates of sustained suppression between the 2 groups at the end of follow-up (Yalcin *et al.*, 2003). As such, therapeutic treatment currently has much to improve upon, and even partially effective treatments, may be used in combination to produce a better outcome. A therapeutic vaccine based on low cost DNA vaccine technology would offer a realistic alternative for the many established carriers who are resident in the poorer countries of the world.

1.5. DNA VACCINATION

Genetic immunisation is a novel vaccine strategy that combines many of the most desirable characteristics of standard vaccine approaches. Although traditional live-attenuated or killed vaccines have proven their effectiveness in the eradication, or minimisation of many microbial infections, current safety requirements and specific pathogens require vaccine actions of significant complexity that will overcome current technological inadequacies.

Increasingly, successful vaccination against many infectious diseases, particularly viral infections, including HSV, and HIV, but also parasitic infections such as malaria, will require the induction of strong, specific CMI, particularly cytotoxic CD8+ T-cell (CTL) responses. Such CTLs may respond early after infection by recognising specific peptides presented in MHC-I molecules on the cell surface, but may also secrete a variety of soluble factors that help to control infection.

Improved vaccination strategies for humoral immunity, especially at mucosal surfaces where most pathogens are first encountered is also desired. Such improvements would not only benefit responses against pathogens, but also for the treatment of both allergic and autoimmune diseases.

1.5.1. Historical Aspects

Since the inception of DNA vaccine technology in the early 1950s, (Stasney *et al.*, 1950), a period of about three decades elapsed before it was demonstrated that the administration of recombinant DNA into an animal resulted in the expression of the protein encoded by that plasmid (Will *et al.*, 1982; Dubensky *et al.*, 1984; Wolff *et al.*, 1990; Gheit *et al.*, 2002). It was subsequently shown that the expression of foreign protein from applied DNA elicited a humoral immune response that was specific for the encoded antigen, (Tang *et al.*, 1992). These results were furthered by observations that immunisation with a DNA plasmid could protect mice against a lethal influenza challenge (Fynan *et al.*, 1993; Ulmer *et al.*, 1993). Moreover, Wang *et al.*, demonstrated that a plasmid vaccine could induce protective immune responses against HIV-1 antigen-expressing targets (Wang *et al.*, 1994). Altogether, the implications of these findings served to establish genetic immunisation as an approach to induce an immune reaction against infectious agents. Since then, it has been shown that DNA vaccines induce strong immune responses against proteins from infectious agents such as malaria (Wang *et al.*, 1998), tuberculosis (TB) (Lowrie *et al.*, 1997), rabies virus (Xiang *et al.*, 1994), HSV (Kriesel *et al.*, 1996), Ebola virus (Xu *et al.*, 1998), HIV (Boyer *et al.*, 1999), and hepatitis B virus (Davis *et al.*, 1994; Tacket *et al.*, 1999).

The strategy of most of these investigations is relatively simple: A DNA plasmid encoding a desired protein is injected into the muscle or skin of an animal, where it enters host cells and directs the synthesis of its polypeptide antigen. Once the plasmid-antigen is processed and presented by transfected host cells, a cellular and humoral immune response against the antigen is provoked. The plasmid's immunogenicity may be enhanced in part by the presence of repeated immunostimulatory motifs that are recognised by the immune system as foreign. The DNA vector is bacterial-derived and equipped with eukaryotic or viral promoter/enhancer transcription elements that direct the high-efficiency transcription of the plasmid-antigen within the nucleus of the host cell.

Increased knowledge of the roles of different T-cell subsets in protection against infectious diseases, and pathology associated with allergic responses has allowed a rational approach to the development of vaccines against these conditions. The application of such knowledge has facilitated the design of vaccination strategies capable of selectively stimulating different classes of immune responses optimal for the treatment of a variety of infectious, and allergic diseases.

Such a vaccine has the possibility of breaking the tolerance that is found in persistent infections. It is thought that if important antigens are delivered to the host by a new pathway that it may be possible to develop an immune response that may clear the infection.

1.5.2. DNA Vaccine advantages

Genetic immunisation exhibits many advantages over traditional vaccines that use live-attenuated or killed pathogen, proteins, or synthetic peptides. Humoral and cellular-immune responses can be achieved in animal models at extremely low dosages of DNA vaccine. Unlike immunisation with proteins, the intracellular synthesis of plasmid protein results in antigen likely to be folded in its native conformation, correctly glycosylated, and normal posttranslational modifications to occur similar to natural infection, favouring the production of relevant neutralising antibodies. In addition, they are safer conceptually than live vaccines because of the inability to revert into virulence, and they do not require the use of toxic chemical inactivation methods. Current techniques in molecular biology enable the easy manipulation of plasmid vectors, which are able to accommodate virtually any gene or its derivatives. At relatively low costs, these recombinant plasmids can be produced at large scale in bacteria and isolated simply using commercially available reagents. DNA vaccines are also considered more temperature stable than conventional vaccines, boasting a longer shelf life. This is of significance because it would impact the requirement of the cold chain, a costly and difficult issue, and thereby enhance vaccine storage and mobility.

1.5.2.1. DNA vaccine safety

The risks associated with DNA plasmid inoculation are currently being assessed in many animal models and Phase I clinical trials. The suspicions that plasmid DNA may cause tumourgenesis, integrate into the host chromosome (Nichols *et al.*, 1995), or induce anti-DNA autoimmune responses in the host (Donnelly *et al.*, 1997) raise concern, yet little evidence has substantiated the occurrence of these phenomenon, particularly in humans or primate experimental models. Mutation rates occurring from the integration of plasmid DNA into the host chromosome have been calculated in animal studies and found to be much lower than the spontaneous mutation rate for mammalian genomes (Nichols *et al.*, 1995; Martin *et al.*, 1999). A study conducted in fish has also confirmed that the administration of DNA plasmids can elicit immunity effectively without the initiation of nucleic-acid autoimmunity or host chromosome integration (Kanellos *et al.*, 1999).

Administration of HIV-1 DNA plasmid constructs has been described as safe and well-tolerated in adult, pregnant, and infant chimpanzees, with the induction of humoral and cellular immunity (Bagarazzi *et al.*, 1998). The first human trial of a therapeutic DNA vaccine for HIV-1 infection generated reassuring results, in fifteen patients, vaccine administration induced no local or systemic reactions, no anti-DNA antibody, nor muscle-enzyme elevations, but increased cytotoxic T lymphocyte activity against HIV surface antigen-bearing targets (MacGregor *et al.*, 1998; Ugen *et al.*, 1998; Boyer *et al.*, 1999). These results suggest that the inoculation of plasmid DNA into animals and humans is

considerably safe and an effective means of generating immune responses against plasmid-encoded antigen.

In another clinical study, twenty healthy adult volunteers demonstrated that intramuscular (*im*) administration of a malaria DNA vaccine of up to three doses of 2500μg plasmid DNA was well tolerated, thereby expanding the safety limits of genetic vaccine dosages in humans (Le *et al.*, 2000).

1.5.3. DNA Vaccination in Alternative Immunotherapies

Another facet of DNA vaccine technology focuses on immune related diseases, such as autoimmunity and cancer (Chen *et al.*, 1999). By manipulating the balance of T helper (Th) 1 and 2 lymphocytes using DNA plasmid immunisation, many of the pathogenic qualities of autoimmune disease may be potentially addressed. Protective immunity against an experimental autoimmune encephalomyelitis (EAE) model has been induced by using a DNA vaccination method that favours the induction of a Th2-type response (Ramshaw *et al.*, 1997). Conversely, suppression of a Th2 response by the induction of a Th1-type response against allergens associated in an IgE antibody-mediated allergic response has been shown to neutralise the dysregulated production of Th2 cytokines and diminish allergic reactions (Raz *et al.*, 1996). These findings demonstrate the functional utility of DNA vaccines in the realm of autoimmune therapy.

1.5.4. DNA Vaccine Delivery

The most popular method of administering DNA vaccines has been parenterally, which includes needle injection into muscle or skin and gas-powered, DNA-covered particle bombardment using a "gene-gun". Although these forms of delivery require either a needle or ballistic device to mechanically force plasmid through or into the skin, non-invasive routes of delivery have been demonstrated, they entail the topical application of pure DNA plasmid to skin or mucosa. Each one of these methods of delivery introduces vaccine to distinct areas of immune surveillance and therefore primes the immune system in distinct ways.

The use of a needle to inject an aqueous solution of DNA plasmid into tissue is a relatively simple and effective way of vaccine administration, resulting in the direct transfection of some cells and the uptake by others in the vicinity of the inserted needle. Injection intradermally (*id*) results in the transfection of mainly skin fibroblasts and keratinocytes, whereas intramuscular (*im*) injection transfects largely myocytes. In gene-gun-mediated delivery, gold particles covered with plasmid DNA are propelled by helium or CO_2 pressure into tissue (Williams *et al.*, 1991; Tang *et al.*, 1992). This method of delivery is very

effective at driving plasmid into the cells of the epidermis and requires far less DNA than needle injection.

Non-invasive methods of plasmid delivery involve the topical application of plasmid to the skin or mucosa. The induction of antigen-specific immune responses has been shown following the application of a plasmid solution to various mucosal surfaces including intranasal (Klavinskis *et al.*, 1999), oral (Etchart *et al.*, 1997), and intravaginal (Bagarazzi *et al.*, 1998). It has also been shown that the topical application of DNA plasmid directly to the skin transfects the superficial layers of the epidermis surrounding hair follicles, generates reporter-gene activity at levels comparable to that of *id* injection (Yu *et al.*, 1999), is dependent on the presence of normal hair follicles, and induces antigen-specific immune responses that display Th2 features (Fan *et al.*, 1999). This technique of delivery may be ideal for targeting genes to the skin for the treatment of cutaneous disorders.

The immunity resulting from each of these methods of delivery are determined usually by the mode and site of plasmid administration. Forms of delivery targeting the skin, including *id* injection, gene-gun bombardment, and topical application, have been shown to elicit a humoral response primarily, characterised by a rapid progression to a Th2-type response, associated with the production of an IgA and IgG1 antibody isotype (Boyle *et al.*, 1997). Conversely, injection into muscle results in the induction of a strong cellular-mediated response, or Th1 type, that primes antigen-specific CTLs and is associated with the production of IgG2a antibody (Sin *et al.*, 1999a).

The extent of protection elicited by these various modes of vaccine administration is determined most likely by the network of antigen-presenting cells (APCs) residing in the target tissue and the quantity of DNA plasmids administered (Takashima and Morita, 1999). APCs are more prevalent in the skin than in muscle, so less plasmid DNA may be required to induce a response of similar magnitude. However, the quality of the immune responses suggests that the APCs transfected in these different locations are functionally distinct and therefore prime the immune response uniquely. These particular features suggest further evaluation of each compartment could be important for future vaccine design.

1.5.5. Direct DNA Injection

Direct DNA injection has been previously shown to produce expression of proteins in animals and humans. Usually the injected material consists of the sequence for the protein of interest coupled to a promoter or enhancer and some sort of expression system. The method usually utilised to obtain the large quantities of DNA required for injection is the insertion of viral DNA into bacteria. This has several consequences: 1) firstly the DNA

itself is slightly different from that found in eukaryotic cells in that it is methylated, which may change the physical shape of the DNA and thus affect regulatory properties, 2) the actual structure of the DNA is different because usually a linear strand of DNA is inserted into a plasmid, and this lacks many of the physical characteristics of virion encapsidated DHBV DNA, such as the covalently linked terminal protein, and the nick-gap structure, and 3) it is devoid of associated proteins which may affect packaging. The mechanism of uptake of the DNA in direct injection is unknown, but may be some remnant of the prokaryotic plasmid transfer system. Apart from the usual injection to express a single protein, multiple proteins and even complete viral particles have been expressed. Direct DNA injection in relation to the hepadnaviruses has been described in 1.2.6 (p.36).

1.5.6. Mechanism of Immune Induction

DNA vaccines elicit strong and long-lasting humoral and cell-mediated immune responses in many animal models. Although there has been much speculation regarding the complex mechanisms underlying DNA vaccine function, these have yet to be fully elucidated. Progressively dissecting the cellular and immunological processes of genetic immunisation that are responsible for the induction of immune responses will lead ultimately to further advances in this technology. At the cellular level, the efficacy of DNA vaccination depends on the interaction between their polypeptide products and the two major groups of cells that mediate immunity: lymphocytes and APCs.

The intracellular transcription and translation of plasmid DNA are thought to mimic the replication of a virus during infection. Both systems must traverse the plasma membrane initially and require the cellular machinery to translate their encoded proteins. In transfected nonhaematopoietic cells, intracellularly synthesised plasmid product is processed effectively via the transporters associated with antigen processing (TAP)-dependent, endogenous-processing pathway. In addition, soluble or secreted vaccine antigen may be phagocytosed by APCs and gain entry into the major histocompatibility complex (MHC) class II exogenous pathway. So, like the viral proteins produced by a replicating virus, plasmid product may gain access to both pathways simultaneously, affecting its presentability to the immune system.

1.5.6.1. Manipulating Immune Responses

Vaccines that elicit prophylactic immune responses are specifically constructed and administered to provide optimal protection at the sites most frequently encountering pathogens. For example, effective mucosal immunity is desired when protecting against infectious agents transmitted by aerosols, such as TB. Ideally, vaccine regimens must be tailored to neutralise pathogens before the onset of infection and disease. Because

experiments in primates suggest that DNA vaccines alone may not be as immunogenic in these species as they are in rodents (Wang *et al.*, 1998), their co-administration with genetic and chemical adjuvants may bolster their immunogenicity and efficacy. In addition, the use of particular adjuvants can help direct the magnitude and direction of prophylactic and therapeutic immune response that target microorganisms at pivotal points within the pathogen/host interaction.

Many strategies involving the combination of DNA immunisation and adjuvants are under investigation. Specifically, vaccine immunogenicity can be modulated by factors that attract professional APCs, provide additional co-stimulation, or heighten the uptake of plasmid DNA. In these ways, the direction of an immune response can be guided toward a cell-mediated, Th1-type response or an antibody-mediated, Th2-type response, driven by the differential expression of cytokine patterns by their distinctive T-cell subsets (O'Garra and Murphy, 1994).

1.5.6.1.1. Cytokine-encoding plasmids

Cytokines are molecules secreted by bone marrow-derived cells that regulate the intensity and duration of the immune response in lymphocytes and other immune cells expressing a particular cytokine receptor.

In 1993, Raz *et al.,* inoculated a group of mice with several DNA plasmids encoding cytokines in an effort to improve the approaches of somatic gene therapy involving the direct administration of cytokines (Raz *et al.*, 1993). Expression of these plasmids was observed to induce systemic immunological effects characteristic of the specific functions of the respective cytokine proteins and also could enhance the immune response to an exogenous antigen that was delivered at a different site.

The co-administration of DNA vaccines with cytokine-encoding adjuvants can manipulate the differentiation and expansion of Th1 and Th2 cytokine producers effectively.
Protection from certain viruses or tumours would require the production of Th1-inducing cytokines, such as IL-2, IL-12, IL-15, IL-18, and IFN-γ, which promote cell-mediated immune responses. Plasmid co-delivery of IL-12 with DNA immunogens can drive the immune responses toward a Th1 phenotype and increase the survival rate of mice, following a lethal dose challenge in an HSV-2 model (Sin *et al.*, 1999b).

Conversely, protection from antibody-mediated pathologies may benefit from the use of Th2-inducing cytokines such as IL-4, IL-5, and IL-10 to drive humoral immunity. It has

been demonstrated that increased levels of antigen-specific antibodies were associated with co-delivery of IL-4, IL-10, with a HIV-1 and SIV construct (Kim *et al.*, 1999).

Another method of enhancing the immune response using genetic cytokine adjuvants is the expansion of the professional APC pool, particularly DCs and macrophages, at the site of inoculation. The expression of the haematopoietic growth factor granulocyte-macrophage colony-stimulating factor (GM-CSF) and a DNA vaccine have been shown to boost the activity of B- and T-helper cells toward rabies glycoprotein and improves the protective response against a lethal challenge (Xiang and Ertl, 1995). This boosting effect of plasmid-expressed GM-CSF on immune responses against vaccine antigen has also been seen for HIV-1 *env* protein constructs (Kim *et al.*, 2000).

1.5.7. CD8+ CTL Restricted Responses

CD8+ CTLs are known to be important mediators of protective immunity against many viruses, intracellular bacteria, parasites, and tumours (Kasper *et al.*, 1995; Zerrahn *et al.*, 1996; Ahmed *et al.*, 2001; Blaszczyk-Thurin *et al.*, 2002; McShane *et al.*, 2002; Nakamura *et al.*, 2003; Tsuji and Zavala, 2003). Such CTLs are normally restricted to recognition of peptides associated with MHC-I molecules and usually recognise small epitopes of 8-10aa in length, which are predominantly derived from the target antigen by proteasome-dependent proteolytic processing.

Artificial recombinant vaccines comprising multiple contiguous minimal CTL MHC-I epitopes can induce CTL responses to each epitope within the polytope construct. This strategy uses relatively small recombinant constructs to induce multiple CTL responses that target multiple antigens and/or induce CTLs that are restricted by multiple HLA alleles (Thomson *et al.*, 1995; Thomson *et al.*, 1998b).

Various proteases may be involved in breaking down the polytope gene product into the individual CTL epitopes, which will be subsequently associated and expressed with MHC-I molecules. It has been shown that each epitope within several polytope constructs made without spacers or linkers may be processed and presented, suggesting that proteolysis and transport of epitopes into the cellular ER is governed primarily by the intrinsic qualities of the epitope rather than by flanking sequences (Niedermann *et al.*, 1996).

1.5.8. MHC-II Restricted T-Cell Responses

The ability of polytope constructs to deliver class II MHC-restricted CD4+ T-cell epitopes was demonstrated by delivering Th-cell epitopes in a polytope construct in a recombinant Vaccinia Virus (An and Whitton, 1997). Whilst this approach was successful, the simple

inclusion of MHC class II-restricted epitopes in cytoplasmically expressed polytopes delivered by non-lytic vectors, such as DNA vaccines or FowlPoxVirus (FPV), is unlikely to reliably generate effective CD4+ T-cell responses *in vivo* (Thomson *et al.*, 1998a). However, ER targeting is an alternate strategy which does not involve cell lysis or antigen secretion, but which significantly enhances the presentation of contiguous class II-restricted T-cell epitopes from polytope constructs, has been shown to be effective (Thomson *et al.*, 1998a).

1.5.8.1. ER-targeted Antigen Processing

ER-targeted antigen processing differs from normal DNA vaccination, by the addition of an ER signal sequence to the beginning of the polytope gene. This allows the polytope proteins to access MHC-II processing compartments in antigen-presenting cells directly from the cytoplasm, thus significantly enhancing CD4+ T-cell responses generated by polytope DNA vaccines. An important design requirement, however, is that the synthetic protein be long enough to delay its removal from the ER following translation (Thomson *et al.*, 1998a). The delay in the ER appears to be important for efficient epitope presentation and may enhance autophagy or may allow unfolded polytope proteins to compete with the invariant chain for binding to newly synthesised class II MHC antigens. Interestingly, the presentation of CD4 T-cell epitopes from polytope proteins does not appear to require the natural flanking sequences, nor does presentation seem to suffer from a lack of conformation-dependent processing signals (Thomson *et al.*, 1998a).

DNA vaccination appears to be the most practically useful, and effective method of therapeutic vaccination that is capable of stimulating a specific cellular immune response. Such stimulation is particularly useful for persistent infections, such as the hepadnaviruses, in which persistence has been shown to be associated with a poor cellular immune response. Because there is evidence of non-cytopathic clearance of hepadnavirus infected cells, a therapeutic vaccine that stimulates the cellular arm of the immune response could be designed to eliminate the infection without excessive side effects, such as massive cell death which would lead to hepatitis.

1.6. EXPERIMENTAL OUTLINE

The aims of this study were to identify critical virus and host factors responsible for recovery from hepadnavirus infection, and to use this knowledge to design and test a therapeutic vaccine, which would promote virus clearance in carriers.

Many factors contribute to the outcome of hepadnavirus infection. These factors can be assigned to two competing forces; the host response aimed at elimination of the virus; and the viral evasion of the response. The host response is complex and multifactorial, which is difficult to analyse in the outbred populations, which are the only available models of hepadnaviral infections. Although the general pathway of the production of specific antibodies and CMI response is well known, it is now clear that the ultimate outcome is also dependent on the exact epitope specificity and effector capability of the response. One of the reasons that one individual develops a different response from another can be explained by the various HLA types. In many infections, not just viral, it has been shown that individuals with certain HLA types either fare better or worse against certain organisms because they either accentuate a specific response or have a repertoire defect that the infecting organism can exploit. Many microorganisms have developed specific mechanisms to facilitate evasion of the host response. These include mutation to alter the immune target antigens, alteration in display of host recognition antigens required for antigen presentation to the immune system, and inhibition of cytokine production.

The host and virus responses are a delicately balanced association, so that relatively minor changes to either may modulate the outcome of infection.

An initial experiment was undertaken to establish experimental conditions that reliably lead to persistent or acute DHBV infection, and to develop a method for nucleotide sequencing which would be useful in studies of specific virus variants.

Viruses from ducks with different patterns of DHBV infection were sequenced and a particular nucleotide substitution in the pre-S gene was identified in association with virus clearance. These strains were cloned and shown to lack infectivity.

Published sequences for the S region of DHBV were analysed to identify epitopes with physiochemical properties associated with antigenicity and these predictions were tested by comparing lymphocyte proliferation responses to short synthetic peptides in naïve, inoculated, and immune ducks.

Seven immunologically dominant peptides were selected for incorporation into a DNA vaccine. The DNA vaccine was tested for immunogenicity and efficacy in ducklings. It was found to confer protective immunity through generation of neutralising antibody and caused a $2\log_{10}$ reduction in the level of viraemia in established carrier ducks.

To ascertain the relative roles of humoral and cellular immunity the ability of ducks to clear DHBV was investigated after neonatal bursectomy or thymectomy.

The experimental procedures undertaken during this investigation are summarised (Figure 6 p.65).

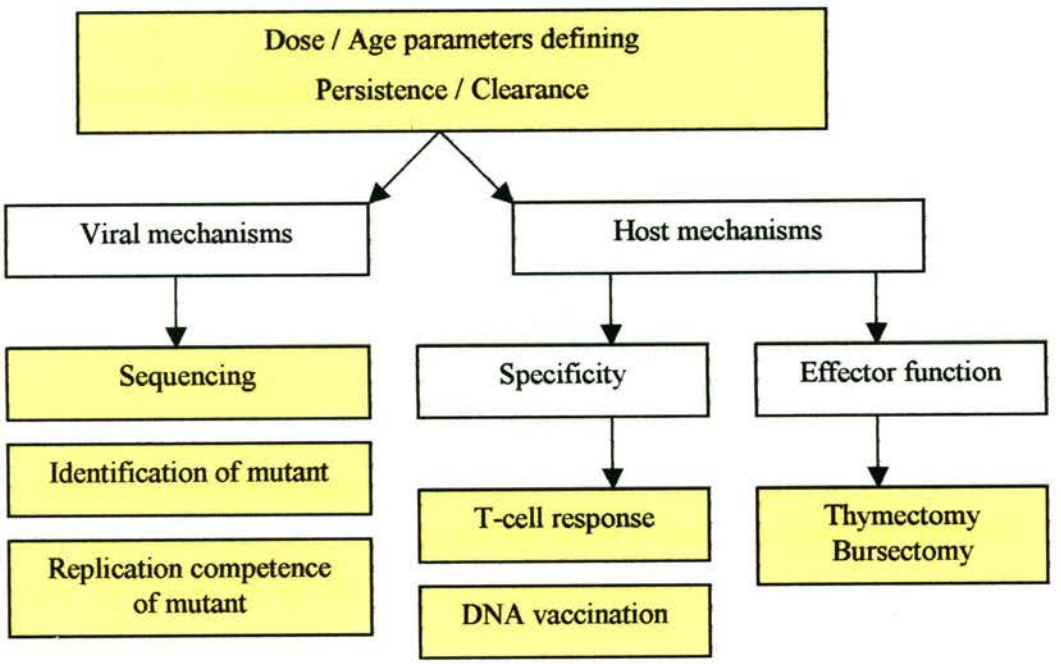


Figure 6. *Experimental Outline.*
Yellow boxes indicate experimental procedures.

On the basis of these experiments a model of DHBV clearance is proposed in which innate cellular immunity causes prolonged down regulation of virus replication, during which a neutralising humoral response develops and prevents ongoing infection of hepatocytes. This model would be consistent with observation on patients treated with antiviral drugs and interferon, and can be tested experimentally in the duck model.

2. METHODS AND MATERIALS

2.1. GENERAL EXPERIMENTAL PROCEDURES

General experimental procedures were used throughout the project, while more specific protocols are described in their own sections.

2.1.1. Experimental Animals

Pekin-Aylesbury crossbred ducks were purchased as unsexed male and female day-old ducklings from a commercial supplier that was known to have DHBV negative flocks (Ingham, Tahmoor, Australia). All ducks were, however, bled on day of hatch to determine if any DHBV DNA was present. No duck was ever found to have DHBV DNA in their serum on day of hatch.

All ducks were housed in specially designed animal house facilities, and were looked after and fed by specially trained animal house attendants, who would monitor the animals on at least a daily basis, and inform the researchers of any slight deviation from normal behaviour. Researchers monitored the animals at least twice a week, although daily visits would normally be undertaken.

2.2. SPECIFIC EXPERIMENTAL PROCEDURES

2.2.1. Extraction of Viral DNA

Viral DNA was extracted from liver and serum samples by a standard method of proteinase K digestion followed by purification using phenol and chloroform (Sambrook *et al.*, 2001). If re-extraction was required for sequencing, then the Casas *et al.* method of digestion using guanidinium hydrochloride followed by glycogen facilitated, isopropanol precipitation was adapted for use (Casas *et al.*, 1995).

Samples were extracted in groups of up to 24, including one DHBV negative duck serum control and one DHBV positive duck serum control. The negative serum served as a control for contamination during the extraction procedure, as well as for the subsequent PCR assays.

Where possible, 50µL of sample was extracted, if there was insufficient sample it was made up to 50µL with PBS for extraction. All the extracted DNA was resuspended in the same volume of TE (0.1mM EDTA, 10mM Tris, pH 8.0) as the original serum sample volume. The pellet was resuspended at RT for approximately 1h prior to use, or stored at –20°C.

2.2.1.1. Proteinase K / Phenol / Chloroform Extraction Method

The extraction buffer was made up as per Table 9 (p.67). An equal volume of buffer was added to serum, or for tissue extraction 275µL of buffer was added to a small cube of liver (3x3x3mm or ~27µL). It was then incubated overnight at 37°C, or for 3hrs at 65°C.

Reagent	Concentration
Tris/HCL pH 7.5	50mM
NaCl	150mM
EDTA	2mM
SDS	1%
Proteinase K	1mg/mL

Table 9. *Composition of Proteinase K Extraction Buffer.*

A volume of phenol (pH 7.5 - 8.0) equal to the total volume of digestion buffer and sample was added, mixed, and centrifuged at 15000rpm for 3mins in a bench microfuge. The supernatant was carefully removed and placed into a clean, labelled eppendorf. This step was repeated if necessary. A volume of phenol / chloroform (1:1 v/v) equal to that of the supernatant was added, mixed, and again centrifuged at 15000rpm for 3mins. The supernatant was carefully removed and placed into a clean eppendorf. A volume of chloroform / isoamylalcohol (24/1) equal to that of the supernatant was added, mixed, and again centrifuged at 15000rpm for 3mins. The supernatant was carefully removed and placed into a clean eppendorf. A 1/10th volume of 3M Sodium Acetate (pH 5.2) was added, then 2 volumes of cold ethanol was added, mixed and incubated at –20°C overnight, or –70°C for 3hrs. It was then centrifuged at 15000rpm in a bench top centrifuge at 4°C for 20-30mins. The supernatant was aspirated and the pellet dried. A volume equal to that of the initial serum extracted, or 100µL for liver, of TE (0.1mM EDTA, 10mM Tris, pH 8.0) was added and stored at –20°C until required.

2.2.1.2. Guanidinium Extraction Method

The adaptations of Casas *et al.* method included using Dithiothreitol instead of 2-mercaptoethanol, and incubation of the specimen with the lysis buffer at 60°C (instead of RT) (Casas *et al.*, 1995). **Procedure**: Four volumes of extraction buffer (Table 2) was mixed with the serum, and glycogen (Boehringer Mannheim, Mannheim, Germany) added to a final concentration of 80µg/mL. The mixture was incubated at 60°C for 10mins.

Reagent	Concentration
Guanidinium thiosocyanate	4M
Sodium citrate (pH 7)	25mM
N-laurylsarcosine (sarcosyl)	0.5% w/v
dithiothreitol	1mM

Table 10. *Composition of Guanidinium Extraction Buffer.*

A volume of cold ethanol equal to the total volume of digestion buffer and serum was added and mixed and centrifuged at 15000rpm in a bench top centrifuge at 4°C for 10mins. The supernatant was aspirated and the pellet washed with 70% ethanol by centrifugation at 4°C for 10mins. The supernatant was aspirated and the pellet dried. A volume equal to that of the initial serum extracted of TE (0.1mM EDTA, 10mM Tris, pH 8.0) was added and stored at –20°C until required.

This method of extraction failed to remove PCR inhibitors which necessitated the dilution of the sample by 1:10, therefore, it was only used when the sample had already been found to be positive for DHBV DNA (by dot blot hybridisation), and PCR was required for sequencing data.

2.2.2. Polymerase Chain Reaction

PCR assays were performed following published recommendations aimed at minimising carry-over contamination (Kwok and Higuchi, 1989). Four physically separate areas, with separate ventilation, were used: a "clean" area for the storage of reagents and the preparation of the PCR reaction mixture; an area for the storage of specimens and extraction of viral nucleic acid; an area where the thermocyclers were kept and used; and an area for the handling and storage of products from PCRs. In the first area all handling of reagents was within a dedicated class II biohazard safety cabinet; in the last area, where possible, products from PCR were handled within a class I biohazard safety cabinet. Each of these areas had equipment and consumables, which were stored and used, only within those areas. Restrictions on workflow were also adopted to minimise contamination.

Reagents for PCR were prepared as a master mix cocktail (Table 11, p.69), aliquots were placed into individual reaction tubes and used immediately, or frozen at -70°C and used within 48 hours. After template addition (equal to 5µl of serum) the tubes were immediately placed into the thermocycler. Amplified DNA was stored at –20°C within 12 hours of cycling.

Reagent	Final concentration		
	Full length	PreS-S	PreCore
10xBuffer	1x	1x	1x
$MgCl_2$	2.5mM	2.5mM	2.5mM
dNTP	200nM	200nM	200nM
Primer (each) forward + reverse	0.4μM	0.4μM	0.4μM
Polymerase	2U /25μL	1U /25μL	2U /25μL
dH_2O	to 25μL	to 25μL	to 25μL

Table 11. *DHBV PCR cocktail contents.*

When possible a full length PCR fragment was produced, which enabled the two ends of the Surface gene, and the preCore region, to be sequenced from the same PCR fragment. The more sensitive PreS-S PCR was used when necessary, and in combination with the preCore PCR.

2.2.2.1. DHBV Full-length PCR assay

A full-length DHBV PCR product (~3kb) (nt 2753-2752) (Figure 7, p.70) was produced from the DHBV_C2fP and DHBV_CrP (Table 12, p.70) primers, and the cocktail (Table 11, p.69). This set of primers was 5' phosphorylated to enable cloning, or ligation to other fragments of DNA. Phosphorylation has no effect on the normal PCR assay. The ends of these primers are next to each other on the DHBV genome but elongate in the opposite direction thus producing a full length PCR product. Cycling conditions consisted of an initial denaturation at 94°C for 2min, thence 30s, annealing at 55°C for 30s, extension at 68°C for 4min, with a final extension at 72°C for 10min after 40 cycles.

The full-length DHBV PCR had a sensitivity of approximately 100-500 vge per reaction, which was equivalent to approximately $1x10^5$ vge/mL in the original serum.

2.2.2.2. DHBV PreS-S PCR assay

(Figure 7, p.70) (Table 12, p.70) (Table 11, p.69)

A 1.1kb PCR amplicon was produced spanning the entire surface gene (nt 686-1824) (Figure 7, p.70), using a single primer from the PreS PCR (DHBV_PreS1_f), developed by Zhang, and a single primer from the S PCR (DHBV_S_r), also developed by Zhang (Zhang, 1994) (see Table 12, p.70). The PCR cocktail is detailed in (Table 11, p.69). Cycling conditions consisted of an initial denaturation at 94°C for 4min, thence 30s, annealing at 60°C for 1min, extension at 72°C for 1.5min, with a final extension at 72°C for 10min after 40 cycles.

The DHBV PreS-S PCR was the most sensitive DHBV PCR used. It was able to detect 1-10 vge per reaction, which was equivalent to approximately $2x10^3$ vge/mL in the original serum.

2.2.2.3. DHBV PreCore PCR assay

A 304bp PCR fragment was produced spanning the two Direct Repeat sites and the PreCore (nt 2456-2760) (Figure 7, p.70) using primers DHBV_PreC_f and DHBV_PreC_r (Table 12, p.70), and the cocktail (Table 11, p.69). The DHBV PreCore was modified from the assay originally developed by Zhang (Zhang, 1994). The numbers of cycles was increased to 40, the magnesium concentration was decreased to 2.5mM, and the amount of polymerase was decreased to 1U. Cycling conditions consisted of an initial denaturation at 95°C for 5min, thence 30s, annealing at 55°C for 1min, extension at 72°C for 1min, with a final extension at 72°C for 10min after 40 cycles.

The DHBV PreCore PCR had a sensitivity of approximately 100-250 vge per reaction, which was equivalent to approximately $5x10^4$ vge/mL in the original serum.

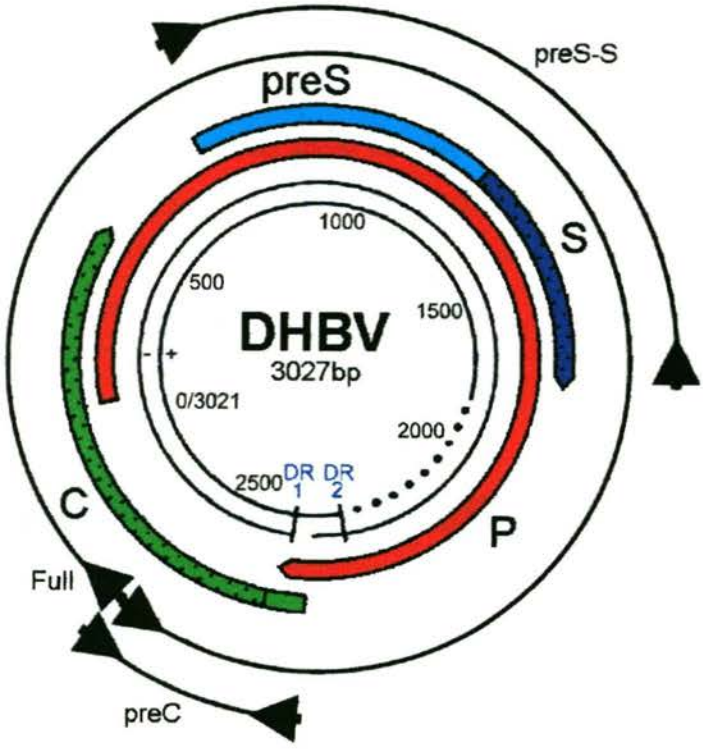


Figure 7. *PCR Amplicons in relation to the DHBV genome.*

Primer Set	Amplicon	Primer	Sequence
Full-length	3kb nt 2753-2752	DHBV_C2fP	TAGAACCTTATTGGAAATCAG
		DHBV_CrP	AAGCGTCTTTAGCATCCCTTACAA
PreS-S	1.1kb nt 686-1824	DHBV_PreS1_f	GGCTCTATGAAGCAGGAATCC
		DHBV_S_r	GGCGTGGTTTTGTCAAAGTT
PreC	304bp nt 2456-2760	DHBV_PreC_f	CGGAATTCGATTGGACGGCTGTTACATACACC
		DHBV_PreC_r	CGGGATCCAAGCGTCTTTAGCATCCCTTACAA

Table 12. *DHBV PCR primers.*

The full-length PCR primers were 5' phosphorylated to enable cloning or ligation. For GS-2000 sequencing the DHBV_PreS1_f primer was 5' labelled with HEX. The preS-S and preC primers were designed by Zhang (Zhang, 1994).

2.2.2.4. Visualisation of PCR bands

The products from the PreCore PCR assay, were run on a 2% (w/v) agarose gel, while the products from the full length and PreS-S PCR were run on a 1% (w/v) agarose gel (Biotech, Perth, Australia, or Promega, Madison, USA), containing 1μg/mL ethidium bromide (in dH_2O). 5μL samples of the PCR reaction (20% reaction volume) were electrophoresed for 20-40mins at 100-140V at room temperature, in the presence of 2x PCR loading buffer. Each gel also contained a marker or DNA ladder (100bp or 1kb PLUS DNA ladder, Life Technologies, Hilden, Germany), and positive and negative controls. Following electrophoresis, DNA bands were visualised by exposure to ultra-violet light in a standard manner (Sambrook *et al.*, 2001). A permanent record of the PCR reaction was made by photographing the gel.

2.2.2.5. PEG Precipitation of PCR products

Procedure: 45μL 2x PEG solution (Table 13, p.71) was added to 45μL PCR reaction and incubated at 4°C for 1hr, centrifuged at 15000rpm for 25min at 4°C. The supernatant was discarded and 300μL of 95% ethanol was added and re-centrifuged at 15000rpm for 25min at 4°C. The supernatant was again discarded and the pellet further washed with 300μL of 70% and re-centrifuged at 15000rpm for 25min at 4°C. The pellet was dried in a heating block at 42°C, and resuspended in 25μL TE (0.1mM EDTA, 10mMTris, pH 8.0). 5pmol (5μL) primer and 11μL PEG precipitated PCR product was sent for sequencing (SUPAMAC, Sydney, Australia) or done *in-house*.

Reagent	Volume (μL)
40% PEG 6000 (w/v)	3338
3M Sodium Acetate (pH 5.2)	1000
1M $MgCl_2$	32.5
dH_2O	629.5

Table 13. *Composition of 2x PEG solution.*

2.2.2.6. Analysis of Sequence Data

Sequence data was visually inspected and corrected for any slight errors or miscalled bases. The sequences were then aligned using ClustalW or PileUp (ANGIS). Any discrepancies from the consensus sequence were again manually inspected and assessed.

2.2.3. Dot Blot Hybridisation

The dot blot hybridisation assay was used as a semi-quantitative measure of DHBV DNA in both serum and liver.

The serum was normally serially diluted (neat, 1:2, 1:4, 1:8). DNA standards of positive and negative duck serum, as well as 200, 100, 50, 25, 10, and 1pg of DHBV DNA (2.2.3.2, p.73)

were placed into column 1. 25µL of sample or standard were denatured with 25µL 1M NaOH and dot blotted onto GeneScreen (Amersham, Buckinghamshire, England) hybridisation membrane using a BioDot® (BioRad, Hurcules, USA) apparatus. The membrane was removed from the apparatus and washed in 2xSSC for 5min, blotted dry and stored in a desiccator until hybridised.

2.2.3.1. Dot Blot Hybridisation

Prehybridisation: The membrane was prehybridised overnight at 65°C, in 20mL prehybridisation solution (2x SSC, BLOTTO, 1% SDS, 25mg/mL Calf Thymus DNA).

Probe preparation: An Amersham MegaPrime® kit was used (Amersham, Buckinghamshire, England) to label 25ng (2.5µL) of full length DHBV DNA (as per Dot Blot Hybridisation Standards, 2.2.3.2, p.73) according to manufacturers instructions. The DNA was denatured by boiling for 5mins in 25.5µL dH_2O, and 5µL random primer solution, cooled to RT before addition of 10µL of 5x Buffer, 2µL Enzyme (Klenow), and 5µL α-^{32}P labelled dCTP (PerkinElmer, Boston, USA, or ICN, Irvine, USA). The reaction was incubated at 37°C for 10min then left at RT for 1hr, 5µL 0.5M EDTA (pH 8.0) was added to stop the reaction. Large labelled DNA fragments were separated using a self made Sephadex G50 column. Duplicate 2µL samples of labelled probe were counted using a RakBeta scintillation counter (LKB Wallac, Stockholm, Sweden), to determine cpm/µL probe.

Hybridisation: The membrane was incubated in prehybridisation solution (2x SSC, BLOTTO, 1% SDS, 25mg/mL Calf Thymus DNA) containing $5x10^6$ cpm of labelled probe for 20hr at 65°C.

Washing and autoradiography of hybridised membranes: The membranes were washed twice with low stringency wash solution (2xSSC, 1% SDS) for 15min, then twice with high stringency wash solution (0.1xSSC, 1% SDS) for 15min and 30min, before being wrapped in cling wrap and placed in an autoradiography cassette with intensifying screens and X-ray film (BioMax MR, Kodak, Rochester, USA), for between 1 and 4 days at –70°C. The X-ray film was processed using a Kodak or DuPont automatic processor.

The sensitivity of the dot blot hybridisation assay was 1pg/25µL which was equivalent to $3.1x10^5$ vge per 25µl or $1.3x10^7$ vge/mL. The specificity of the dot blot hybridisation assay was good, as no DHBV negative duck serum or HBV positive human serum ever produced any result.

2.2.3.2. Dot Blot Hybridisation Standards

The standards for the dot blot membranes were full-length DHBV PCR fragments (2.2.2.1, p.69), purified by PEG precipitation (2.2.2.5, p.71), followed by column purification (Qiagen, Melbourne, Australia). All steps were checked by running on an agarose gel to confirm a single clean band of DNA of the correct size. The DNA concentration of the resultant solution was determined by spectophotometry (2.2.4, p.73), and the solution was diluted such that 25µL of standard contained 200, 100, 50, 25, 10, or 1 pg. DHBV positive and negative duck serums were also included in the standards, to provide specificity controls.

2.2.3.3. Dot Blot Hybridisation Values

The semi quantitative values given to serum and liver samples were based on comparison of the size and density of the sample dot with that of the standards in the dot blot hybridisation assay as described in Table 14 (p.73).

Value	vge/mL	Comparison
0	$\leq x10^6$	Not detected
1	$1.25x10^7$	Sample (neat) = 1pg standard
2	$1.25x10^8$	Sample (neat) = 10pg standard or Sample (1:8 dilution) = 1pg standard
3	$1.25x10^9$	Sample (neat) = 100pg standard or Sample (1:8 dilution) = 10pg standard
4	$1.00x10^{10}$	Sample (1:4) = 200pg standard Sample (1:8) = 100pg standard
5	$>2.01x10^{10}$	Sample (1:8 dilution) > 200pg standard

Table 14. *Dot blot hybridisation values.*

2.2.4. DNA concentration by Spectrophotometry

DNA concentration of a solution was determined using a spectrophotometer (DU640, Beckman, Palo Alto, USA). The absorbance of a sample containing DNA was determined at wavelengths 260, 280, and 320nm, when compared to a control solution consisting of the sample diluent (normally dH_2O, or TE). The ratio of A_{260}/A_{280} was used to determine the purity of the sample, optimally around a value of approximately 1.8. The A_{320} value was used as a background control. The sample was diluted in such a manner that the A_{260} value was between 0.1 and 1.0. Three A_{260} readings were taken, and averaged. The average A_{260} was then multiplied by the dilution factor and by 50 to obtain the amount of DNA in the original sample as µg/mL (Sambrook *et al.*, 2001).

2.2.5. Calculating the Mass of DNA in a DHBV genome

The mass of a viral genome equivalent of DHBV was calculated from the average of the 4 nucleotides (A, C, G, and T) in Daltons, which was then converted to grams and multiplied by the number of base pairs in the DHBV genome (Figure 8, p.74).

$MW_{(ave)}$ 1bp = 635 Daltons and 1 Dalton = 1.66×10^{-24}g

therefore : 1bp = 635 Daltons x 1.66×10^{-24}g
= 1.05×10^{-21}g

so : 1vge = 3027bp = 3027bp x 1.05×10^{-21}g/bp
= 3.19×10^{-18}g

Figure 8. *Calculations to determine the mass of a vge of DHBV DNA.*

2.2.6. Calculation of DHBV DNA concentration as vge/mL

The quantification of DHBV DNA in serum was achieved by dot blot. Viral Genome Equivalents (vge) were determined by visually comparing dots produced by 25µL samples of serial dilutions of serum with the dots produced by the DNA standards. From this comparison, calculations were performed to determine the concentration of DHBV in serum as vge/mL (Figure 9, p.74).

eg. DHBV051094

25µL of 1:8 dilution serum ≡ 10pg DNA standard
(visual comparison to standards from X-ray film)

therefore : 25µL undiluted serum = 10pg x 8 (Dilution Factor) = 80pg DNA

so: 1mL undiluted serum = 80pg / 0.025mL = 3200pg
= 3200×10^{-12}g / 3.19×10^{-18}g/viral genome
= 1.0×10^{9}vge/mL

Figure 9. *Calculations to determine the DHBV vge/mL of a serum sample.*

2.2.7. Preparation of DHBV Positive Duck Serum Pools

Pooled DHBV positive duck serum was produced by injecting day old ducklings with 200µL of the Australian strain of DHBV, isolated by Freiman and Cossart (Freiman and Cossart, 1986). Several serum pools, quantitated by dot blot hybridisation, were used throughout the experiments as detailed in Table 15 (p.74).

Serum Pool	Viral titre (vge/ml)
DHBV051094	1.4×10^{9}
DHBV200197	2.0×10^{10}
DHBV200499	2.0×10^{10}
DHBV201299	2.5×10^{10}

Table 15. *DHBV serum pools used throughout the experiments*

1 ID_{50} of serum pool DHBV051094 was 100µL of a $10^{-5.5}$ dilution (corresponding to 450vge) for ducklings when injected intraperitoneally at day 1 or day 4 (Dr. Karen Vickery, personal communication).

2.2.8. Preparation of DHBV Negative Duck Serum Pools

Six week old ducks were obtained from a DHBV negative farm and subjected to veterinary health checks for one week. Ducks were anaesthetised by *iv* pentobarbitone, and exsanguinated by heart puncture. Blood was collected, placed into 50mL centrifuge tubes and allowed to stand overnight at RT. The tubes were then centrifuged at 5000 rpm in a Beckman JA-14 rotor for 5min (Beckman, Palo Alto, USA). The serum was pipetted off and pooled, then frozen and stored at -20°C. The serum was tested by both dot blot hybridisation and PCR to ensure that it was DHBV negative. The same batch of negative serum was used throughout the experiments.

2.2.9. Cell Counting

This technique is used to determining cell numbers. The haemocytometer consists of two chambers, each of which is divided into nine 1.0mm squares, which are divided into 16 smaller squares. A cover glass is supported 0.1mm over these squares so that the total volume over each square is 1.0mm x 0.1mm or $0.1mm^3$, or $10^{-4}cm^3$. Since $1cm^3$ is approximately equivalent to 1mL, the cell concentration per mL will be the average count per square $x10^4$.

Haemocytometer counts are subject to various sources of error (Table 16, p.75). Careful attention to detail can reduce the overall error to approx. 15%. It is assumed that the total volume in the chamber represents a random sample. These will not be a valid assumption unless the suspension consists of individual well separated cells. Cell distribution in the haemocytometer chamber depends on the particle number, not the particle mass. Thus, cell clumps will distribute in the same manner as single cells and can distort the final result. Unless 90% or more of the cells are free from contact with other cells, the count should be repeated with a new sample. A sample will not be representative if the cells are permitted to settle before a sample is taken. Always mix the cell suspension thoroughly before sampling. In order to fill the haemocytometer chamber properly by capillary action, the cover slip, chamber and pipette used to fill the chamber must be scrupulously clean. The chamber and cover slip are cleaned first with distilled water and then with absolute alcohol, and wiped dry.

Error source
Unequal cell distribution in the sample
Improper filling of chambers
Failure to adopt a convention for counting cells in contact with boundary lines or with each other
Statistical error

Table 16. *Sources of Haemocytometer error.*

The average of 3 large, 1mm squares was used to calculate the cell concentration. The cell concentration is determined as being the average number of cells counted multiplied by the dilution factor divided by the volume (Figure 10, p.76).

(a) $C = (N_{ave} \times DF) / vol$

(b) $C = (N_{ave} \times DF) \times 10^4$

Figure 10. *Formulae for the calculation of cell concentration.*
(a) General formula (b) Formula for the modified Neubauer rulings haemocytometer. C= cell concentration (cells/mL), N_{ave}= average number of cells counted, and vol= volume counted (mL), and DF= dilution factor.

2.2.9.1. Cell viability determined using Trypan Blue exclusion

Cell viability was determined by diluting the sample in 1x Trypan blue (Table 17, p.76). Non-viable cells were stained as blue.

Reagent	Concentration
Trypan Blue	0.3% (w/v)
NaCl	0.15 M
dH_2O	To volume

Table 17. *Composition of Trypan Blue.*

2.2.9.2. Avian White Blood Cell counting using Natt and Herrick's solution

The Natt and Herrick's method was used to enumerate total leukocytes (Natt and Herrick, 1952). Leukocytes and lymphocytes stain darkly while erythrocytes and thrombocytes are lightly stained.

A volume of 10µL of blood was mixed with 990 µL of Natt and Herrick's solution. The solution was well mixed and further diluted by 1:10 to facilitate easier counting. The leukocyte counts were averaged and cell counts per mL were calculated as follows: Total leukocyte/mL = average number of leukocytes x dilution factor x 10^4.

Dissolve chemicals in order described (Table 18 p.76), bring volume to 1L with dH_2O. After standing o/n, filter through fine filter paper (Watman No. 2). Solution should have a pH of 7.3.

Component	Amount
NaCl	3.88g
Na_2SO_4	2.50g
$Na_2PO_4.12H_2O$	2.91g
KH_2PO_4	0.25g
Formalin (~37% Gluteraldehyde)	7.50mL
Methyl Violet 28	0.10g

Table 18. *Components of Natt and Herrick's solution.*

2.2.10. Tissue Processing for Histology

Samples were immediately placed into 10% formalin, and left for 24-36hrs. The samples were then placed into labelled plastic mounting blocks in 70% ethanol. The samples were dehydrated overnight by slowly increasing the percentage of ethanol to 100%. The samples were mounted into paraffin blocks, and sections of 10μm were sliced and placed onto silane-coated slides (2.2.10.1, p.77). Slides were stained with Haemotoxylin and Eosin (Sigma, St. Louis, USA).

2.2.10.1. Silane Coated Slides

Coated slides were produced by cleaning glass slides with pyroneg, followed by washing with water, then dH_2O, and finally ethanol for 10min, and air dried. The slides were placed into 2% 3-aminopropyltriethoxysilane (Sigma, St. Louis, USA) in acetone for 2min, then fresh acetone for 2min, and finally running tap water for 2min, air dried and placed into a dustproof container.

2.2.11. Preparation of the DHBV Protein Vaccine

A sAg based vaccine was produced in a similar manner to that which has previously been shown to provide effective immunity from DHBV challenge (Vickery *et al.*, 1989).

DHBsAg was purified from serum containing high titre DHBV by a previously described method (Marion *et al.*, 1983a). Serum (0.5mL) was layered over 7ml of 10% (w/v) sucrose in TNE in Beckman Quick-Seal centrifuge tubes. The tubes were spun in a Beckman 70.1 Ti rotor at 45000rpm at 4°C for 1hr in a Beckman L8-M ultracentrifuge (Beckman, Palo Alto, USA). The viral pellet was resuspended overnight in 250μL TNE. The volume was made up to 1mL by adding TNE containing CsCl to a density of 1.2g/ml and then layered over a discontinuous gradient of CsCI 0.5 ml (1.4g/ml) and 0.5ml (1.25g/ml) in TNE. The tubes were filled with CsCl (1.1g/ml) in TNE and centrifuged in an SW55Ti rotor at 45000rpm for 48hrs at 10°C. Fractions of 200μL were collected from the bottom of the tube with a homemade fraction collector. These fractions were tested for solution density in an Abbe Refractometer and for absorbance at 280nm in a spectrophotometer (DU640, Beckman, Palo Alto, USA). Fractions in the density range of 1.13-1.19 gCsCl/mL have previously been found to contain viral particles by electron microscopy (EM) (Vickery *et al.*, 1989). These fractions contained a corresponding higher concentration of protein. The fractions containing peak viral absorbency were then pooled and centrifuged through a second discontinuous CsCI gradient. The fractions were collected and their refractive index and absorbency measured. Fractions containing viral antigen were pooled and found to have a refractive index of 1.3450 corresponding to a density of 1.17 g/ml. The pooled fractions

were then dialysed against PBS for 24 hours, changing the PBS 4 times. The purified DHBsAg was stored in aliquots at –20°C.

The DHBV sAg protein vaccine differed from that originally described (Vickery *et al.*, 1989), in that it was inactivated by treatment with 1:4000 formalin for 36hrs at 37°C (Tabor *et al.*, 1983), prior to use. The amount of protein vaccine to be inoculated was dispersed into TitreMax adjuvant (SIGMA, St. Louis, USA), by repeated introduction into a 2mL syringe, such that a 200μL inoculum would contain the appropriate amount of purified DHBsAg. A 1mL syringe with 26G needle was used for inoculation.

2.2.12. Bacterial Media

All bacterial cultures were grown on or in Luria-Bertani (LB) media (1% Tryptone, 0.5% Yeast extract, 1% NaCl, pH 7.0). All bacterial work was carried out in a C2 cabinet, or on a bench within 30cm of a lit Bunsen burner, for sterile conditions.

2.2.12.1. LB broth

10g tryptone, 5g yeast extract, and 10g NaCl, was dissolved and made up to 950mL with dH_2O. The pH was adjusted to 7.0, and the volume made up to 1L with dH_2O. Autoclaved for 20min at 121°C, stored at 4°C for up to 1 month. If antibiotics were required to produce selective LB media, they were added to the required concentration just before use.

2.2.12.2. LB Agar plates

LB broth was made up as per 2.2.12.1, (p.78). Prior to autoclaving agar was added to a concentration of 15g/L. After autoclaving for 20min at 121°C, the solution was allowed to cool to 50-55°C, and if required antibiotics were added to produce selective LB plates, at the concentration required. The agar solution was poured into standard 100mm Petri-dishes (Interpath, Sydeny, Australia), half filling the dishes, and allowed to cool and set. The set plates were stored upside down at 4°C until required, for up to 2 weeks.

If necessary, X-Gal was added to the plates, just prior to use. The plates were warmed up to 37°C for approximately 30mins. 40μL of 40mg/mL X-Gal stock solution was added, and spread evenly over the plate. The plates were protected from light by wrapping in aluminium foil, and let dry for another 15mins before use.

2.3. METHOD DEVELOPMENT: DNA SEQUENCING

2.3.1. Introduction

The ability to sequence long stretches of DNA rapidly and accurately has become an essential technique in molecular biology. This is clearly evident from the growth of

GenBank, a genetic sequence database, which has grown from only 5 million nucleotides in 1984 (Burks *et al.*, 1985), through 85 million in 1991, almost doubling in size every 2 years (Burks *et al.*, 1992), and now 29 billion nucleotides and doubling every 10 months (Benson *et al.*, 2004). The database currently holds 23 million sequences from over 140,000 distinct organisms. Most of the sequence data that is now being obtained comes from automated DNA sequencers.

2.3.1.1. History

DNA was originally discovered as "Nuclein" around 1870 (Miescher, 1871), termed Nucleic Acid (Altmann, 1889), and found to be composed of 4 nucleotides (Kossel, 1893). It was observed to induce pathogenic transformation of *Pneumococcal* types (Griffith, 1928), and was proposed to be genetic material (Avery *et al.*, 1944). Its double helical structure was elucidated in 1953 (Watson and Crick, 1974). Although its structure was defined, most of the sequencing work before 1965 was carried out using RNA and chromatographic techniques, which finally yielded an 80bp yeast tRNA (Holley *et al.*, 1965). Around 1970, the discovery of restriction enzymes (Arber and Linn, 1969) and DNA polymerases (Patel *et al.*, 1967), made DNA sequencing possible (Sanger and Coulson, 1975). Methods based on primed synthesis (Wu and Taylor, 1971) and gel electrophoresis separation led to the first sequence of a genome, the 5.4 kb of bacteriophage ϕX174, in 1976 (Sanger *et al.*, 1977a). The introduction of chemical degradation (Maxam and Gilbert, 1977) and dideoxy chain termination (Sanger *et al.*, 1977b) methods dramatically increased the rate of sequencing, with the analysis of the 40 kb bacteriophage T7 (Dunn and Studier, 1983) by chemical degradation, and the 16.5 kb human mitochondria genome (Anderson *et al.*, 1981) by the dideoxy chain termination method. The dideoxy chain termination method forms the basis of current automated fluorescent sequencing instruments.

2.3.1.2. The Sanger Dideoxy Chain Termination Method

The dideoxy chain termination method of Sanger (Sanger *et al.*, 1977b) is based on the use of chain terminating dideoxy nucleoside triphosphates which are base analogues of the deoxynucleoside triphosphates that are incorporated into a growing DNA chain (Figure 11, p.80). Ordinarily the growth of DNA chains proceeds from the 5' to the 3' end by addition of a new nucleoside triphosphate to the 3' position of the previous pentose ring. Since the dideoxy triphosphates lack the 3' as well as the 2' hydroxyl groups in the pentose ring, chain growth cannot occur, and DNA synthesis is terminated. These agents can be used for sequencing in the following way. The primer is hybridised to the template in a reaction mixture containing all 4 deoxy nucleoside triphosphates, one in the radioactive form, (α^{32}P-deoxynucleoside triphosphate), one dideoxy analogue (eg. ddATP), and DNA polymerase. When T residues are encountered in the template strand at the 3' end of the heterogeneous,

newly synthesised strands, there is a chance of inserting either the deoxy or the dideoxy nucleoside triphosphate. If the deoxy ATP is incorporated, DNA synthesis will proceed until the next position where A is to be inserted. The chain may grow further if deoxy ATP is inserted or terminate if the dideoxy analogue ddATP is inserted. In another growing molecule, that same position may be filled with the deoxy ATP, and synthesis would proceed to another A position where again there is a chance of inserting either dideoxy ATP or deoxy ATP. The result is the synthesis of a series of DNA fragments, all radioactive, and representing all possible lengths from the 5' end to each position of A. In the mixture of molecules, chain termination occurs at all A positions, and therefore the preparation will contain newly synthesised fragments, all radioactive, and ending at each A residue. Likewise, chain termination is done with the other three dideoxy nucleoside triphosphates, giving newly synthesised chains ending with their corresponding nucleoside triphosphates, eg. C, G, and, T. Products of the reactions are examined by gel electrophoresis (PAGE), the autoradiograph is read from the bottom going upwards in the 5'→3' direction.

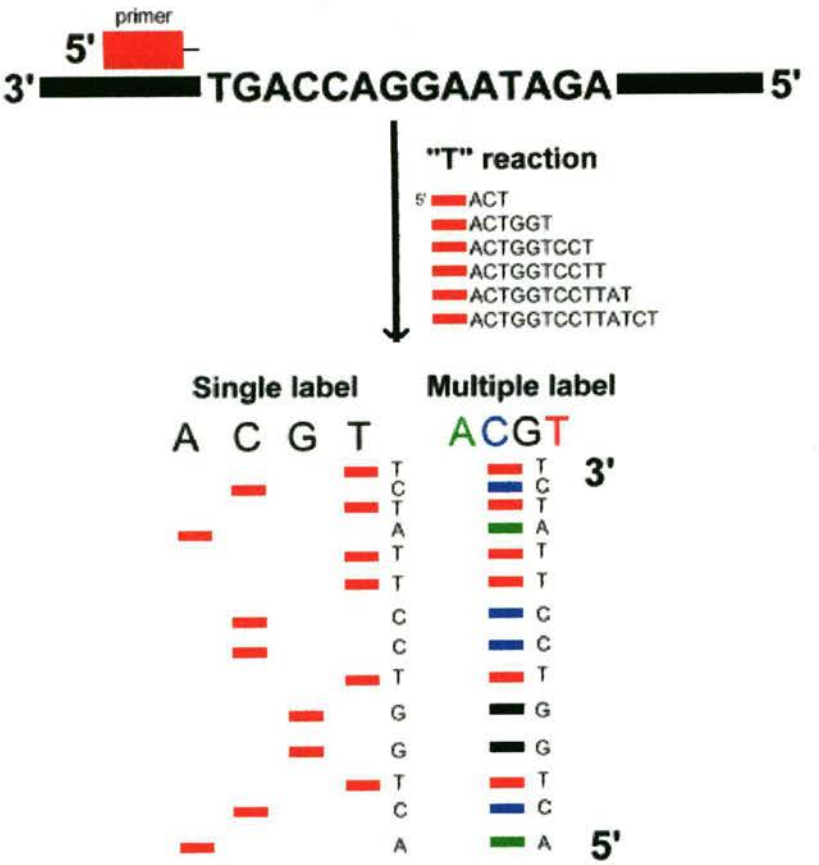


Figure 11. *The Sanger Dideoxy Chain Termination Sequencing Technique.*
Chain termination occurs with the incorporation of a dideoxy nucleotide, and when run on a polyacrylamide gel a band is visualised for that termination. The sequence is read from the bottom up. If multiple labels are used, as in dye terminator chemistry, then all four reactions can be run on the same lane.

2.3.1.3. Dye-Primer Chemistry

Dye-primer chemistry was originally designed to detect the short fragments of DNA generated by sequencing reactions. The primer is simply modified to aid detection, originally this was done with radioactive ^{32}P labelled deoxy nucleosides, which were detected by autoradiography, but may also include other labels such as DIG, and fluorescent dyes (Figure 12, p.81). In dye-primer sequencing, four separate reactions are carried out for each sample, and loaded into 4 separate lanes on a gel. Dye-primer tends to have a slightly higher accuracy at longer read lengths than dye terminator chemistry, however it requires a different primer to be labelled for each different tract of DNA to be sequenced.

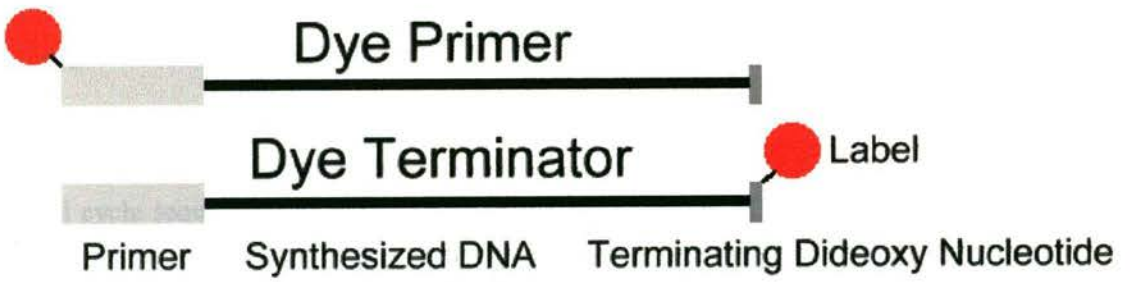


Figure 12. *Location of the label for detection of DNA.*
Dye Primer labelling involves labelling the primer, while Dye Terminator labelling involves labelling the terminating dideoxy nucleotides. The label may be fluorescent, radioactive, or a protein eg. DIG.

2.3.1.4. Dye-Terminator Chemistry

Dye-terminator chemistry is more flexible because it is the dideoxy nucleosides that are labelled (Figure 12, p.81). Fluorescent labels are most commonly used as they are the easiest to detect by automated methods, however other labels such as ^{32}P may also be used for manual sequencing. If the different dideoxy nucleotides are labelled with a different label then all four reactions can be combined into one, and run on a single polyacrylamide gel lane (Figure 11, p.80).

2.3.1.5. Fluorescent Nucleoside Triphosphates

Fluorescence detection of the DNA fragments is usually accomplished by covalently attaching a fluorophore (molecule that fluoresces when exposed to UV light) to the primer used in DNA sequence analysis (Smith *et al.*, 1985; Smith *et al.*, 1986). A different fluorophore is used for each of the reactions specific for the bases A, C, G, and T. The fluorophores are differentiated on the basis that they will reflect a different frequency (or colour) of light, eg. A may be green, and C blue. The combined reaction mixture is electrophoresed down a single polyacrylamide gel lane; the separated fluorescent bands of DNA are detected and analysed. The use of fluorescence detection is intimately related to the development of dye synthetic chemistry necessary to create appropriate fluorescent oligonucleotide primers. It is necessary to have fluorescent dyes that have high quantum

efficiencies and effective detection optics. For example, background reduction can be improved by adjustment of the plane of polarisation of the incident laser light and the glass should be of high optical quality with little or no fluorescence (Ansorge *et al.*, 1987).

Automated DNA sequences based on laser induced fluorescent dye primer chemistry have been reported by several research groups (Connell *et al.*, 1987; Prober *et al.*, 1987; Hunkapiller *et al.*, 1991; Du *et al.*, 1993). Fluorescence chemistries for automated primer-directed DNA sequencing (Hawkins *et al.*, 1992) are important because the dye labelled terminators are constituents of an overall package (Lee *et al.*, 1992) which must be completely compatible, including such aspects as the laser (fluorescence excitation) wavelength and the detection optics.

2.3.1.6. Thermal Cycle Sequencing

Thermal cycle sequencing is a method of dideoxy sequencing in which a small number of template DNA molecules are repetitively utilised to generate a sequencing ladder (Carothers *et al.*, 1989; Murray, 1989; Lee, 1991). A dideoxy sequencing reaction mixture (template, primer, dNTPs, ddNTPs, and a thermostable DNA polymerase) is subjected to repeated rounds of denaturation, annealing, and synthesis steps, similar to PCR, using a commercially available thermal cycling machine (Figure 13, p.83).

Cycle sequencing offers a number of advantages over manual protocols: (i) since the reactions are carried out by an automated thermal cycler, a large number of sequencing reactions can be performed simultaneously; it is also convenient to set up a large number of sequencing reactions for this protocol; (ii) since chain-termination reactions are repeated 30 times or more, the sequence ladders generated are of high intensity; (iii) a small quantity of template DNA is adequate to generate high-intensity sequence ladders (as low as 10 fmol or 6 ng of a 1-kb template are adequate); (iv) DNA sequence can be obtained from a crude DNA sample, eg. from individual plaques or colonies (Krishnan *et al.*, 1991); (v) cycle sequencing is not limited to only the PCR amplified DNA templates; it can also be used for generating DNA sequence from conventional templates such as phage M13 single-stranded DNA, supercoiled double-stranded plasmid DNA, or phage DNA; and (vi) several modifications of this protocol would allow determination of DNA sequence directly from a very low copy number sample (as low as 1 molecule per sample). Cycle sequencing also offers two very important features. With cycle sequencing, it is easier to control strand annealing and random priming reactions that generate background in sequence ladders. Second, random priming events can be minimised by designing appropriate primers and performing sequencing reactions at a stringent annealing temperature of 55-60°C and an extension temperature of 72°C.

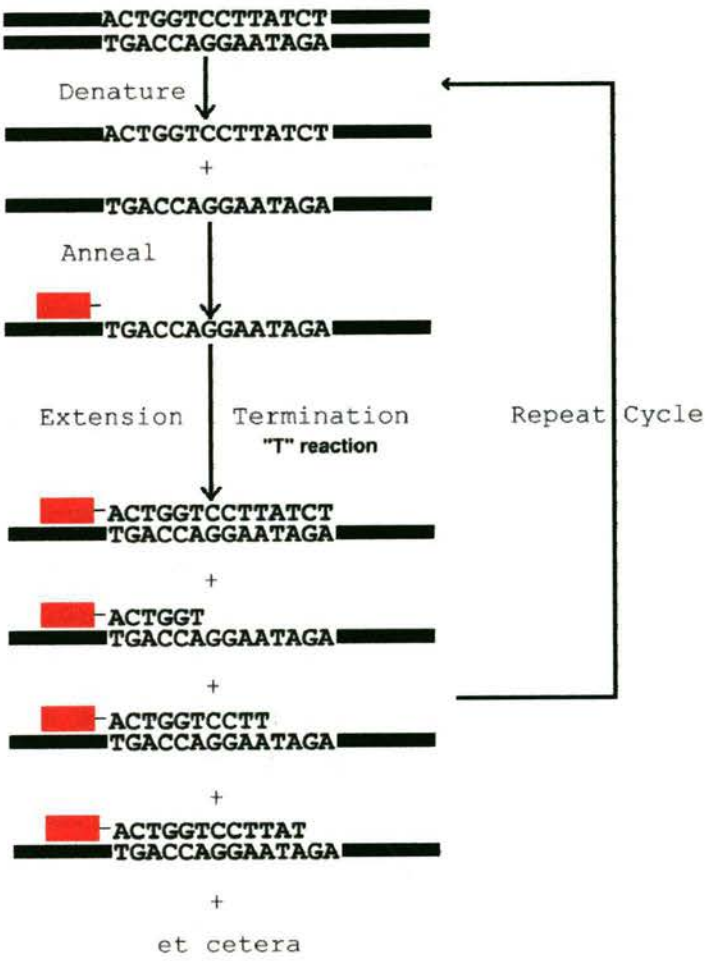


Figure 13. *Cycle sequencing explanation.*

Cycle sequencing is based on a combination of Sanger dideoxy chain termination sequencing and PCR. Thermophillic polymerases are used in the termination reaction, the products are then heat denaturated and reannealed to primers and termination is repeated just as for PCR. This effectively increases the amount of labelled product, greatly increasing the sensitivity of the reaction.

2.3.1.7. Manual Sequencing

Manual sequencing is a method by which no automation is used in the sequencing technique. It has been made redundant by the Human Genome Research Project, which has invested vast sums of money into automation to increase the speed and reliability of sequence data through automation of the time consuming and repetitive nature of DNA sequencing. Simply considered, manual sequencing is the radioactively labelled dideoxy chain termination method of Sanger, in which all steps are completed by manual handling.

2.3.1.8. Automated Sequencing

DNA sequencing is a time and labour-consuming task, which is full of repetitive steps, thus making it amenable to automation. Automation allows simple tasks to be performed by machines rapidly and continuously. A number of new DNA sequencing techniques, which include the application of fluorescent dyes in combination with automated DNA sequencers,

have been developed (Smith *et al.*, 1986; Ansorge *et al.*, 1987; Prober *et al.*, 1987; Brumbaugh *et al.*, 1988). Automated DNA sequencing can be a misleading phrase; it actually means automated analysis of DNA sequencing reactions. The enzymatic process to produce the DNA sequence is the Sanger dideoxy chain terminator system, however it is the electrophoretic separation and detection of the products of the Sanger method that have become automated. Manual sequencing utilises large gels and electrophoretic separation for a specified time, followed by autoradiography. The sequence is read from the bottom of the gel to the top, because in a given period of time the smaller fragments will have migrated farther than the larger ones. The autoradiogram presents a detailed view of the separation achieved at a certain time-point. All automated sequencers also utilise electrophoresis, but in a fundamentally different way. Automated sequencers, instead of looking at the whole gel at one point in time (as in an autoradiogram), look at one point of the gel over time. They measure the time it takes a band to traverse a specified distance in the gel. Smaller bands traverse the gel more rapidly than larger ones, and arrive at the detection window in a shorter period of time. Thus, the output is very different from the "ladder" observed in the manual sequencing autoradiogram. Instead, an electrophoretogram is produced, presenting the detected bands as peaks on the Y axis, and time of electrophoresis on the X-axis. Each peak is then identified as an A, T, G, or C depending upon the detection system (Figure 14, p.84).

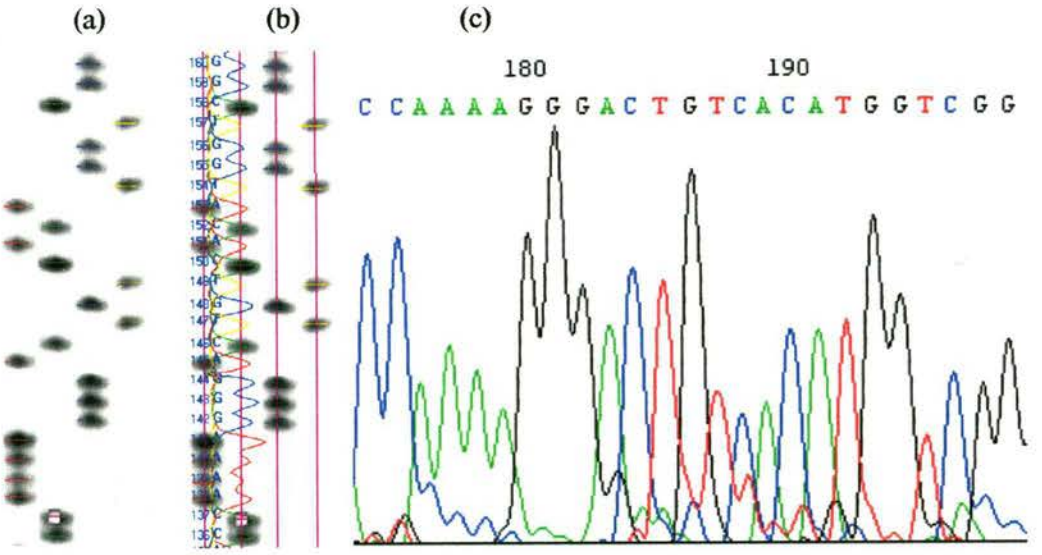


Figure 14. *Raw sequencing data output.*

Comparison of visual output of Manual sequencing, Corbett sequencing, and ABI automated sequencing. The same sequence data is shown as (a) Manual sequence data, (b) Corbett sequencing, which is a combination of manual and automated sequencing methods, note the visual bands corresponding to the Sanger dideoxy nucleotide chain termination, overlapped with the chromatophraphic representation of the various bands, and (c) Chromatographic output of the ABI automated sequencing system.

2.3.1.9. Manual Versus Automated Sequencing

Manual sequencing was originally considered the "Gold Standard" by which all other sequencing methods have been judged. It has now been superseded by automation of most of the manual handling and physical manipulations. However, the critical skill of sequencing requires the interpretation of the results obtained. Automated sequencing is increasingly reliant on computer programs to interpret the data obtained. This can lead to error, as most base calling programs, despite increasingly sophisticated algorithmical engines, sometimes misidentify a base. In general, automated sequencing still needs to be manually verified to validate any mutation or sequence variation.

2.3.1.10. Sequencing Errors

PCR can give rise to two types of discrepancies between the target sequence (to be amplified) and that of individual PCR products: Point mutations and mosaic alleles, generated by *in vitro* recombination between different amplified products.

When estimated by a fidelity assay using M13, the frequency of base substitution errors (1/ 10,000) and frame shift errors (1/ 40,000) of Taq polymerase is considerably higher than for Klenow polymerase (1/ 29,000 base substitution errors, 1/ 65,000 frameshift errors) and T4 DNA polymerase (1/ 160,000 base substitution errors, 1/ 280,000 frameshift errors) (Eckert and Kunkel, 1989; Eckert and Kunkel, 1990). However, since the processivity and rate of the DNA polymerase are affected by changes in $MgCl_2$, buffer components, dNTP concentrations and the temperature profile of the cycle, and because these assays were not performed under the same conditions as a standard PCR, the absolute numbers may not apply directly to PCR. The actual error rate in the PCR, estimated by sequencing of individual PCR products after 30 cycles starting from 100-1000 ng of genomic target DNA, suggested that two random PCR products might be expected to differ once every 400-4000 bp (Saiki *et al.*, 1988). The tenfold range in this estimate is due to differences between different studies and different amplified regions. The mosaic PCR products are the result of partially extended DNA strands that can act as primers on other allelic templates in later cycles. Both of these artefact products are likely to accumulate primarily at the endpoint of PCR because of insufficient enzyme to extend all available templates and an abundance of DNA strands for annealing.

Both types of errors have to be considered when PCR products are cloned and allelic sequences inferred from individual PCR products. In direct sequencing, by contrast, these artefact PCR products will not be visible against the consensus sequence on the gel. For example, even when starting from a single DNA copy like that found in a single sperm, a mis-incorporation that arises in the very first PCR cycle will only appear with, at the most,

25% of the intensity of the consensus nucleotide, given that all templates have equal probability of being replicated (Gyllensten and Erlich, 1988). Thus, direct sequencing is to be preferred, unless the primer sequences do not allow sufficient specificity to amplify only a single target, or the individual allelic sequences cannot be determined due to genetic polymorphism (heterozygosity) at multiple positions between the primers. Allelic variants may be separated prior to the sequencing using denaturing gradient gel electrophoresis (Myers *et al.*, 1988; Gyllensten, 1989; Gyllensten and Erlich, 1989; Gyllensten and Erlich, 1990), or the polymorphic positions at the ends of the amplified fragments may be used to selectively amplify or sequence one allele at a time (Gyllensten and Erlich, 1988). The relatively high error rate of Taq polymerase may however, create problems when individual products are to be used for expression studies, or analysis of mutation frequencies. Unless a population of linear PCR products can be used in the expression system, several molecules have to be cloned and sequenced to identify the unmodified clones.

In general, one of the most serious errors in PCR is that of carryover of product from previous amplification reactions into unamplified samples. Since an amplification reaction of 100 µl may result in 10^{12} copies of a DNA fragment, 0.1 µl will contain 10^9 copies, or 10^3 copies more than that found in an unamplified sample of 3 pg of genomic DNA. To prevent product carryover, preparation of reagents and reactions should be isolated from analysis of the PCR products. This can be achieved by using several sets of pipetteman with disposable positive-displacement tips and by separating physically the preparation of reactions and the analysis of products.

2.3.1.11. Commercial Approaches to Automated Sequencing

Applied Biosystems Incorporated (ABI) was one of the first companies to produce automated sequencers. It took advantage of the Sanger dideoxy nucleoside chain termination method, in combination with fluorescent chemistry to produce a range of machines that enabled fast and efficient DNA sequencing. Its dominance of the scientific field is almost total and has become the new standard by increasing its reliability, reproducibility, and most importantly the length of useable sequence per reaction. Most sequencing reactions are now able to return 500 or more base pairs of sequence.

2.3.1.12. Corbett Sequencing

Corbett Research is Australian company specialising in the manufacture of scientific instrumentation for Life Science research. Corbett Research products include a full range of thermal cyclers and Automated DNA Fragment Analysis and DNA Sequencing systems. One of these systems is the Gel-Scan 2000 (GS-2000), a real-time gel electrophoresis system. Samples are loaded onto an ultra-thin vertical gel, a laser scans the base of the gel

and detects DNA fluorescence. During the run a 2-dimensional gel image is built up on the screen, similar to a manual sequencing gel autoradiograph. The GS-2000 utilises dye primer chemistry and therefore uses four lanes of a gel per sample, ie. one lane per nucleotide. It is considered an automated sequencing system because after the gel is run a computer program is used to automatically determine the sequence from the 2-dimensional gel image. The major advantage to this system is that it enables many sequencing reactions to be done using the same primer, it has a high throughput of specific reactions. This would permit rapid sequencing and mutant determination of a relatively large number of samples in a given time, if the placements of the mutant or changes were known. Another advantage of this system is that to scan for a known mutation, ie. C→A it simple requires only two of the four sequencing reactions to be performed and run on the gel, increasing again the number of samples that can be run and analysed on a given gel by two fold.

The GS-2000 was considered to be an excellent investment to investigate and detect mutations and sequence variation for a single section of genome with a large number of samples.

As such, it would be an invaluable tool for the epidemiological study of virus variation. HBV with its many overlapping reading frames is relatively restricted in its ability to successfully mutate, in either increasing its functionality, eg. increased infectivity or replication, or to evade immune pressure. It has been observed that the virus tends to mutate in selected sites of the genome to selected bases, which produce changes in only one of the reading frames, when under certain circumstances, such as drug or immune pressure. If these regions are known then a method such as restriction enzyme digestion may be used to observe whether a sequence has changed, however, sequencing provides the extra information of what the sequence has changed to, and thus provides information on the effect of the change on the associated protein/s.

2.3.2. Aim

(1) To establish and evaluate a method for automated sequencing of DHBV DNA using the Corbett GS-2000S machine.

2.3.3. Experimental Design

Automated cycle sequencing using a Corbett's Research GS-2000 gel scanner, was developed and several parameters optimised. The parameters included the type and amount of template required, the amount of labelled primer, the number of reaction cycles, the annealing / extension temperature, and the amount loaded on the gel.

Plasmid DNA of known sequence was used for optimisation purposes. It was used directly, and also compared with PCR fragments from the same plasmid. Both were used as the basis to determine the parameters required for efficient and accurate DNA sequence data.

2.3.4. Materials and Methods

2.3.4.1. Corbett Sequencing Method

The Corbett GS-2000 sequencing machine was designed to be used with any dye primer chemistry sequencing kit. The manufacturers recommended the Amersham Life Science Thermo Sequenase fluorescent-labelled primer cycle sequencing kit (personal communication) (Amersham, Buckinghamshire, England). They also recommended that the standard kit protocol was effective in producing an accurate and reproducible result. However, optimisation of reaction conditions was attempted to obtain the best results possible for the selected primer reaction.

The primer chosen for the Corbett GS-2000 sequencing method was the forward primer of the DHBV PreS-S PCR. This primer was chosen because it was known to be very effective in the PCR reaction and would provide sequence data for the start of the DHBV Surface gene, which is where we would expect immune pressure to drive mutants. The primer used in the sequencing reaction had to be labelled with a HEX dye (available Research Genetics, Huntsville, USA).

Plasmid DNA of known sequence was used directly, or PCR fragments from the plasmid, were used as the basis to determine the parameters required for efficient and accurate DNA sequence data.

2.3.4.1.1. Sequencing Reaction

PCR fragments were produced (2.2.2, p.68) and PEG precipitated (2.2.2.5, p.71). The resuspended PCR sample was run on a gel for visual confirmation, and concentration determined by spectrophotometry (2.2.4, p.73); it was then diluted with dH_2O to 10-500 ng/μL. Plasmid DNA was produced by either MINIprep or MAXIprep (Qiagen, Melbourne, Australia), and was then diluted with dH_2O to 0.25-2 μg/μL. The sequencing reactions were carried out using the Amersham Life Science Thermo Sequenase fluorescent labelled primer cycle sequencing kit (RPN 2436, Amersham, Buckinghamshire, England) (11.4.2, p.A4).

The procedure followed was similar to normal PCR (2.2.2, p.68), in that the cocktail was made up in the clean room to avoid contamination of the cocktail with extraneous DNA, and all subsequent steps were performed in the PCR room.

The 4 reagent tubes (A, C, G, and T), and the HEX labelled DHBV PreS1f primer were removed from the –20°C freezer and allowed to thaw. All were thoroughly mixed prior to use, and stored on ice. A cocktail of each sequencing reaction was produced (Table 19 p.89).

Reagent	Concentration	µL
Template DNA		
PCR product	10-500 ng/µL	5
Plasmid	0.25-8 µg/µL	5
Fluorescent primer	0.5-20 pmol/µL	1
A, C, G, or T reagent	4x	2

Table 19. *Contents of each cycle sequencing reaction.*

Each tube was overlaid with 1 drop of paraffin oil. The tubes were cycled with conditions specified in Table 20 (p.89). After cycling was completed the oil was removed and the sequencing cocktail aspirated into a new Eppendorf™ tube. If required the sample was ethanol precipitated by addition of 2 volumes of 95% ethanol, and incubation at –20°C for 15 mins, before centrifugation at 1500rpm for 15 mins in a bench centrifuge at 4°C, the supernatant was removed and the remaining DNA pellet dried. Either 1 volume or 5µL of formamide loading dye was added. The samples were then denatured into single strands of DNA by incubation for 2 minutes at 90°C and placed on ice prior to gel loading.

Cycle	Temperature (°C)	Cycle 1	Cycle 2-n	Cycle n+1
Denaturation	95°C	2:00	0:30	0:30
Annealing / Extension	50-70°C[a]	0:30	0:30	0:30
Post cycling	4°C			0:00

([a]) Only 1 temperature was used for the Annealing / Extension phase in any single reaction.
n= number of cycles (10-40).

Table 20. *Cycling conditions for DHBs gene sequencing on the GS-2000.*

2.3.4.1.2. Gel Formation

Utmost care was taken to thoroughly clean the glass sequencing gel plates with pyroneg and tap water, to reduce the amount of background and non-specific fluorescence detected by the gel scanner. Gloves were not worn during the cleaning of the glass, because the powder in the gloves fluoresces under the gel scanner. It was also important to wash hands carefully before cleaning the glass as to remove any oil from the hands, which will later smear and streak the glass. Gloves had to be worn after the glass is cleaned because of the acrylamide used for the gel. The plates were rinsed several times with tap water, several times with dH_2O, and dried with lint free wipes, 100% ethanol was used to polish the plates, before finally being dried with lint free wipes.

The gel pouring apparatus, spacers, and comb were also rinsed. The gel pouring apparatus was assembled by placing the heavier back plate into the pouring apparatus face up, placing

the spacers at both side edges, closing the front clamps the front plate was finally placed face down on the back plate. The 5% gel was prepared with 3mL of x10 sequencing gel solution (Table 21 p.90), 4mL of 40% Acrylamide:bis-Acrylanmide (19:1) (Sigma, St. Louis, USA) and 23mL of dH_2O in a small beaker.

Components	Final Conc	1L	30mL (1 gel)
Urea	5 M	420 g	12.6 g
10x TBE	0.6x	60 mL	1.8 mL
dH_2O (fill to)	-	815 mL	24.6 mL

Table 21. *x10 Sequencing Gel Solution.*
After adding urea and TBE, the solution was placed onto a heating block stirrer until dissolved, adjusted to final volume, filtered and autoclaved. Stored at RT. The solution was not used if precipitate was present.

The gel was sucked into a 50mL syringe and degassed by placing under negative pressure, ie. the tip was temporarily sealed with a melted yellow pipette tip and the plunger drawn. The syringe was tapped a few times on the bench, the pressure released, air was evacuated, and the procedure repeated.

Ammonium PerSulphate (APS) and TEMED were used to increase the speed of polymerisation. A 10% APS solution was freshly made by adding 1 mL dH_2O to 100mg APS. A 150µL aliquot of 10% APS and 15µL TEMED were carefully mixed into the gel solution. Polymerisation occurred within a few minutes.
The gel was slowly poured onto the back plate, leaving approx. 3mL in the syringe. Very carefully, the front plate was lowered down onto the back plate, ensuring that no bubbles were trapped in the gel. If bubbles did appear, the front plate was carefully lifted and lowered again. When completely down, the well former (comb) was inserted backwards to the level indicator, ie. with the teeth pointing away from the gel. The remaining gel was poured onto the comb to seal the area and produce a good even well shape. Polymerisation occurs only under anaerobic conditions. The lid of the gel forming apparatus was put in place and securely clamped. The gel was left for approx. 1hr before being removed.

The gel was then unclamped, the well former removed, the excess gel wiped away with paper towel, and the glass plates washed with pyroneg and tap water. The plates were rinsed with tap water, then dH_2O, dried with KimWipes, and polished with 100% ethanol. The gel was then placed into the bottom buffer tank of the GS-2000, the top buffer tank was screwed in at the top, all knobs were tightened, but not overly. The tanks were filled with 0.6x TBE to the indicated levels. The well cavity was flushed with TBE to evacuate any water that was present.

2.3.4.1.3. Gel Running

The GS-2000 was turned on, temperature set to 45°C, pre-run for 30mins at 900V and then flushed with TBE, to remove excess urea.

The shark tooth comb was inserted into the well cavity, with the teeth only just into the bottom of the gel. The wells are formed between the teeth of the shark tooth comb. The denatured samples (0.25-8µL) were added with a specialised flat duck billed pipette tip, and pulse loaded for 40s. The wells were again flushed, to remove any excess sample that would cause trailing of the bands. The gel was run for 5hrs at 1500V.

2.3.4.2. Analysing Corbett Sequence Data

The .FLF file was converted to a .TIFF file, before being analysed by DNAscan™® (Scanalytics™, Bilerica, USA). The lanes were manually marked on the screen using the software before the program interpreted the bands. Any ambiguous base calls were manually checked and corrected if necessary. The sequence was then output as a text sequence file to be analysed further with ANGIS.

2.3.4.3. Corbett Sequencing Optimisation

The Corbett sequencing technique was optimised for several parameters (Table 22 p.91). The type and amount of template is an important factor in that the samples to be sequenced were direct from PCR fragments, while the use of plasmid was excellent for optimisation because one single batch could be used for the entire optimisation procedure reducing the sample-to-sample variation in the starting material.

Condition	Values tested
Type / amount of template	
PCR fragment	10, 25, 50, 75, 100, 200, and 500 ng/µL
Plasmid product	0.25, 0.5, 0.75, 1, 2, 4, 6, and 8 µg/µL
Labelled primer concentration	0.5, 1, 2.5, 5, 7.5, 10, 15, and 20 pmol/µL
Number of reaction cycles	10, 15, 20, 25, 30, 35, and 40 cycles
Annealing / Extension temperature	50, 55, 58, 60, 62, 64, 68, and 70°C
Amount of sample loaded on the gel	0.25, 0.5, 1, 2, 2.5, 4, 5, 6, and 8 µL

Table 22. *Range of values tested during optimisation of the Sequencing reactions.*

The labelled primer concentration is just as, if not more important in the sequencing reaction as it is in the normal PCR reaction. This is because the label that is detected by the GS-2000 is directly attached to the primer, so that too little primer and the signal is weak and or not present, while too much primer will saturate the early reads of the sequence.

The number of cycles for the cycle sequencing reaction is important, as too few cycles will also lead to the signal becoming too weak or not present. The dynamics of the reaction mean that the greater the number of cycles the greater the percentage of short fragments produced

which could lead to saturation of the early sequence read, which may smear bands together, making it more difficult to accurately interpret. Too many cycles also increase the number of mismatched primer pairings, which could lead to inaccurate sequence data.

The annealing and extension temperature of the cycle sequencing reaction are extremely important and should be as high as possible to allow for the most stringent primer annealing conditions. Stringent conditions should minimise the non-specific binding, and increase selectivity, but should be low enough to allow efficient extension of the DNA fragment so that the signal produced is strong enough to be read.

The amount of sample loaded onto the gel is determines the signal strength; too much and the signal is saturated, which blurs the bands and prevents early sequence data from being obtained.

Purification of the sequencing reaction is required to remove as much of the non-extended primer as possible, which produces a dense black smear, but the gel is also sensitive to the amount of salt placed into the wells, so the reaction should be as clean as possible to allow the wells to run straight and parallel. The samples were loaded after ethanol precipitation or straight from the sequencing reaction.

2.3.5. Results

The sequencing reactions were compared for band compactness, separation, sharpness and amount of background. Visual inspection of the gel provided a good basis of the quality of the sequence data that could be obtained after computer interpretation. The better the gel looked visually, the less manual interpretation was required. Comparison of the partial gel pictures provides a good indication of sequencing quality (Figure 68 - Figure 71, p. 6-9).

2.3.5.1. Optimised Cycle Sequencing protocol

The final optimised cycle sequencing protocol is given below.

The PCR fragments were PEG precipitated (2.2.2.5, p.71). The concentration of the resuspended sample was determined by spectrophotometry (2.2.4, p.73), and diluted with dH_2O to a final concentration of 200 ng/µL.

The 4 reagent tubes (A, C, G, and T), and the HEX labelled DHBV PreS1f primer were removed from the –20°C freezer and allowed to thaw. All were thoroughly mixed prior to use, and stored on ice. A cocktail of each sequencing reaction was produced (Table 23, p.93).

Reagent	Concentration	µL
Fluorescent primer	5 pmol/µL	1
Template DNA PCR product	200 ng/µL	5
A, C, G, or T reagent	4x	2

Table 23. *Contents of each optimised cycle sequencing reaction.*

Each tube was treated as per Sequencing Reaction (2.3.4.1.1, p.88). The tubes were cycled with specific conditions (Table 24, p.93). The optimised gel formation method was as described in Gel Formation (2.3.4.1.2, p.89). The optimised gel running method was as described Gel Running (2.3.4.1.3, p.91). The gel was loaded with 2µL of denatured sample.

Cycle	Temperature (°C)	Cycle 1	Cycle 2-29	Cycle 30
Denaturation	95°C	2:00	0:30	0:30
Annealing / Extension	60°C	0:30	0:30	0:30
Post cycling	4°C			0:00

Table 24. *Sequence cycling conditions.*

2.3.5.2. Comparison of GS-2000 and Automated Sequencing

The accuracy of the sequence obtained from the GS-2000 was calculated by comparison of the same template under the same conditions. The sequences were then aligned using PileUp or ClustalW (Appendix 11.6.1, p.A42), and the differences before (Figure 15, p.94) and subsequent to manual editing (Figure 16, p.95) were calculated (Table 25, p.93) for the fist 200 nucleotides of the sequence data.

Bases	Automated	GS-2000 Before edit	GS-2000 After edit
Incorrect			
Reverse	1	8	4
Duplicate +	3	27	10
Outright	0	0	0
Missing			
Duplicate -	5	186	67
Outright	2	22	7
Incorrect+Missing total	11	243	88
Total bases	~1200	~2400	~2400

Reverse: indicates that two or more bases have been mixed up ie. instead of CT the sequence was called TC. Duplicate: refers to where there are two of the same bases sequentially, + then denotes an extra base called, while – a base missed.

Table 25. *Calculated Sequencing Error Rates and Types.*

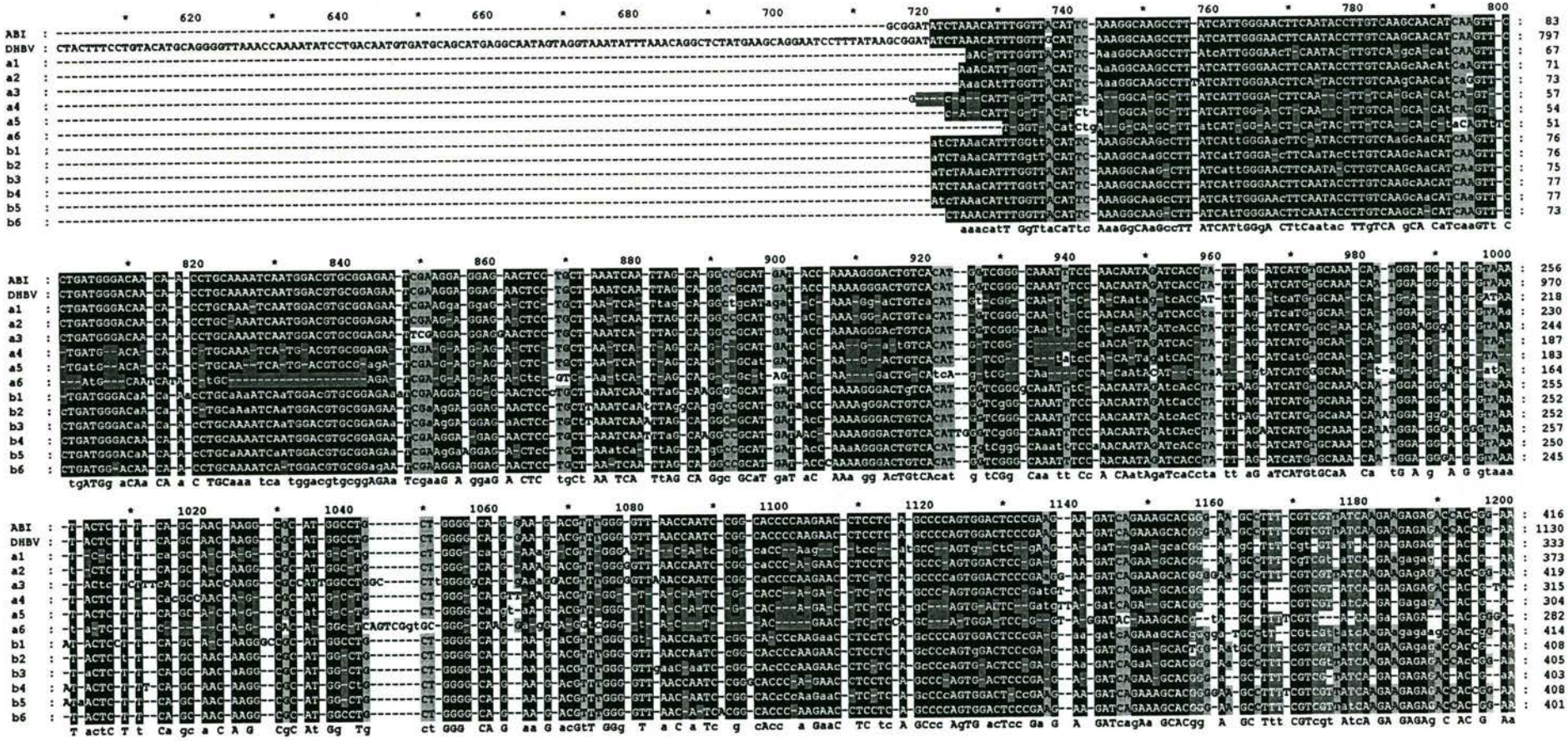


Figure 15. *Sequence alignment obtained from the GS-2000 of the Pre-S region of DHBV before manual editing.*

(a) PCR product, (b) Plasmid, DHBV: sequence of cloned Australian DHBV (Triyatni *et al.*, 2001), ABI: Sequence obtained from an ABI 377. DASH: represents a missing base, BLACK: at that point there are no other bases called, GREY: another bases has been called at that spot different to the consensus.

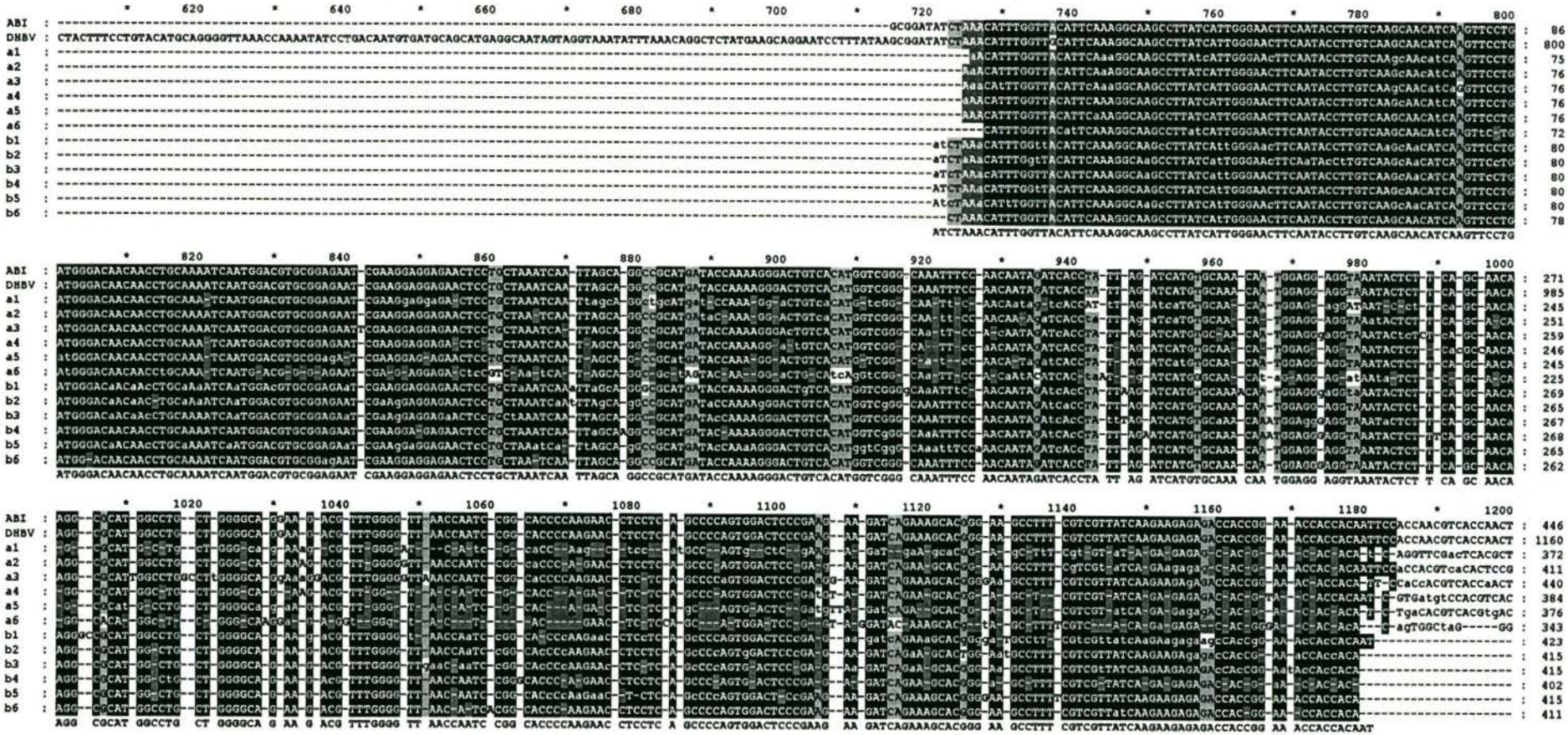


Figure 16. *Sequence alignment obtained from the GS-2000 of the Pre-S region of DHBV after manual editing.*

(a) PCR product, (b) Plasmid, DHBV: sequence of cloned Australian DHBV (Triyatni *et al.*, 2001), ABI: Sequence obtained from an ABI 377. DASH: represents a missing base, BLACK: at that point there are no other bases called, GREY: another bases has been called at that spot different to the consensus.

NOTE: After manual editing there are fewer errors and a more consistent sequence.

2.3.5.3. Assessment of Sequencing Techniques

Although the GS-2000 sequence data was slightly less accurate than the automated method (Table 25, p.93), it is still capable of producing excellent results. However, the time required to do the sequencing and then analyse the data obtained was much more labour intensive than for the automated method.

This would not be a problem for targeted sequencing, in which a specific mutation is to be found. The initial phase of the project endeavoured to find some sequence variation in the Surface gene, which is approximately 1.1kb long. This would require the use of many different primers along the length of the gene, which would all require separate optimisation.

So the advantages of the automated sequencing methods (longer reads, and shorter editing analysing time) were considered to be of use when sequencing the large areas of the genome for unknown mutations. If mutations were found to occur and specifically looked for then the GS-2000 sequencing method could be employed.

2.3.6. Discussion

The sequence data obtained by the Corbett's GS-2000 sequencer was considered acceptable for general use. It did however, have a slightly higher rate of errors than the automated ABI system, but it was still a reasonably low error rate.

Combined with sequence alignment, which makes errors much more visually observable, the Corbett sequencing method would provide a good basis from which to analyse a large number of samples for sequence variation at specific points in a genome.

The advantage of the Corbett's GS-2000 sequencer is the cost per sequencing reaction, which is approximately a third of the automated ABI sequencing. This cost benefit is obtained when five or more sequence reactions are done at the same time, because they can be done as a batch and run on a single gel. This makes the time required a lot more productive, as the entire method was very labour intensive and required a lot of time in setup preparation and cleaning up afterwards.

It was decided that all future sequencing be a combination of the Corbett's GS-2000 sequencing, and automated ABI sequencing depending on which would the most efficient at the time. A larger proportion of the sequence data was obtained using the automated ABI sequencing method because often only a few samples were ready to be sequenced at any one time, and that they required slightly less time to manually edit, which allowed more time for sequence data analysis.

2.3.7. Conclusions

A method for DNA sequencing using the GS-2000 was established and found to be comparable to the automated method.

Although more labour intensive, the method would be useful in situations in which batch orientated processing and selective sequencing of specific areas is required.

3. PERSISTENCE-CLEARANCE EXPERIMENT

3.1. INTRODUCTION

The mechanisms which determine whether the outcome of hepadnavirus infection will be acute self-limited clearance or persistence are still unclear. It has been observed that the age at which the infection occurs plays a large role in the outcome. Ducks infected or inoculated at a young age tend to develop a persistent infection, while older ducks (3 weeks plus) tend to develop a self-limiting acute infection. However, older ducks can become persistently infected with a large enough dose. A few young ducks have been observed to clear infection, and so it should be possible to manipulate the dose age combination to produce both outcomes, ie. clearance, or persistence. By evaluating the response of ducks that clear with those that do not, any pattern that predicts clearance or persistence should be evident.

It has also been observed that in individual ducklings early onset of high level viraemia generally leads to chronic infection, while low level viraemia developing later tends towards an acute infection (Vickery and Cossart, 1996).

We have previously shown clearance in ducks infected at 11 days of age (Freiman *et al.*, 1990) but already at this age the logistics of holding ducks are considerable. In this chapter we are investigating the conditions needed to achieve clearance in the experimentally more convenient younger ducks, and charting the kinetics of viraemia during the critical early phase of infection.

3.2. AIMS

(1) To establish experimental conditions which reliably lead to acute DHBV infection in neonatal ducks. Two parameters were tested: age at inoculation, and virus dose.

(2) To determine whether the pattern of viraemia early in infection predicts the final outcome of infection in neonatal ducks.

3.3. MATERIALS AND METHODS

3.3.1. Ducks

Pekin-Aylesbury crossbred ducks, as described in Methods and Materials (2.1.1, p.66), were used.

3.3.2. Duck Hepatitis B Virus strain

Positive serum pool DHBV051094 (containing $1.4x10^9$ vge/mL) was used for this experiment (Methods and Materials, 2.2.7, p.74). This serum had an ID_{50} of ~450 vge when *intraperitoneally* injected into 1 or 4 day old ducks (Vickery and Cossart, 1996).

3.3.3. Age and Dose of inoculation for duck groups

Ducklings were randomly divided into 3 groups and inoculated with DHBV positive serum at day 1, 4, and 7, respectively (Table 26, p. 99). The dose is shown in Viral Genome Equivalents (vge) rather than ID_{50} because the ID_{50} progressively increases with age (Vickery and Cossart, 1996).

inoculation	Dose (vge)	No. ducks
Day 1	$2.8x10^3$	6
	$2.8x10^4$	6
Day 4	$2.8x10^3$	7
	$2.8x10^4$	7
Day 7	$2.8x10^4$	7
	$2.8x10^5$	7

Table 26. *Dosage of DHBV given to 1, 4, and 7 day old ducks.*

The doses for the Day 1 and Day 4 groups were $2.8x10^3$ and $2.8x10^4$ vge which were approximately 6 and 60 ID_{50} respectively, and were chosen to ensure that the majority of ducks become infected, but low enough so that some of the ducks would be able to clear the infection. The Day 7 groups were inoculated with a one $\log_{10}$ larger dose (Table 26, p.99), because of their increased resistance to infection. DHBV051094 was diluted in PBS, such that a 200µL inoculum would contain the vge dose of DHBV for inoculation.

3.3.4. DHBV DNA detection

The ducks were bled three times a week for seven weeks (0.1-1.0mL was drawn from the external jugular vein using a 1mL syringe with 26G needle, depending on the size of the duck). The blood was allowed to coagulate overnight, spun for 1-5min, 13000rpm at RT, the serum was removed and stored at –20°C until required. Two liver samples were obtained at euthanasia; one sample (3x3x3mm, $27mm^3$) was used for extraction while a second larger aliquot was stored at –20°C until required.

The level of DHBV DNA in serum samples was estimated by dot blot hybridisation as described in (Methods and Materials, 2.2.3, p.71). The limit of detection for the dot blot hybridisation assay was approximately 1 pg of DHBV DNA (~$3x10^5$ vge) in a 25µL sample which is equivalent to ~$1x10^7$ vge/mL, but allowed semi-quantitation up to >$2x10^{10}$ vge/mL.

If sufficient serum remained, samples negative by dot blot hybridisation, as well as all prebleed samples were assayed by PCR as described in Methods and Materials (2.2.2, p.68). Liver samples from the ducks were DNA extracted, dot blot hybridised, and assayed by PCR as described in Methods and Materials (p.66). The limit of detection for the PCR assay was less than 10 vge in a 5µL sample which is equivalent to ~$2x10^3$ vge/mL, which is approximately 4 $\log_{10}$ greater than dot blot hybridisation.

3.4. RESULTS

3.4.1. DHBV DNA detection

Samples were initially tested by dot blot hybridisation to obtain semi-quantitative data in the range of $1x10^7$ to >$2x10^{10}$ vge/mL (Methods and Materials, 2.2.3.3, p.73). In negative samples this was augmented by PCR to increase sensitivity (which had a lower level of sensitivity of $2x10^3$ vge/mL, Methods and Materials, 2.2.2.2, p.69).

The outcome of infection in the various groups is shown in Table 27 (p.100). If DHBV DNA was detected in any sample (serum or liver) at any experimental time point by either dot blot hybridisation or PCR, the particular duck was classified as "infected".

			DHBV positive		DHBV negative
inoculation	Dose (vge)	No.	Serum	Liver	
Day 1	$2.8x10^3$	6	5	5	1
	$2.8x10^4$	6	6	6	0
Day 4	$2.8x10^3$	7	4	6	1
	$2.8x10^4$	7	7	7	0
Day 7	$2.8x10^4$	7	7	7	0
	$2.8x10^5$	7	5	5	2

Table 27. *Number of Ducks DHBV positive in the Serum and Liver following inoculation with DHBV on Days 1, 4, and 7.*

The doses used for the day 1 and day 4 ducks were ~6 and 60 ID_{50}.

The sequential results of DHBV DNA detection in serum and liver for individual ducks are shown in Table 28 (p.101) (Day 1 inoculation groups), Table 29 (p.102) (Day 4 inoculation groups), and, Table 30 (p.103) (Day 7 inoculation groups).

Day	Dose (vge)	Legband	Sex	Day 0	4	6	8	11	13	15	18	20	22	25	27	29	32	34	36	39	41	43	L
					3	5	7	10	12	14	17	19	21	24	26	28	31	33	35	38	40	42	
		P19	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		P20	F	0	0	0	0	0	5	2	2	3	4	3	4	4	5	5	4	4	4	4	5
1	$2.8x10^3$	P21	M	0	0	0	1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		P22	F	0	0	0	0	3	5	4	4	3	3	4	4	5	5	4	5	5	5	4	5
		P23	F	0	0	0	4	5	5	5	4	4	4	4	5	5	5	5	5	5	5	5	5
		P24	F	0	0	0	0	4	5	5	5	4	4	2	2	4	5	3	4	4	4	4	5
		P13	M	0	0	0	5	4	5	5		4	4	4	5	5	5	5	5	5	5	5	5
		P14	M	0	0	0	0	4	5	4	5	5	5	5	5	5	5	5	5	5	5	5	5
1	$2.8x10^4$	P15	M	0	0	0	5	4		5		4	5	5	4	5	5	5	5	5	5	5	5
		P16	M	0	0	0	0	5		5	5	5	5	2	4	5	5	5	5	5	4	5	5
		P17	F	0	0	0	0	5	4	4	4	4	4	4	5	5	5	5	5	5	5	5	5
		P18	M	0	0	0	0	4	5	5	4	1	1	3	1	3	4	3	4	4	4	4	5

Table 28. *Dot blot hybridisation and PCR results for ducks inoculated with either $2.8x10^3$, or $2.8x10^4$ vge of DHBV when 1 days old.*

Dark shaded numbers indicate days post inoculation. Dot blot results are the numerical value (0=not detected (≤$x10^6$vge/mL), 1=$1x10^7$vge/mL, 2=$1x10^8$vge/mL, 3=$1x10^9$vge/mL, 4=$1x10^{10}$vge/mL, 5>$2x10^{10}$vge/mL). Shaded blocks indicate DHBV PCR results: red = positive (>$2x10^3$ vge/mL), green = negative (<$2x10^3$ vge/mL), clear = not tested. Sex: M= male, F= female. Empty blocks indicate that no sample was available for that day.

Day	Dose (vge)	Legband	Sex	Day 0	4	6	8	11	13	15	18	20	22	25	27	29	32	34	36	39	41	43	L
					0	2	4	7	9	11	14	16	18	21	23	25	28	30	32	35	37	39	
		W18	M	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		W19	F	0		0	0	0	5	5	4	4	4	4	4	5	5	5	5	5	5	5	5
		W20	M	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	$2.8x10^3$	W21	F	0		0	0	0	2	5	4	4	0	4	4	5	5	5	5	5	5	5	5
		W22	F	0		0	0	0		5	0	0	0	0	0	0	4	5	5	5	5	5	5
		W23	M	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		W24	F	0		0	0	0		5	0	0	2	2	2	5	5	5	5	5	5	5	5
		W11	M	0		0	0	0	5	5	5	5	3	3	4	5	5	5	5	5	5	5	5
		W12	M	0		0	0	0	0	5		4	4	4	4	5	5	5	5	5	5	5	5
		W13	F	0		0	0	0		0	0	3	0	3	2	5	4	0	4	0	4	1	5
4	$2.8x10^4$	W14	M	0		0	0	0	5	5	4	4	3	2	2	5	5	5	5	5	5	5	5
		W15	M	0		0	0	3	5	4	4	0	0	0	0	0	0	0	0	0	0	0	0
		W16	F	0		0	0	0	5	5	5	4	1	3	4	5	5	5	5	4	5	5	5
		W17	M	0		0	0	0	1	5	4	4	2	3	3	5	5	5	4	4	0	2	5

Table 29. *Dot blot hybridisation and PCR results for ducks inoculated with either $2.8x10^3$, or $2.8x10^4$ vge of DHBV when 4 days old.*

Dark shaded numbers indicate days post inoculation. Dot blot results are the numerical value (0=not detected ($\leq x10^6$vge/mL), 1=$1x10^7$vge/mL, 2=$1x10^8$vge/mL, 3=$1x10^9$vge/mL, 4=$1x10^{10}$vge/mL, 5>$2x10^{10}$vge/mL). Shaded blocks indicate DHBV PCR results: red = positive (>$2x10^3$ vge/mL), green = negative (<$2x10^3$ vge/mL), clear = not tested. Sex: M= male, F= female. Empty blocks indicate that no sample was available for that day.

Day	Dose (vge)	Legband	Sex	Day 0	8	11	13	15	18	20	22	25	27	29	32	34	36	39	41	43	L
					1	4	6	8	11	13	15	18	20	22	25	27	29	32	34	36		
		B33	M	0		0	0	0	0	0	0	1	5	5	5	5	5	5	4	5	5
		B34	F	0		0	0	3	4	0	1	0	3	4	4	5	5	5	5	5	5
		B35	F	0		0	0	0	4	4	0	0	0	0	0	0	4	2	3	4	5
7	$2.8x10^4$	B36	M	0		0	0	0	0	0	0	5	5	5	5	5	5	5	5	5	5
		B37	M	0		0		0	0	0	0	0	0	0	0	0	0	0	0	0	5
		B38	M	0		0	0	0	5	5	3	4	1	0	4	5	5	5	5	5	5
		B39	M	0		0	0	5	3	4	2	4	4	5	5	5	5	5	5	5	5
		B26	F	0		0	0	4	1	0	0	0	0	0	3	3	5	3	4	4	5
		B27	M	0		0		5	5	5	5	5	5	5	5	5	5	5	5	5	5
		B28	F	0		0	0	4	4	0	2	0	1	2	3	4	4	4	3	4	5
7	$2.8x10^5$	B29	F	0		0		0	0	0	0	0	0	0	0	0	0	0	0	0	0
		B30	M	0		0		5	0	0	0	3	3	4	4	5	5	5	5	5	5
		B31	F	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		B32	M	0		0	0	0	5	0	0	0	0	1	4	5	5	5	5	5	5

Table 30. *Dot blot hybridisation and PCR results for ducks inoculated with either $2.8x10^4$, or $2.8x10^5$ vge of DHBV when 7 days old.*

Dark shaded numbers indicate days post inoculation. Dot blot results are the numerical value (0=not detected ($\leq x10^6$vge/mL), 1=$1x10^7$vge/mL, 2=$1x10^8$vge/mL, 3=$1x10^9$vge/mL, 4=$1x10^{10}$vge/mL, 5>$2x10^{10}$vge/mL). Shaded blocks indicate DHBV PCR results: red = positive (>$2x10^3$ vge/mL), green = negative (<$2x10^3$ vge/mL), clear = not tested. Sex: M= male, F= female. Empty blocks indicate that no sample was available for that day.

3.4.1.1. Group Results

In most infected ducks, DHBV DNA was detectable by PCR 2-4 days before dot blot hybridisation became positive.

For the Day 1 groups, 5 of 6 ducks of the $2.8x10^3$ vge subgroup (equivalent to 6 ID_{50}), and all 6 ducks of the $2.8x10^4$ vge subgroup (equivalent to 60 ID_{50}) became infected. For this age group, the ID_{50} was less than $2.8x10^3$ vge, or less than 200µL of a $1x10^{-5}$ dilution, which correlates closely with the original determination of the infectivity of the DHBV041094 positive serum pool for day old ducks.

In the Day 4 groups, 6 of 7 ducks from the $2.8x10^3$ vge subgroup, and all 7 ducks of the $2.8x10^4$ vge subgroup became infected, as determined by DHBV DNA. Two ducks (W20, and W23, both $2.8x10^3$ vge), were only PCR positive in the liver, and may have eventually cleared the infection completely, given further time. The ID_{50} for this group was less than $2.8x10^3$ vge.

In the Day 7 groups, all 7 ducks of the $2.8x10^4$ vge subgroup, while only 5 of 7 ducks of the $2.8x10^5$ vge subgroup became infected. The ID_{50} for this group was less than $2.8x10^4$ vge.

The persistence of DHBV in ducks infected at an early age is quite remarkable; only four ducks cleared DHBV DNA from the liver.

3.4.1.2. Individual Duck Results

In Figure 17 - Figure 22 (p.105-110) DHBV DNA results have been graphed to describe the course of infection in individual ducks. They are presented group by group.

DHBV was never detected in either the serum or liver of four ducks: P19 (Day 1, $2.8x10^3$ vge), W18 (Day 4, $2.8x10^3$ vge), B29, and B31 (both Day 7, $2.8x10^5$ vge). They were completely dot blot hybridisation negative, and were found to be PCR negative in the liver and at various time points, two ducks were male and two female.

In a further two male ducks; W20 and W23 (Day 4 $2.8x10^3$ vge), DHBV DNA was not detected in the serum throughout the experimental period. In the liver these ducks were only DHBV DNA positive by the more sensitive PCR assay. Fourteen serum samples from each duck, were assayed by PCR (not enough serum was available to test day 11, 14, and 43), and all were found to be PCR negative.

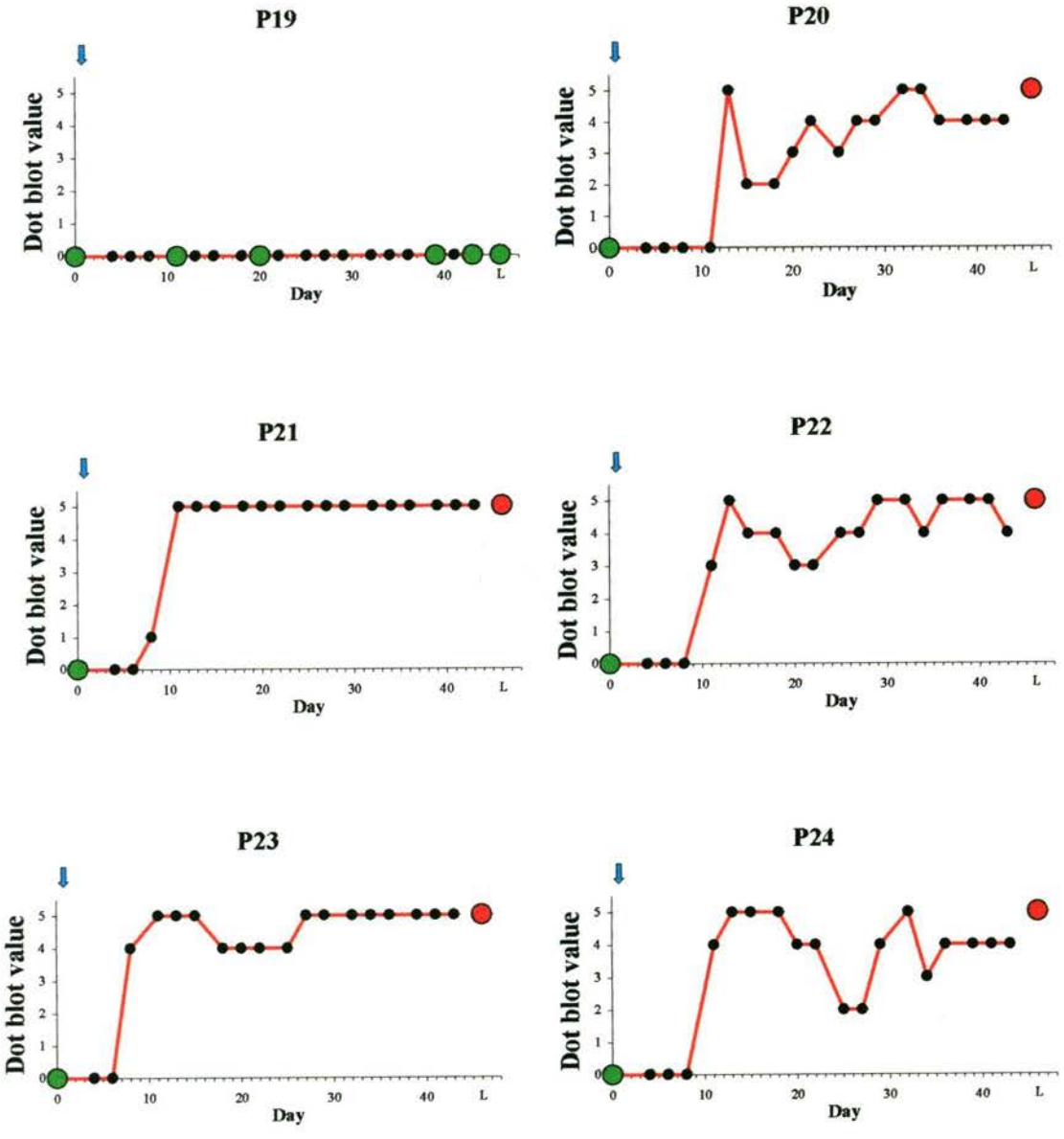


Figure 17. *Graphic results for ducks injected on day 1 with 2.8x10^3 vge.*

Dot blot results are the plotted numerical value: 0=not detected (≤x10^{6}vge/mL), 1=1x10^{7}vge/mL, 2=1x10^{8}vge/mL, 3=1x10^{9}vge/mL, 4=1x10^{10}vge/mL, 5>2x10^{10}vge/mL.

The blue arrow indicates when the ducks were inoculated.

PreS-S PCR results are indicated by large data points: green = PCR negative (<2x10^3 vge/mL), red = PCR positive (>2x10^3 vge/mL), small black = not tested.

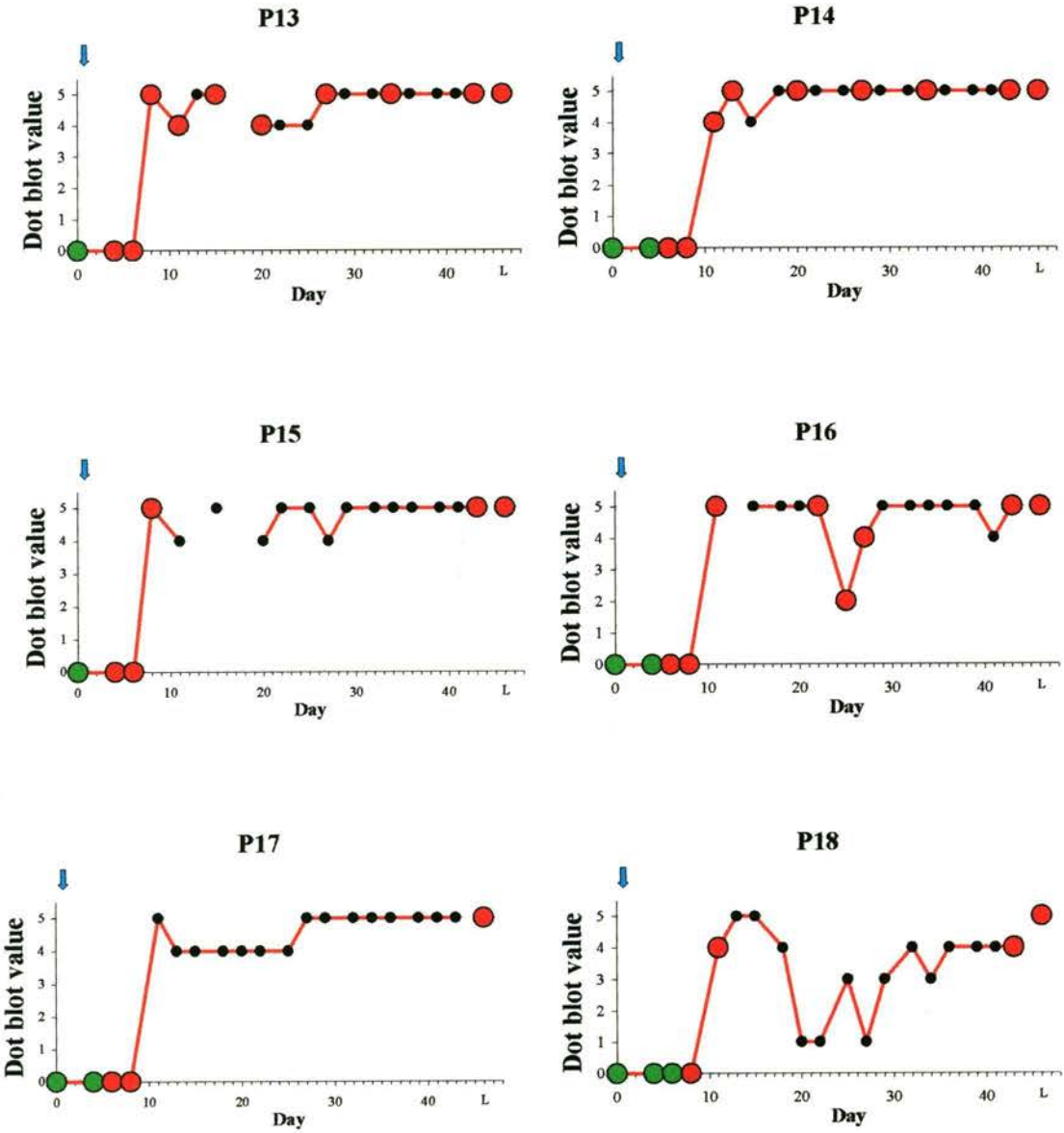


Figure 18. *Graphic results for ducks injected on day 1 with $2.8x10^4$ vge.*

Dot blot results are the plotted numerical value: 0=not detected ($\leq x10^6$vge/mL), 1=$1x10^7$vge/mL, 2=$1x10^8$vge/mL, 3=$1x10^9$vge/mL, 4=$1x10^{10}$vge/mL, 5>$2x10^{10}$vge/mL.

The blue arrow indicates when the ducks were inoculated.

PreS-S PCR results are indicated by large data points: green = PCR negative (<$2x10^3$ vge/mL), red = PCR positive (>$2x10^3$ vge/mL), small black = not tested.

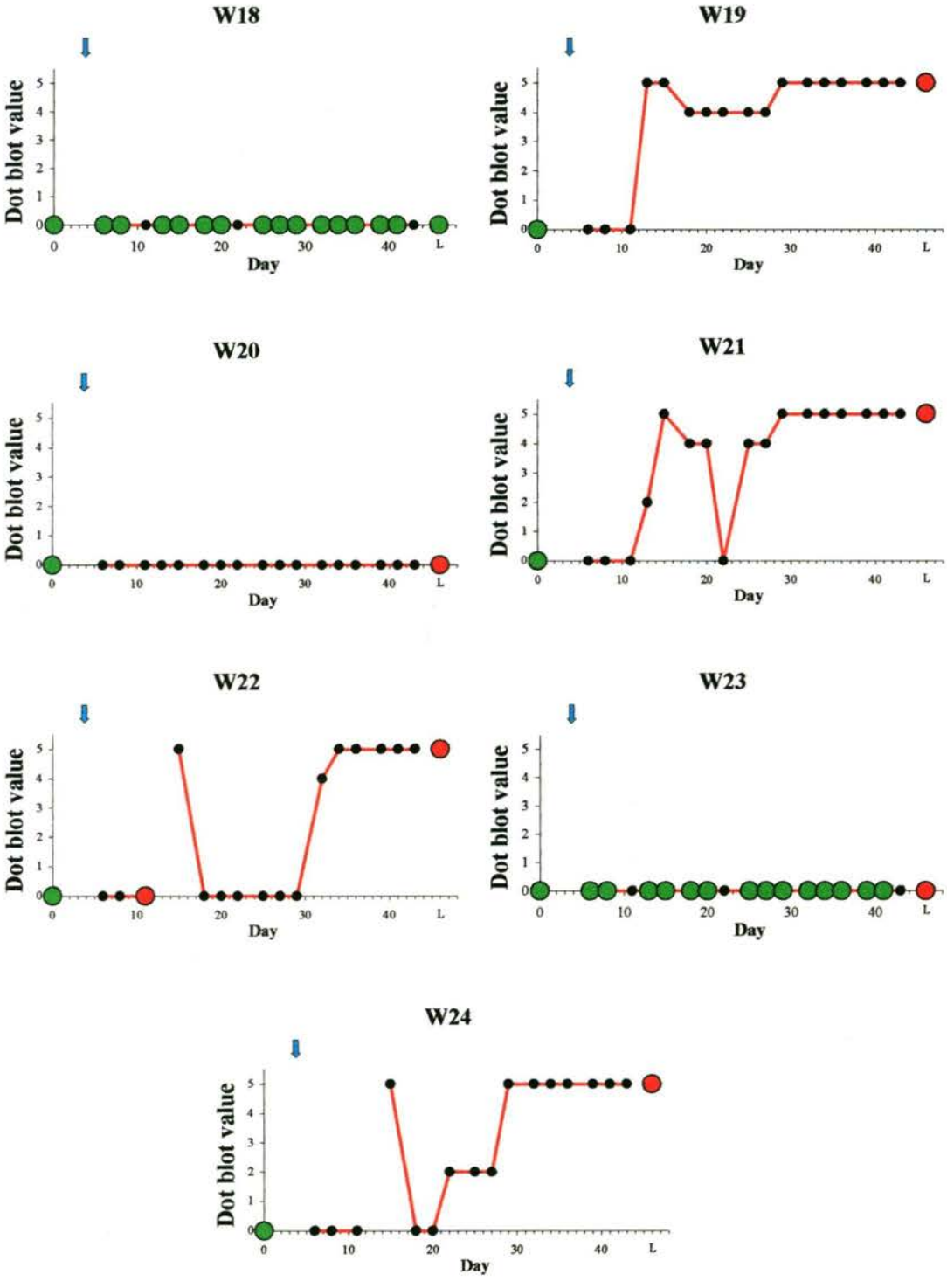


Figure 19. *Graphic results for ducks injected on day 4 with $2.8x10^3$ vge.*

Dot blot results are the plotted numerical value: 0=not detected ($\leq x10^6$vge/mL), 1=$1x10^7$vge/mL, 2=$1x10^8$vge/mL, 3=$1x10^9$vge/mL, 4=$1x10^{10}$vge/mL, 5>$2x10^{10}$vge/mL.

The blue arrow indicates when the ducks were inoculated.

PreS-S PCR results are indicated by large data points: green = PCR negative (<$2x10^3$ vge/mL), red = PCR positive (>$2x10^3$ vge/mL), small black = not tested.

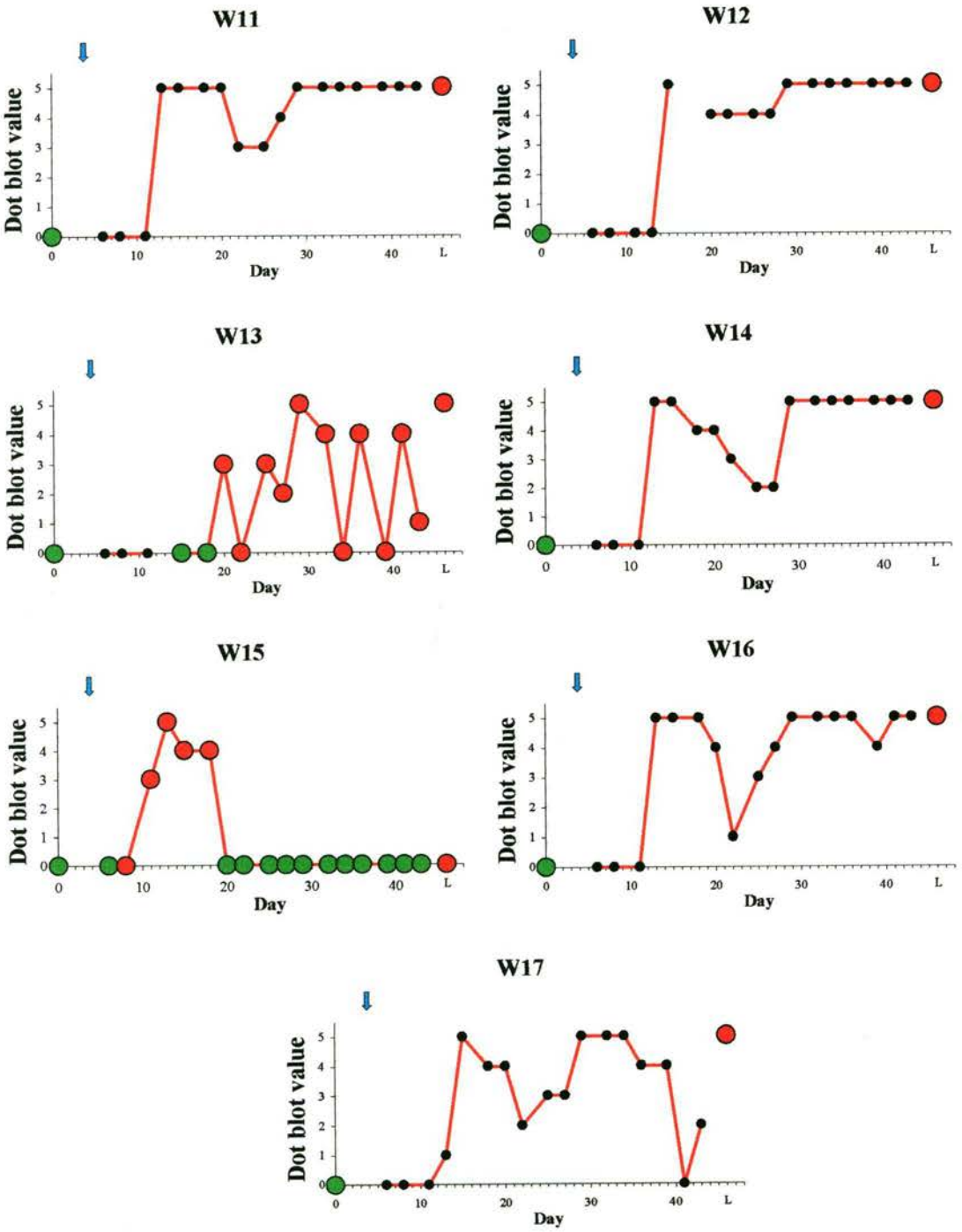


Figure 20. *Graphic results for ducks injected on day 4 with $2.8x10^4$ vge.*

Dot blot results are the plotted numerical value: 0=not detected (≤x10^6vge/mL), 1=1x10^7vge/mL, 2=1x10^8vge/mL, 3=1x10^9vge/mL, 4=1x10^{10}vge/mL, 5>2x10^{10}vge/mL.

The blue arrow indicates when the ducks were inoculated.

PreS-S PCR results are indicated by large data points: green = PCR negative (<2x10^3 vge/mL), red = PCR positive (>2x10^3 vge/mL), small black = not tested.

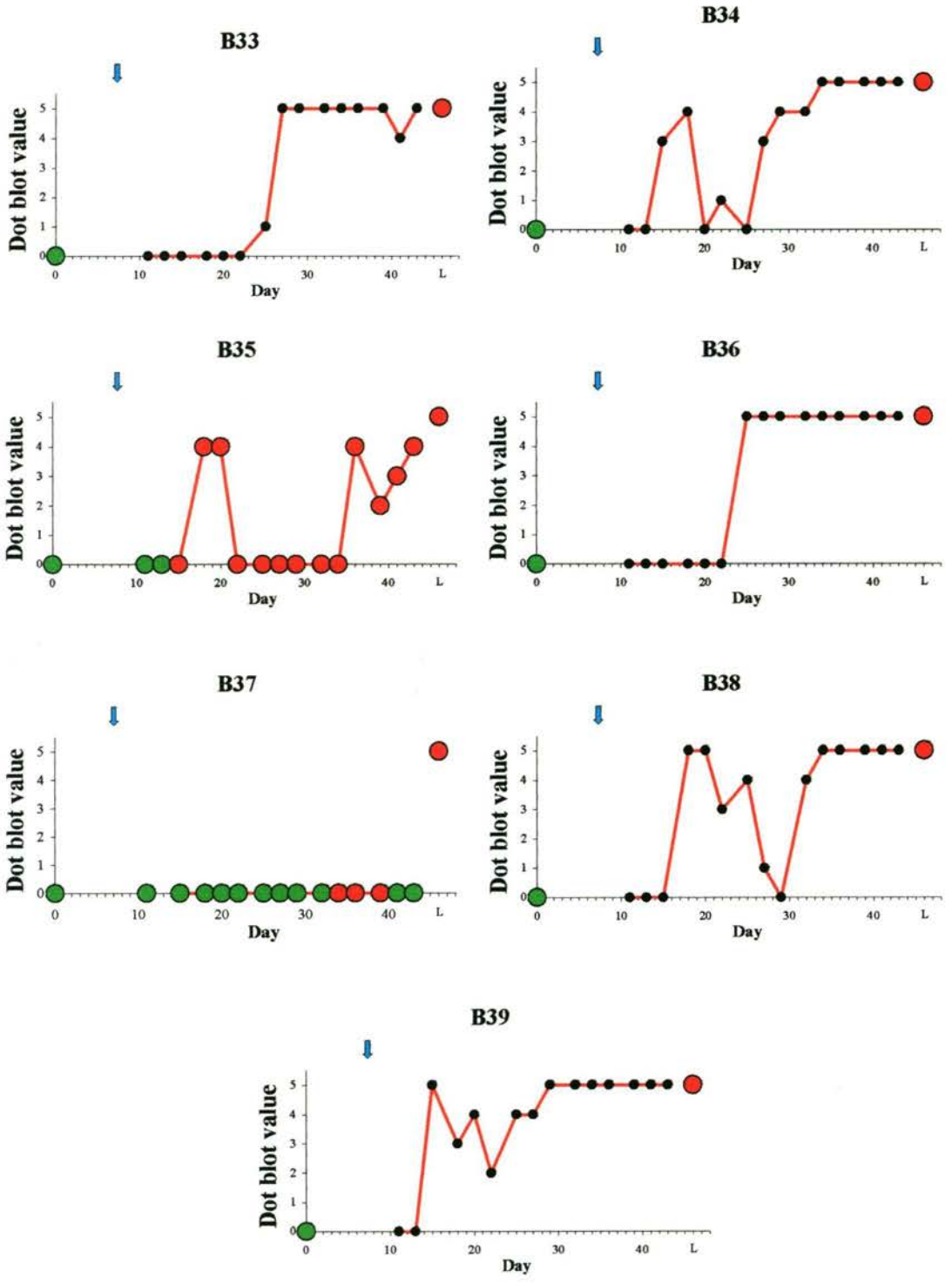


Figure 21. *Graphic results for ducks injected on day 7 with 2.8x10^4 vge.*

Dot blot results are the plotted numerical value: 0=not detected (≤x10^{6}vge/mL), 1=1x10^{7}vge/mL, 2=1x10^{8}vge/mL, 3=1x10^{9}vge/mL, 4=1x10^{10}vge/mL, 5>2x10^{10}vge/mL.

The blue arrow indicates when the ducks were inoculated.

PreS-S PCR results are indicated by large data points: green = PCR negative (<2x10^3 vge/mL), red = PCR positive (>2x10^3 vge/mL), small black = not tested.

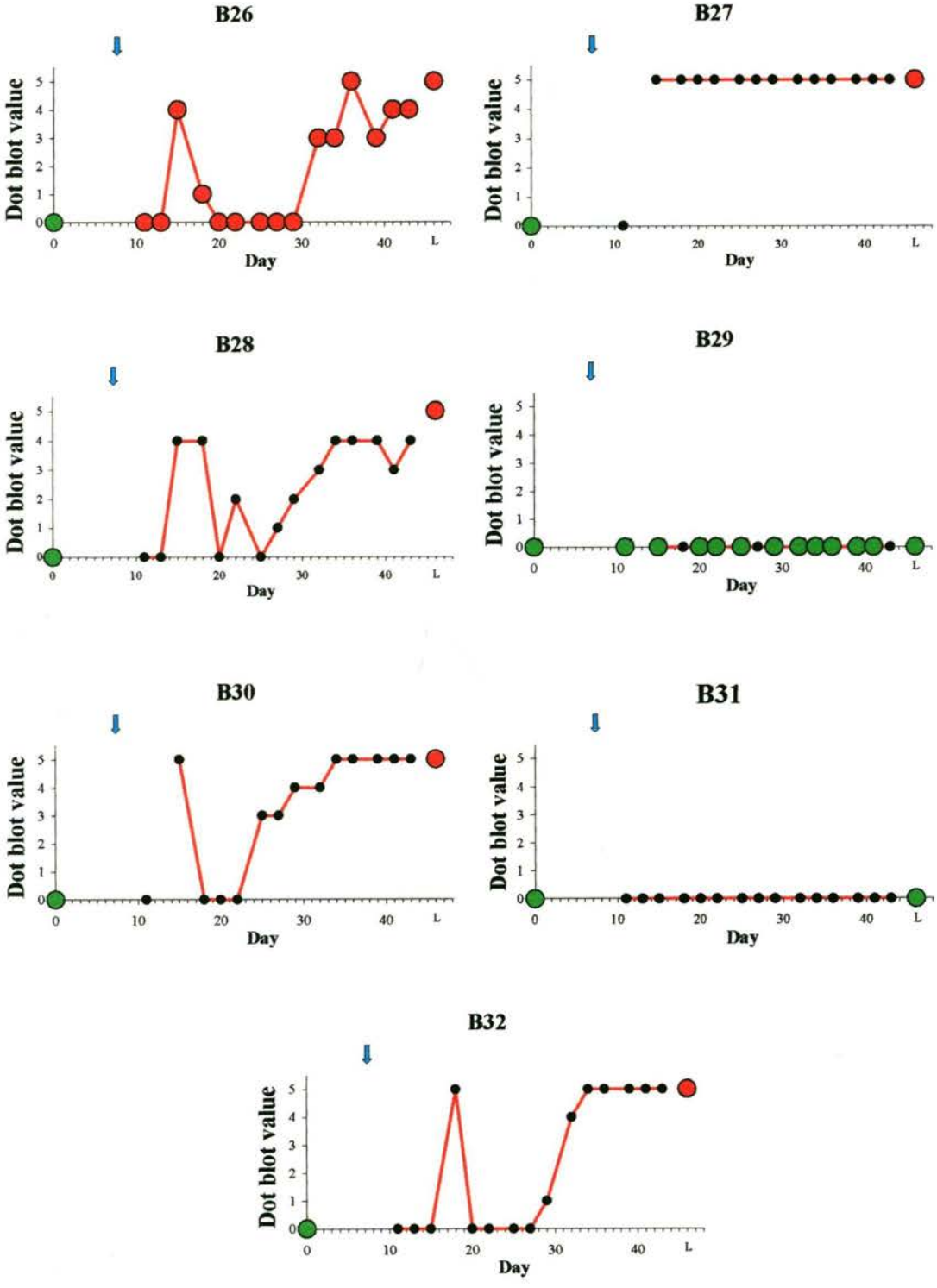


Figure 22. *Graphic results for ducks injected on day 7 with $2.8x10^5$ vge.*

Dot blot results are the plotted numerical value: 0=not detected (≤x10^6vge/mL), 1=1x10^7vge/mL, 2=1x10^8vge/mL, 3=1x10^9vge/mL, 4=1x10^{10}vge/mL, 5>2x10^{10}vge/mL.

The blue arrow indicates when the ducks were inoculated.

PreS-S PCR results are indicated by large data points: **green** = PCR negative (<2x10^3 vge/mL), **red** = PCR positive (>2x10^3 vge/mL), small black = not tested.

An additional male duck, W15 (Day 4, $2.8x10^4$ vge), had a peak of viraemia detectable by dot blot hybridisation, followed by clearance from the serum by dot blot hybridisation and PCR. Despite clearing DHBV DNA from the serum, PCR, but not dot blot hybridisation, revealed the presence of DNA in the liver.

One male duck, B37 (Day 7, $2.8x10^4$ vge), which was strongly positive for DHBV DNA in the liver, had no detectable levels in the serum by dot blot hybridisation. PCR showed that three consecutive samples (days 34-39) contained DNA.

In Duck W13 (Day 4, $2.8x10^4$ vge), which developed a fluctuating viraemia, DHBV DNA was found much later than the rest of the group at 16 days *pi* (day 20) (Table 29, p.102). This duck had several episodes of high viraemia, remained constantly PCR positive until the end of the experiment, and was DHBV DNA positive in the liver by dot blot hybridisation.

All the other ducks, remained PCR positive from the date of first detection of viraemia until the end of the experiment.

3.4.2. Infection Kinetics

Most Day 1 group ducks showed the characteristic rapid rise in viraemia, but ducks inoculated later (Day 4 and 7 groups) (ducks W22, W24, B34, B35, B38, B26, B28, B30, and B32) (6 female, 3 male), exhibited a previously unreported biphasic pattern. This pattern consists of a short but high serum viral DNA level followed by several logs reduction for a short period of a few days, then a subsequent rebounding and persistence.

Of the 11 ducks that developed early onset of viraemia, ten went on to develop persistent with a high level viraemia. However, although developing an early onset of high level viraemia duck W15 (Day 4, $2.8x10^4$ vge), went on to clear the infection from the serum and was only positive in the liver by PCR suggesting that this duck was clearing the infection.

In contrast, all four ducks that produced undetectable or low level viraemia, cleared DHBV from the serum and in the liver.

The incubation period before initial viraemia is detected, has been summarised (Table 31 p.112).

inoculation	Dose (vge)	7-8 days pi	9-10 days pi	11-12 days pi	16-18 days pi	Not detected
Day 1	$2.8x10^3$	2	2	1	-	1
	$2.8x10^4$	2	4	-	-	-
Day 4	$2.8x10^3$	-	2	2	-	3
	$2.8x10^4$	1	4	1	1	-
Day 7	$2.8x10^4$	2	-	2	2	1
	$2.8x10^5$	4	-	1	-	2

Table 31. *Days post inoculation to first detection of DHBV DNA in serum by dot blot hybridisation.*

NB: Table indicates days post inoculation (*pi*). ie. 7-8 days *pi* for the Day 1, 4 and 7 groups is day 8, day 11, and day 15 respectively. DHBV DNA was generally detected by PCR 2-5 days previous to dot blot hybridisation detection.

The three inoculation groups (Day 1, 4, and 7) can be directly compared with the $2.8x10^4$ dose, which was given to all groups. As the age of inoculation increased, onset of viraemia was delayed (Figure 23, p.112). Significantly more of the Day 1 ducks were viraemic by 10 days *pi* than the Day 7 group ($P = 0.021$).

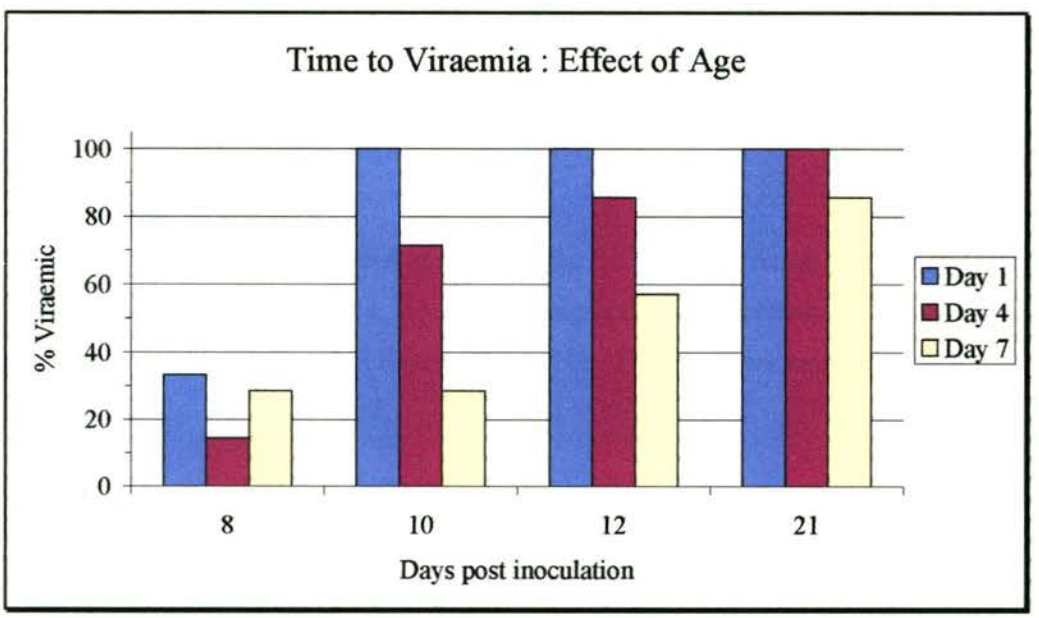


Figure 23. *Effect of age at inoculation on time to viraemia.*
The dose of $2.8x10^4$ vge is compared across all three groups.

When the low dose groups are combined and compared with the high dose groups a pattern emerges in that the higher doses appear to produce a shorter incubation period (Figure 24, p.113). Unfortunately, due to the low numbers of ducks used in the experiment, the results are non-significant; however, for 10 days *pi* they are only just non-significant (p=0.055).

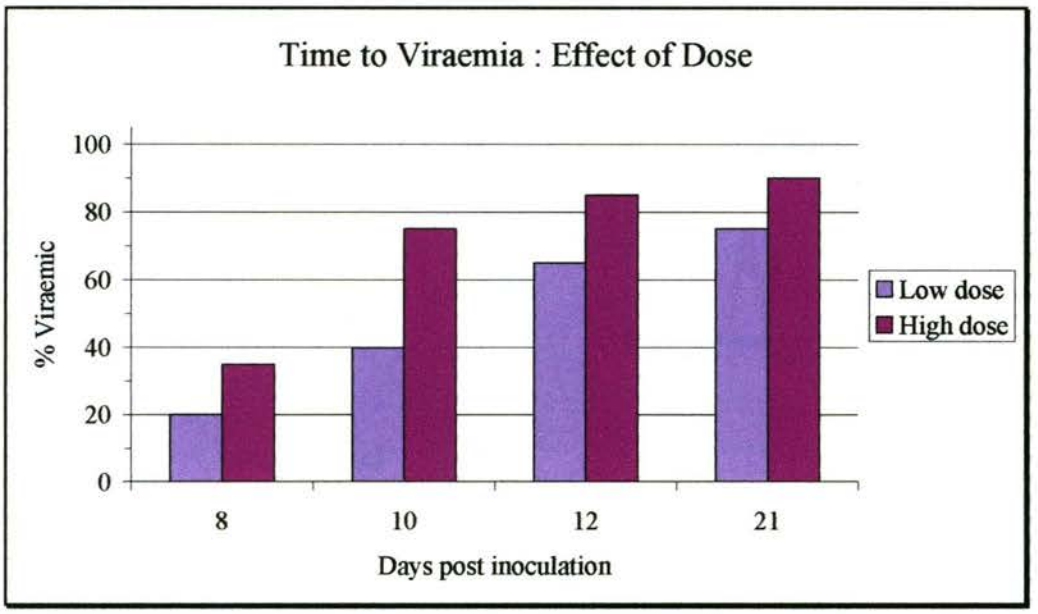


Figure 24. *Effect of dose on time to viraemia.*
The low dose of all three groups combined (Day 1, 4, and 7) is compared to the high dose of all three groups combined.

3.4.3. Overview of Results

In ducks that remained DHBV DNA positive in the liver, five different patterns of viraemia are evident: (a) classic persistence, (b) self-limiting acute, (c) biphasic, (d) fluctuating, and (e) non-viraemic, demonstrated in Figure 25 (p.114). These are summarised in Table 32 (p. 114).

Clearance from the liver following viraemia was only found in ducks showing pattern (b).

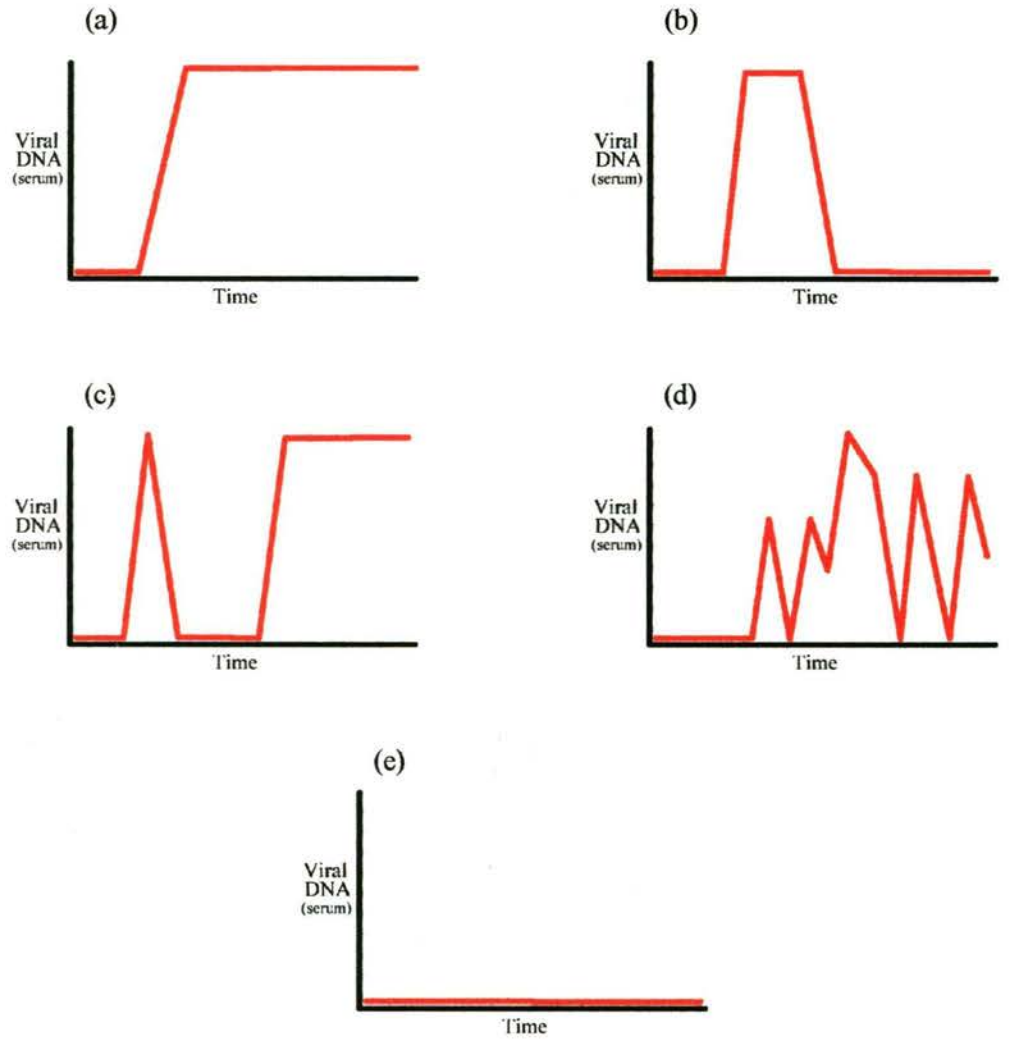


Figure 25. *Five different patterns of early DHBV infection.*

(a) Classic persistence, (b) Self-limiting acute, (c) Biphasic, an acute infection followed by persistence, (d) Fluctuating viraemia, in which the host appears to clear the virus many times only for it to rebound, and (e) non-viraemia.

Inoculation	Dose (vge)	Total ducks	(a) Persistent	(b) Cleared	(c) Biphasic	(d) Fluct.Vir	(e) Uninfected
day1	$2.8x10^3$	6	5	-	-	-	1
	$2.8x10^4$	6	6	-	-	-	-
day4	$2.8x10^3$	7	2	2	2	-	1
	$2.8x10^4$	7	5	1	-	1	-
day7	$2.8x10^4$	7	3	1	3	-	-
	$2.8x10^5$	7	1	-	4	-	2

Table 32. *Summary of DHBV infection outcome.*

Outcome of infection is based on DHBV DNA presence in serum and liver at euthanasia as depicted in Figure 25 (p.114). (a) Persistent infection: serum and liver positive, (b) Cleared: serum negative, liver positive or negative, (c) Biphasic: Single peak of viraemia followed by persistence, liver positive, (d) Fluctuating viraemia: several peaks of viraemia, liver positive, (e) Uninfected ducks: Negative in both serum and liver throughout the experiment.

3.5. DISCUSSION

Following the original description of the experimental transmission of DHBV (Mason *et al.*, 1980), many studies have confirmed that experimental transmission with DHBV is easily achieved, producing high level viraemia in ducks infected at an early age (Mason *et al.*, 1983; Tagawa *et al.*, 1985; Fukuda *et al.*, 1987; Marion *et al.*, 1987; Freiman *et al.*, 1988a).

The outcome of DHBV infection is related to several factors: the dose of the inoculum, age of duck at inoculation, the route of administration, the DHBV isolate, and the duck strain. The size of the inoculation dose is an important variable with a high dose producing quick viraemia and persistence, while a smaller dose is associated with low or non viraemia, and acute self-limiting infection. The age at inoculation is important because infection at a young age leads to persistence while inoculated adults tend towards an acute infection. The infectious dose depends on route of administration, with an *intravenous* inoculation, requiring fewer virions to produce an infection than *intraperitoneal* inoculation. The DHBV isolate may affect the infectivity dose, however for the current experiment a single DHBV isolate was utilised. The final factor that may play a role is the genetic composition of the duck strain used, with ducks from different suppliers having different susceptibility. The one source of ducks was used through the present study.

Experimentally, the outcome can be manipulated by either the dose used and/or the age at which the ducks are infected (Vickery and Cossart, 1996; Jilbert *et al.*, 1998). In older ducks, a larger DHBV dose normally produces persistence, while a lower dose leads to a higher proportion of self-limited acute infection (Vickery and Cossart, 1996; Jilbert *et al.*, 1998). This dose relationship is also evident in other hepadnaviruses such as Woodchuck Hepatitis B Virus (Cote *et al.*, 2000). In the neonatal period infection almost invariably leads to persistence even at very low virus doses.

In this study we investigated the inter-relationship between dose and age in more detail.

3.5.1. Dose

In earlier studies we determined the ID_{50} of the serum pool DHBV051094 for day old ducks, based on dot blot assay of the ducks 5 weeks after *intra peritoneal* inoculation (Vickery and Cossart, 1996). For our present study, we selected doses that were predicted to infect most ducks, while allowing a few to clear the infection. Although both transient and persistent infections were observed, the proportion of transient infection was lower than anticipated. This is partially due to the detection of residual viral DNA by PCR analysis of the serum and liver rather than the less sensitive dot blot hybridisation method used in earlier studies. The PCR assay is approximately 4 $\log_{10}$ more sensitive than the dot blot hybridisation assay, and

is able to detect the small amount of virus that is still being produced, and finally, the long lasting cccDNA in the liver.

Previous studies in adult ducks (Vickery and Cossart, 1996; Jilbert *et al.*, 1998), have determined that reduction of DHBV to undetectable levels by dot blot hybridisation was associated with detection of anti-DHBs antibody. After a short period, the serum would then become and remain PCR negative. Although non-viraemic, the duck may not yet have completely cleared the infection, as the liver may be dot blot hybridisation negative, the liver may still remain PCR positive for many months. Similar findings have been reported for both humans and woodchucks (Kajino *et al.*, 1994; Penna *et al.*, 1996).

The original ID_{50} was based on ducks inoculated on day 1 with dot blot hybridisation results of the liver at 5 weeks (35 days) of age. The infectivity of the serum pool DHBV051094 for Day 1 ducks was originally calculated as 1 ID_{50} being 100µL of a $10^{-5.5}$ dilution. This calculation was based on groups of five ducklings and analysed by the method of Reed and Muench, which equalises chance variations and defines an accurate end point (Reed and Muench, 1938). The serum pool used for this experiment was identical to that used to determine the ID_{50} in the original experiment, and has been stored at between –20 and –70°C. In the present study the ID_{50} could be estimated by using the dot blot hybridisation results, as was used for the original calculation, however this would just be an approximation, as there are only two dilutions to compare. The dose approximates that of the original study, considering the low number of ducks and doses used in this experiment and that the ducks were also about two weeks older when tested. Another important consideration is that the difference in infectivity between the original and the present experiment may also be attributed to the genetic differences in the ducks available after an eight-year difference. Although the ducks were obtained form the same hatchery as those of the original experiment; the hatchery has undertaken a program of selective breeding to select for ducks of commercial benefit during the period between the experiments.

The delicate balance of the infectious dosage is evident when comparing the two doses used on the Day 4 ducks. The lower dose, ($2.8x10^3$ vge), ended up producing either persistent, or non-viraemic infection (two ducks had only PCR detectable DHBV DNA in the liver). The non-viraemic infection with low level DNA present in the liver, has been shown to eventually clear completely (Jilbert *et al.*, 1998). While the higher dose, although producing persistence in 5/7 ducks, produced two ducks that were evidently attempting clearance of the infection (W13, and W15), with temporary high level viraemia, that was eventually cleared from 1 duck. The lower dose in Day 4 ducks ($2.8x10^3$ vge) produced more biphasic and

cleared ducks than persistent infections, while the higher dose ($2.8x10^4$ vge) produced mainly persistent infection with a higher level of viraemia.

3.5.2. Age at inoculation

The susceptibility of ducks to DHBV decreases rapidly after hatching and by day 11 a significantly higher dose is required to produce an infection (Freiman *et al.*, 1990). Ducklings injected on day 1 were 100% (20/20) infectable and 17/20 remained viraemic for greater than 6 months, while the same dose only persistently infected 1/7 ducks when injected at 3 weeks of age (Omata *et al.*, 1984). When ducks inoculated on day 1 and day 26 are compared, it has been shown that the ID_{50} for the 26 day old ducks is approximately $3log_{10}$ larger (Vickery and Cossart, 1996). While four month old ducks have been shown to be require $5log_{10}$ higher doses than day 1 ducks, and even such a dose only caused persistent infection in 1/3 ducks while the other two where only transiently infected (Jilbert *et al.*, 1998). This age related decrease in susceptibility is also paralleled in the woodchuck model (Cote *et al.*, 2000).

In this experiment the increasing age of ducks was associated with a lower frequency of persistently infected ducks. Eleven of the twelve ducks inoculated on Day 1 were persistently infected, with 1 duck remaining uninfected. In Day 4 ducks, 7/14 produced classic persistent infection, while only 4/14 of the Day 7 ducks were found to have the classic persistent infection pattern. The older ducks tended to develop a biphasic pattern in which an initial spike of viraemia was followed by several days of dot blot hybridisation negative serum samples, after which viraemia returned and persisted until the end of the experiment. This biphasic pattern may be due to an initial immune response that was able to contain the infection temporarily, but ultimately failed to eliminate it; however, it might also reflect selection of an "escape" mutant able to evade the host response.

3.5.3. Incubation period

The incubation period of virus infections is usually related to the size of the infecting dose, and this has been shown for HBV (Barker and Murray, 1972). Similar observations have been reported with DHBV (Tagawa *et al.*, 1985; Jilbert *et al.*, 1996). Our experimental results confirmed such observations, as the ducks given the larger dose developed viraemia sooner than ducks given the lower dose (Figure 24, p.113). However the increase in incubation time with increasing age at inoculation has a greater influence than can be explained simply by the difference in the ID_{50} and has been attributed to other factors such as decreased permeability of the more mature hepatocyte or to the increased maturation of non-specific immunity. Decreased permeability of the more mature hepatocytes is unlikely as these were baby ducks with rapidly dividing hepatocytes, even so, hepatocytes from older

ducks can be easily infected *in vitro*, and *in vitro* infection is enhanced by maintaining the differentiated cell state (Galle *et al.*, 1989). Other immune mechanisms may be more developed in the older ducks than the young ducks, such as there may be a greater number of mature T- and B-cells which are able to produce a greater response quicker, which may contain the infection until it can be eliminated.

Although a similar level of viraemia was reached by the three age groups, the younger the ducks were inoculated, the sooner they became viraemic (Figure 23, p.112). A non-specific mechanism involved may be the physical growth pattern of the ducks in which the weight of the ducks in the first four days of life is relatively stable but doubles every week for about four weeks and then slows until about 3-4 months old when they reach their maximum size. This rapid growth after the first few days is associated with a rapid increase in hepatocytes. The virus may be quickly taken up by the new hepatocytes, while the slower growth rate of the Day 1 ducks means fewer hepatocytes to take up the virus. This may also relate to the cell cycle, in which it has been observed that rapidly dividing, or mature hepatocytes are more easily infected, and the very young ducks have lower numbers of these cells. Another possibility is of a physical dilution, in which the multiplicity of infection is much higher for the day old ducks (which have fewer hepatocytes) as it is for the older ducks (which have undergone weight and hepatocyte gain).

3.5.4. Kinetics of Infection

In almost every case, the amount of virus in the serum increased exponentially to a level of approximately $2x10^{10}$ vge/mL, regardless of initial dose, incubation period, or age of ducks. This high level of virus in the serum has been correlated with infection of >95% of all hepatocytes (Jilbert *et al.*, 1988), and occurs before the specific immune system is able to mount a reasonably large response.

The DHBV infection can be classified into five distinct patterns:

(a) The classic persistent infection pattern, in which a high level viraemia is maintained throughout, was found in representatives of each experimental group. Both innate and specific immune responses are ineffective.

(b) The self-limiting acute infection was found in older ducks, which were given the lower virus dose. This requires a combination of innate and specific immune responses but the exact mechanisms remain speculative.

(c) The newly observed biphasic pattern may reflect a successful down regulation of viral replication by innate immunity, which is not supported by an adequate specific response. It would however, also be consistent with emergence of a virus escape mutant. It is a

combination of the acute and persistent infection in which the initial viraemia is controlled but cannot be eliminated leading to persistence.
(d) The fluctuating viraemia most likely reflects a partially effective immune response that either cannot be sustained, or is avoided by the virus.
(e) The uninfected pattern (no viraemia, or liver infection), can be produced by ducks that are either not susceptible, or have been able to mount an extremely effective immune response.

In two ducks infection did not conform to the dogma that a high titre early viraemia lead to persistent infection. Duck W15 (Day 4, $2.8x10^4$ vge), had a high viraemia early during infection, at a level which was found to predict persistence in all other ducks, but was subsequently able to clear the infection from the serum, and only residual DHBV DNA was found in the liver. The other duck was B37 (Day 7, $2.8x10^4$ vge), in which very low level of DHBV DNA was found in the serum for a few days, but at the end of the experiment the liver was found to contain high levels of DHBV DNA. This might be due to selection of defective genome, which was able to persist and accumulate in the liver, but was unable to export virions into the circulation. This possibility is explored in Chapter 4.

These exceptions show that prediction of clearance is not as evident, or well defined, in very young ducks as for the adults (Vickery *et al.*, 1989; Vickery and Cossart, 1996), and suggests that both virus and host related mechanisms are involved.

3.5.5. Persistent Infection

The persistence of perinatal infections in ducks is likely to be attributed to the mechanism of tolerance. It was originally believed that the secondary lymphoid organs of ducklings are devoid of lymphocytes until two days before hatching, but has since been shown that several waves of immune cells pass through the secondary lymphoid organs before hatching. At hatching the secondary lymphoid organs are functional (Hashimoto and Sugimura, 1976b), but tolerance to the virus leading to persistence is readily achievable eg. Duck Plague Virus (Burgess and Yuill, 1982).

The relatively stable level of DHBV DNA in serum of high titre persistent infection demonstrates that viral loss is equal to viral replication and virion production. The consistently high levels of DHBV DNA of both surface and core antigen in the liver, suggest that viral production is maintained at high levels indefinitely. The spleen plays an important role in sequestering virus form the circulation (Freiman *et al.*, 1987; Jilbert *et al.*, 1987b), but there may also be excretion in the bile or through the kidneys. Other non-specific immune mechanisms may also be able to remove at least some of the virions from the bloodstream. The virions may be taken up by new hepatocytes which are replacing hepatocytes lost

through natural old age, or a cytotoxic immune response. Hepatocytes already infected may take up more virus producing a superinfection (Chuang *et al.*, 1994; Zhang and Summers, 1999), but the efficiency of this reaction is low as the viral cellular receptor is down regulated in infected cells (Breiner *et al.*, 2001). A combination of the above factors is most likely the reason that the level of viraemia is relatively stable and maintained during the course of persistent infection.

3.5.6. Biphasic pattern

This previously unrecognised pattern of infection occurred only in the older ducks inoculated at 4 and 7 days of age. The trough could arise by rapid removal of virus from the circulation, which subsequently fails, as this mechanism becomes saturated, or from direct inhibition of virus synthesis by a mechanism which is only transiently effective.

Virus removal form the serum is often associated with antibody production and the generation of immune complexes. The timeline is consistent with antibody production as the fall in serum DHBV DNA is observed 10 days or more post inoculation. The antibody-antigen complexes, are subsequently removed by the kidneys, or antibody assisted endocytosis. If antibody is the main pressure that forces the removal of virus from the serum then the rebound may be due to the production of mutants that are able to escape from this antibody pressure. These mutants may be able to avoid the antibody-mediated destruction, but still able to infect hepatocytes, which would lead to a new round of infection and replication, which would result in the rebound observed.

Another reason for the viral rebound could be the very high level of virus production in the liver where almost all the hepatocytes are infected. The serum level of $\sim 10^{10}$ vge/mL may be beyond the capacity of the B-cells to produce enough antibody. If the antibody production is unable to match the viral production anergy may occur, which could result in persistence. As the duck increases in size over the first few weeks the number of hepatocytes also increases, and if the antibody production is able to keep up with virion production then the DHBV virions should not be able to infect new hepatocytes. However, if antibody production is insufficient new hepatocytes may be infected which results in increased virion production compounding the problem.

Another hypothesis for the trough in viraemia proposes that infection induces immune mediating agents such as IFN-γ that reduce viral production. The effectiveness of these mediators to remove the cccDNA from the cell nucleus is somewhat uncertain as the down regulation of viral products does not necessarily lead to decreased cccDNA. Down regulation of viral products may lead to, or promote the development of tolerance, and the

failure to completely clear the infection. The inability of these mediators to rid every cell of viral DNA, and the prolonged production of these mediators, which constitute this immune response, may lead to a depletion of its effectiveness. If the mediators are depleted or their concentration reduced to levels that are unable to contain viral production, then the unaffected pool of cccDNA may rapidly enable virion production to rebound to initial levels.

3.5.7. Clearance mechanisms

The mechanisms involved in viral clearance have not been fully elucidated. In DHBV the classic explanation for removal of infected cells by antigen-specific cytotoxic T-cells is not supported by histological studies of the liver which show only minimal cell damage or regeneration and little lymphocyte infiltration. Lymphokines and possibly other mediators are believed to play a large role in non-cytolytic clearance of virus. In the transgenic mouse model of human HBV it has been shown that cytotoxic T lymphocytes are able to use a non-cytopathic mechanism for the elimination of viral DNA from infected cells, achieved by cell mediators (Guidotti *et al.*, 1994), later determined to be IFN-γ and TNF-α (Guidotti *et al.*, 1996b). The lack of reagents for identification of duck lymphokines has retarded investigation of this mechanism in DHBV.

Another mechanism that plays an important role in clearance or persistence is the emergence of escape mutants. Other viruses that have shown the mechanism of escape mutants are human HCV, and BVDV (Bovine pestivirus). The hallmark of these RNA viruses is their plasticity (Domingo *et al.*, 1985; Domingo, 1992). The absence of an efficient exonuclease to correct misincorporated bases results in a high frequency of base substitutions, approaching one error for every 10,000 nt polymerised. The term quasispecies was coined to describe the concept of genomic variability (Eigen, 1971). Many genomes in the quasispecies will not be viable because of the lethality of certain base substitutions. RNA viruses use this strategy to generate genomes with potentially greater fitness and ability to survive under certain altered environmental conditions. The RT replication mechanism of hepadnaviruses means that they too can take advantage of this mechanism. The consequences of this process are seen in the form of neutralisation escape mutants, or the selection for viruses that are antigenically different from vaccine strains (Donis *et al.*, 1991; Paton *et al.*, 1992). In BVDV the gp53/E2 protein is the target of neutralising antibodies and becomes a source of antigenic hypervariability. This variability constantly changes the protein and thus enables it to escape the immune response (Donis, 1995).

Although the hepadnaviruses are not RNA viruses, their replication cycle involves the use of an endogenous reverse transcriptase (Summers and Mason, 1982). However they are very much constrained in their variability by the distinct overlapping reading frames (Sprengel *et al.*, 1985; Uchida *et al.*, 1989), ie. a nucleotide change in one position of the genome may

effect two proteins. If there is a change in the sequence of one ORF, such as the surface protein, cause by immune pressure, it may cause a change in the overlapping polymerase protein. Such a change in the polymerase protein may well be lethal as it could affect the virus replication cycle.

Investigation of the role of virus variation in determining the different outcomes in HBV has shown that many different point mutations have been identified in patients and associated with different clinical outcomes (Carman, 1997). Regions of the genome which encode viral structural antigens (such as the surface protein of DHBV), or regulatory regions (such as the preCore) have been intensively studied, and functional analysis of the mutants has shown substantial differences in replicative capacity and/or antigenic structure. This falls short of demonstrating a cause and effect relation because of the lack of a suitable experimental model.

Persistence may be due to the selection of a sub-population of the initial inoculum. If sequence variability does occur, then the serum from infected ducks should contain several subspecies of virions, some of which could have increased infectivity, and/or replication rate, or may contain a different epitope to which the host cannot mount an effective immune response, as epitopes may be HLA class restricted (Penna *et al.*, 1991). As such, a subspecies of the heterogeneous inoculum may evade immune system and develop tolerance which may lead to persistence by selection of a more replication competent sub-species, which while then become the majority of the viral species in the bloodstream. In a recent human investigation, the sequence of the HBV genome before, during and after acute exacerbations was examined. Most exacerbations were preceded by an upsurge of serum HBV identical to the pre-existing HBV strain. After exacerbation however, about half of the patients were repopulated by a different viral variant, which was likely a result of immune selection (Liu *et al.*, 2003). Classic escape mutants emerge in liver transplant patients by treatment with hyperimmune hepatitis B immunoglobulin (Carman *et al.*, 1996; Fischer *et al.*, 2001b; Germer *et al.*, 2003).

If virus variation is a major mechanism of persistence in the DHBV system, it should be possible to verify this by identifying mutations of interest and testing their effect on infectivity and pathogenicity.

In the next chapter we investigate the role of antigenic variation in determining the pattern of viraemia and outcome of infection in DHBV. Because of the known association between specific immune responses to the surface gene and viral clearance this gene was targeted for study.

4. DNA SEQUENCE CHANGES DURING CLEARANCE OF DHBV

4.1. INTRODUCTION

In millions of carriers worldwide hepatitis B persists in stable equilibrium with its host. Over the long term some of these carriers (about 5% per annum) do clear the virus at least from the serum without ever developing symptoms of hepatitis. In patients under observation in liver clinics seroconversion from HBe positive (when the virus is replicating at high levels) to anti-e (with low or absent viraemia) is characteristically associated with an inflammatory "flare" in the liver. A proportion of carriers, variously estimated at 20-40%, proceed along a different, apparently inexorable path of liver destruction, and eventually develop cirrhosis and/or hepatocellular carcinoma. There have been many attempts to fit these observations into a unified hypothesis involving cell mediated immune responses to different viral antigens, but in practice the only useful prognostic indicators remain ongoing viraemia which is linked to liver damage as shown by ALT elevation.

During hepadnavirus infection the virus population is not homogeneous, but consists of quasispecies, distinguishable by gene sequence and often by phenotypic characters including antigen production and specificity, viral enzyme activity, infectivity and immunogenicity (Blum, 1993; von Weizsacker *et al.*, 1995; Mathet *et al.*, 2003).

It has been hypothesised that recovery from infection can be achieved either by selection of defective mutants (when little liver damage ensues) or by emergence of highly replicative, highly immunogenic variants which stimulate cell mediated immune clearance (and induce hepatitis). Immunological selection directly affects replication because of the overlap of the polymerase open reading frame with that of the core, and the surface genes (also X gene in mammalian hepadnaviruses).

Interpretation of the significance of mutations in HBV is hampered by the intrinsic difficulties of human studies with their limited scope for manipulation of conditions, ready

availability of only secreted particles from serum, and the complexity of a virus replication system where both episomal and integrated genomic material may be transcriptionally active in the same cell. The duck virus, which does not integrate, has been widely used to elucidate the functional significance of hepadnavirus mutants because it can be manipulated experimentally *in vivo* as well as *in vitro*. Knowledge of the effects of mutation on viral polymerase, packaging and infectivity are mainly derived from mutagenesis experiments using the DHBV system. To date this has not been extended to study of naturally occurring mutation during the course of infection in individual birds or flocks, although DHBV strains with distinctive sequence differences from the prototype have been isolated from wild ducks and different commercial flocks.

From the previous chapter it is evident that there is a wide range of outcomes within groups of ducks inoculated under the same circumstances (age at inoculation, virus strain, virus dose, route of administration). Although differences in the dose and age at inoculation are important factors, in which younger ducklings and larger doses tend to develop persistent infection it does not account for the variability of all of the outcomes. One of the factors that many infectious agents have utilised to escape the immune response is by varying their genetic material. This mechanism may lead to a change in amino acid sequence of the viral protein or affect the interaction of viral and cellular regulatory mechanisms.

It was evident from ducks that produced a biphasic infection that the return of viral DNA to the serum and establishment of persistence was at a time when a specific immune response should have been generated. We decided to investigate changes to the S region of the viral genome because we had previously observed that immune responses to S were good predictors of virus clearance.

4.1.1. DNA Sequencing Methodology

The data presented below were obtained by automated sequencing. The background to sequencing techniques and details of development of an alternative method are given in Methods and Materials (2.3, p.78).

4.2. AIM

(1) To investigate whether DHBV viral clearance is associated with the appearance of specific mutations

4.3. EXPERIMENTAL DESIGN

Samples from seven ducks representing the characteristic infection patterns described in the previous chapter were selected for study (Chapter 3, p.98). The initial inoculum was sequenced several times to determine the heterogeneity of the viral population.

Duck samples were limited because of an Ethics Committee restriction on bleeding frequency and sample size. Samples were chosen to cover a relatively broad spectrum of the infection, but were also chosen either before or after large changes in the viral DNA level in the serum.

Two areas of the DHBV genome were selected for sequencing; the Core gene as a control, and the Surface gene, where changes may affect the immune response (Figure 26 p.125). Where possible, the full-length PCR product was sequenced ensuring that both the core and surface sequence data would be obtained from a single genome. Otherwise, individual PreS-S and Core PCR reactions were carried out directly on the extracted serum, and these products sequenced.

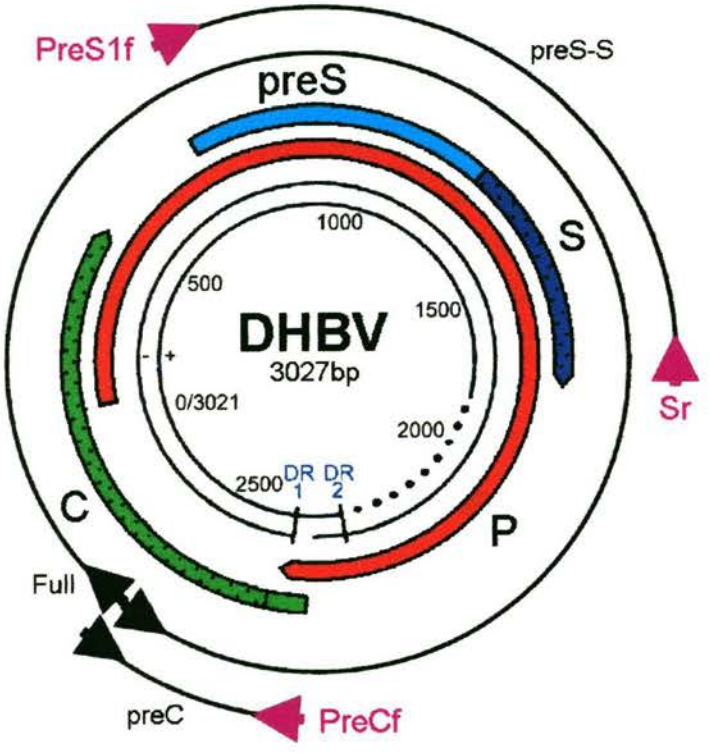


Figure 26. *Regions of DHBV sequenced and primers used.*

The location of the primers used and the direction of sequence data obtained is indicated by the **magenta arrowheads**. PreS1f (nt 686 forward), Sr (nt 1824 reverse), PreCf (nt 2760 reverse). The black lines around the outside of the genome represent the PCR fragments that were used to obtain sequence data as described in Methods and Materials (2.2.2, p.68).

The sequences obtained were manually edited and aligned (Appendix 11.6.1, p.A42) to make observation of changes more visually observable.

4.4. MATERIALS AND METHODS

4.4.1. Persistence-Clearance Ducks

Seven ducks were selected from the Persistence-Clearance experiment (Chapter 3, p.98), for detailed sequence study, two classic persistent (P13, P14, both Day 1 $2.0x10^5$ vge), two biphasic (B26, Day 7 $2.0x10^6$ vge, and B35, Day 7 $2.0x10^5$ vge), two acute self limiting (W15, Day 4 $2.0x10^5$ vge, and B37, Day 7 $2.0x10^5$ vge), and one fluctuating viraemia (W13, Day 4 $2.0x10^5$ vge). The serum samples selected from the course of infection for each of the ducks is represented graphically in the Results section (Figure 27, p.129).

4.4.2. PCR and Sequencing

During the course of the entire experimental period the serum that was used as the original inoculum was also sequenced (15 times): full length (4 times), PreS-S (8 times), and preCore (3 times) PCR fragments.

Full-length PCR could not be produced for all samples. The PCR fragment from which sequence data was obtained for each sample is summarised (Table 33, p.127).

Direct PCR sequencing was performed. Serum samples were extracted by the Phenol/Chloroform Proteinase K method (2.2.1.1, p.67), or by the Guanidinium method (2.2.1.2, p.67). Liver samples were extracted by the Proteinase K Phenol/Chloroform method only.

PCR fragments were obtained as described in (2.2.2, p.68). Production of full-length PCR fragments was initially attempted, and if unsuccessful, generation of PreS-S and PreCore fragments was attempted.

The 1.1kb Surface gene PCR fragment was sequenced from both ends using the PreS1f and Sr primers (Figure 26, p.125). In most cases the sequence data obtained overlapped by only a few bases because of the distance that these primers are apart from each other. The 304bp PreCore region was sequenced using the PreCf primer.

Duck	day	Full	PreS-S	PreCore
P13	6			-
	11			
	27			
	43			
	L			
P14	6			-
	11			
	27			
	43			-
	L			
W13	20			
	29			
	34			-
	39			-
	41			
	L			
W15	13			
	18			
	L			-
B26	15			
	25			
	27			-
	36			-
	L			
B35	15			
	25			
	27			-
	36			-
	L			
B37	36			-
	L			
DHBV051094				

Table 33. *PCR fragment used for sequencing data.*

Surface and Core region sequence data was generated from every duck in which the full-length PCR fragment was obtained. **Light shading**: no sequence data available. **L**: Liver (day 43). DHBV051094: initial inoculum, italic number: number of times the inoculum was sequenced.

4.5. RESULTS

The higher sensitivity of the PreS-S PCR enabled sequence data for the Surface gene to be obtained for all samples. However, due to the lower sensitivity of the PreCore PCR, not all of the selected serums have data for this region (Table 33, p.127). Examples of the edited sequence data output appear in the Appendix (11.5.1, p.A11-A25). The PreC PCR covers the 'nick' region (that may not be completely double stranded) and thus has a lower amplification efficiency.

The sequencing of the Surface gene of the original inoculum serum was performed on the full length, and PreS-S PCR fragments, at least 4 and 8 times respectively, and no difference was ever seen. For the PreCore region the original inoculum serum was sequenced at least 3 times, and no difference was ever seen.

From the three areas that were sequenced (Surface forward and reverse, and Core forward), it was apparent that the DHBV genome is highly conserved, which is evident in the multiple sequence alignments (Appendix 11.5.2, p.A26-A36), which show a highly conserved genome, with few changes.

4.5.1. Clearance Sequencing Results

Only one type of sequence variation was seen in the three areas sequenced (Surface forward and reverse, and Core forward). It was a double substitution of T⇒A at nt 731 and 732. This mutation was found in two ducks infected on day 4 with $2.0x10^5$ vge, these ducks however exhibited different patterns of infection. Duck W15 showed an acute self-limiting infection, while duck W13 had viraemia that fluctuated (Figure 27 p.129). In both cases the appearance of the mutation was not a distinct change in the whole population, but rather appeared as peaks in conjunction with the wild-type sequence, suggesting a quasi-species relationship.

In duck W13 (Day 4 $2.0x10^5$ vge) viraemia was first detected by PCR and dot blot hybridisation on day 20 (16 days post inoculation). From day 20 until the end of the experiment duck W13 remained PCR positive. However, it had several episodes of being dot blot hybridisation negative: day 22, 34, and 39, which were 18, 30, and 35 days post inoculation respectively. Immediately before and after each of these episodes, relatively high levels of DHBV DNA were present (dot blot hybridisation values of at least 3, ie. $\sim 1x10^9$ vge). Five samples, three of which were during peaks of viraemia (days 20, 29, and 41), and two of which were during episodes when dot blot hybridisation negative (days 34, and 39) were sequenced, as well as the liver (day 43). The initial peak at day 20 (16 days post inoculation) was found to only contain wild type virus, while 7 days later the day 29 serum sample (25 days post inoculation) and all subsequent serum samples (days 34, 39, and 41) (30, 35, and 37 days post inoculation respectively) were found to contain the mutation. The liver (day 43) was found to only contain the wild-type virus.

In duck W15 (Day 4 $2.0x10^5$ vge) viraemia was first detected by PCR on day 8 (4 days post inoculation), and by the next bleed (day 11) it was detectable by dot blot hybridisation. Viraemia lasted until day 18 (10 days), and by next bleed (day 20) was both PCR and dot blot hybridisation negative. The liver (day 43) was dot blot hybridisation negative, but PCR positive. Two samples in the peak of viraemia were sequenced (day 13 and 18), as well as the liver (day 43). The mutation was discovered in the day 13 (9 days post inoculation) sample, while both the day 18 (14 days post inoculation and just before clearance) and liver sample (day 43) contained only the wild-type virus.

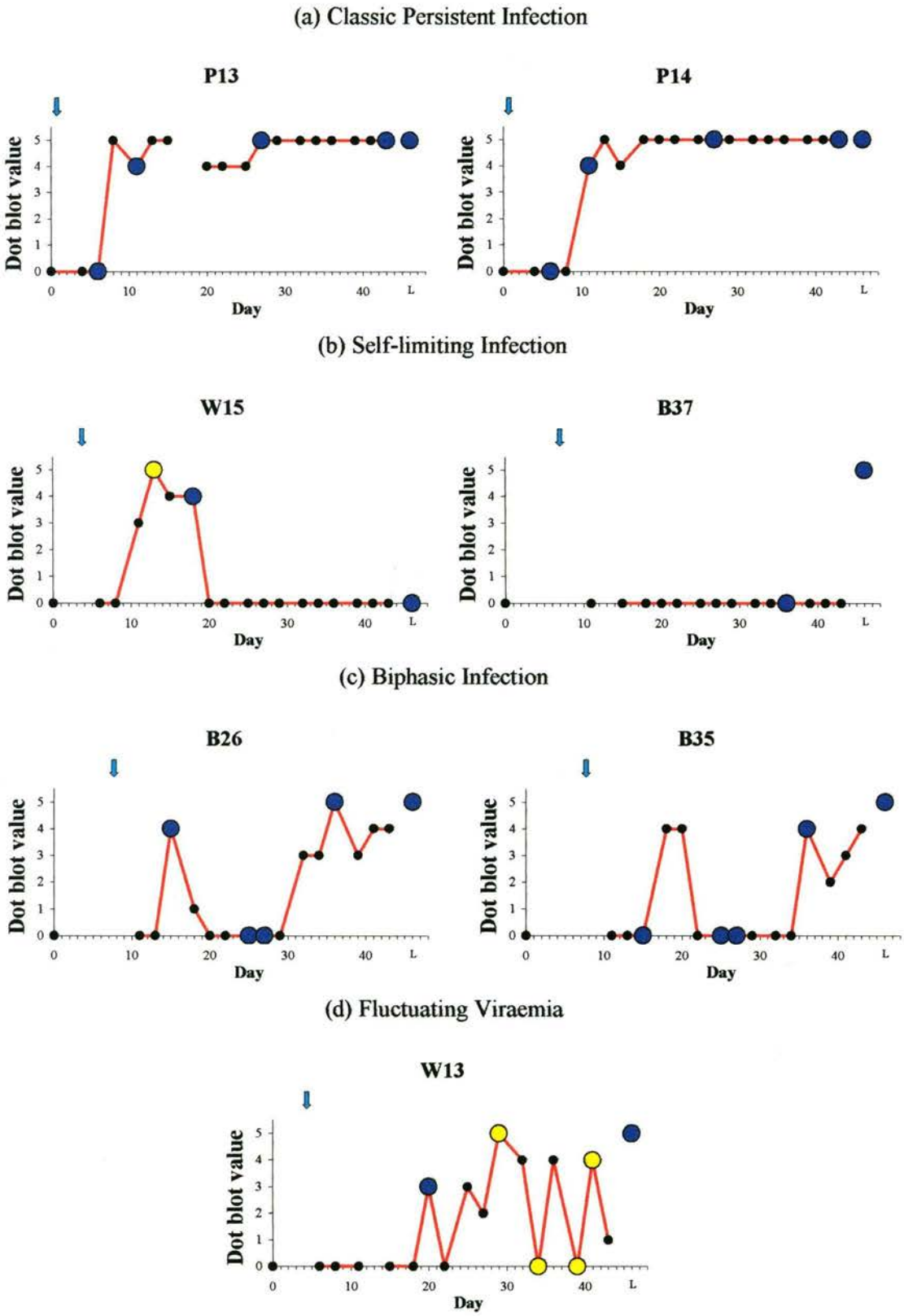


Figure 27. *Results of DNA sequencing from the Persistence-Clearance Experiment.*

Dot blot results for selected ducks from the Persistence-Clearance Experiment. Dot blot results are the numerical value: 0=not detected ($\leq x10^6$vge/mL), 1=$1x10^7$vge/mL, 2=$1x10^8$vge/mL, 3=$1x10^9$vge/mL, 4=$1x10^{10}$vge/mL, 5>$2x10^{10}$vge/mL; L = liver sample. The blue arrow indicates when the ducks were inoculated. Large dots indicate the samples DNA sequenced: Blue= Wild type, Yellow= Mutant.

In an attempt to determine the relative amounts of the wild and mutant virus limiting dilutions (10^{-3} to 10^{-6}) were made and amplified. Even with re-amplification, in both cases where the mutation was found, it was not found as a single predominant species of DHBV, but rather, in conjunction with the wild-type species. Limiting dilutions were not successfully sequenced, so no data is available on the frequency of the mutation in relation to the wild-type population.

4.5.2. Description of Mutation

The double T⇒A substitution at nt 731 and 732 would encode a silent nucleotide change at amino acid 13 (ATT⇒ATA), and a Tryptophan (W) to Arginine (R) substitution at amino acid 14 (TGG⇒AGG) of the Surface protein. Due to the overlapping reading frame this sequence change also affects the Polymerase protein in which a single substitution of Leucine (L) to Lysine (K) would occur at aa 188 (TTG⇒AAG). The mutation is described in more detail in Chapter 6 (p.150).

4.6. DISCUSSION

Samples were obtained from ducks exhibiting the five patterns of viraemia. For each duck individual samples for sequencing were chosen either before or after large changes in the viral DNA level in the serum. It was considered that these large fluctuations could have been the result of the selection of a mutant population that was either rapidly removed or was able to rapidly escape the immune response. The initial inoculum was sequenced several times to determine its composition and the heterogeneity of the viral population within it. There was never any evidence that the initial inoculum contained subspecies of virus, as all of the sequence data was quite clean, but the limited number of samples sequenced means that subspecies comprising less than 5-10% of the population would not be detected.

The stability of the DHBV genome is evident from the limited variation of sequence in the current study. This has also been demonstrated *in vitro* (Stevens *et al.*, 1995), and is similar to neonatal infection in humans (Ridge *et al.*, 1996; Cacciola *et al.*, 2002), or in an immunocompromised host (Samuel and Kimmoun, 2003).

A region of DHBV that has been shown to be highly immunogenic to both the humoral and cellular arms of the immune system is the surface gene (Vickery *et al.*, 1989; Vickery *et al.*, 1999a; Vickery *et al.*, 1999b). The surface gene encodes the surface protein, which would be expected to be under immune pressure from both the adaptive humoral and CMI arms of the immune response. This was the basis for investigating the surface gene for sequence changes. The other region investigated was the beginning of the core gene (preCore), which

is relatively conserved in the avihepadnaviruses and was considered to be stable enough to act as a sequencing control. The region of the preCore that was sequenced included the two Direct Repeats (DR) which although not absolutely essential are required for efficient replication. There were no changes discovered in the preCore region of the DHBV genome in this experiment.

The only mutation discovered in the surface gene was found in ducks (W13 and W15) and is located at nt 731 and 732 which is at the very start of the surface ORF gene (Figure 28 p.131). The overlapping genome of DHBV means that these nucleotides are also translated into the polymerase gene, which might affect the replicative capacity of the mutant genome.

The changes that the mutation would have on the surface ORF protein may decrease the immunological recognition of the protein, which would allow the virus to persist. The changes may also affect the attachment of the virion to the viral cell receptor, as the exact region responsible for attachment has not been fully mapped to the DHBV surface protein. Although a different region has been shown to be important in neutralisation (aa 83-107, as counted from the second ATG in the Surface ORF, or aa119-143 from the first ATG) (Sunyach *et al.*, 1999), this does not exclude an additional role for our mutated region.

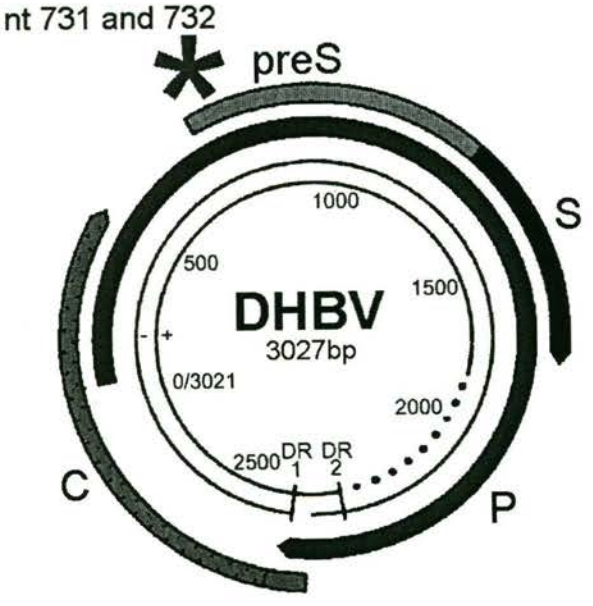


Figure 28. *Location of the mutation in relation to the entire DHBV genome.*
The relative location of the mutation (*) discovered in two ducks attempting to clear the DHBV infection. The mutation is a double substitution of T =>A at nt 731 and 732 affecting both the Surface and Polymerase genes.

The sequence variation of both the self-limiting acute and the fluctuating viraemia indicates that the change of sequence is associated with clearance of the virus, or at least attempted clearance. The exact origin of this mutation is difficult to discover, as direct PCR

sequencing of the initial inoculum was unable to discern any trace of the mutant, however this does not discount the possibility that it was in the starting population. As, even if the mutant was present in as much as 1% of the whole virus population finding evidence of the mutant would require several hundred sequencing reactions which is practically difficult to achieve. The Surface ORF gene of the starting inoculum was sequenced at least twelve times, with no changes discovered, this would indicate that if the mutant was present in the starting inoculum it was present in less than 10% of the total population.

An interesting consideration is that individual hepatocytes of the ducks liver may be co-infected with both the wild type and mutant DHBV genomes. It has been shown that heterogeneous mutant populations simultaneously exist in Korean hepatitis B patients (Keum *et al.*, 1998), and also in persistent infection (Zoulim *et al.*, 1996). As such it would be possible for virions to be produced that contain the less immunogenic surface protein, but the cell would still contain the replication efficient polymerase. More likely the co-infection would lead to the production of a virion that has both mutant and wild type surface antigen. This may lead to a situation in which the wild type antigen may allow antibody attachment but leave enough mutant antigen to bind to the viral cell receptor, and penetrate the cell and continue the infection. This reduced antibody attachment may also lead to antibody-mediated endocytosis, which may then infect immune cells. The mutant virus could not be identified in the liver samples, but these were obtained a week after the mutant was found in the serum. Moreover the pool of DHBV DNA may have contained a large pool of variants, which would make it difficult to identify the presence of a minor species. Preferential export of one or two strains from this quasispecies, would yield a simpler picture in the circulation.

The preCore/Core gene represents what should be a relatively strictly regulated region, which is unlikely to exhibit too much sequence variation. The core gene was used as the basis of a control for the sequencing reaction, and was sequenced using the DHBV PreCf primer. The preCore region was only sequenced in one direction (forward), this was considered sufficient because it was relatively short PCR fragment (256 bp), which was well within the length of what should be clean sequence data.

Sequence variation associated with hepadnaviruses has been observed when they were under various pressures. One type of pressure that hepadnaviruses has been shown to escape from is drug therapy (nucleoside analogues). Drug escape mutants have been seen for human HBV (Bain *et al.*, 1996; Ling *et al.*, 1996; Bartholomeusz *et al.*, 1997; Doo and Liang, 2001; Ono *et al.*, 2001; Lok *et al.*, 2002; Yu and Keeffe, 2003), and are generally associated with the YMDD motif of the polymerase protein, and caused by a small amino acid change (Ling *et al.*, 1996). The same mutational changes in the duck polymerase produce drug escape

mutants that have the same properties as the human equivalents (Fischer and Tyrrell, 1996; Seigneres *et al.*, 2001), again showing that the duck model reflects that which is found in humans (Zoulim *et al.*, 2002). CMI escape mutations are also possible; most acute infections are associated with a multi-specific response, but if the response is much narrower and the virus is able to mutate it may escape the CMI response. Chronic patients that had a narrow CMI response were found to respond well to a wild-type epitope of the PreCore protein; however, when the HBV genomes present in the serum were sequenced it was found that most had mutational changes. The changes found in this region were substitutions that were not as immunogenic as the wild-type in the individuals (Bertoletti *et al.*, 1994).

The selection of a sequence variant is usually the result of selection of an advantageous clone within the quasi-species repertoire. The characterisation of quasi-species in chronic HBV infection is well documented for humans (Dong *et al.*, 2002; Huangfu *et al.*, 2002; Jeantet *et al.*, 2002). The clones present within these quasi-species populations appear to be the result of prolonged persistence with the accumulation of mutations; that provide an immunological advantage. The effect of quasi-species in perinatal infection is not as clear, as it has been observed that HBV genomic heterogeneity may not be primarily involved in the evolution of the infection, or failure of neonatal HBV immunoprophylaxis (Cacciola *et al.*, 2002). The effect of quasi-species during acute human infection is also unclear, as it is difficult to obtain sequential samples soon after infection. These quasi-species are obviously important in DHBV infection, as mutants were observed soon after inoculation, and were associated with attempted clearance.

Immune pressure plays an important role, but even vaccination and the presence of antibodies before infection is not absolutely protective as vaccine associated escape mutants have been discovered (Lu and Lorentz, 2003; Shizuma *et al.*, 2003). These escape mutants can be associated with a little as a single amino acid change (Karthigesu *et al.*, 1994; Yamamoto *et al.*, 1994; Carman *et al.*, 1996). Other antibody escape mutations have been produced in the duck model system in which a neutralising antibody was used to place pressure on the virus (Sunyach *et al.*, 1997), this is similar to the situation in humans that are give prophylactic immunoglobulin (Shields *et al.*, 1999).

The region of the DHBV genome that the mutational changes were found is at the very beginning of the Surface ORF, and also the spacer region of the Polymerase protein, which is in the overlapping reading frame. Further analysis and discussion of the theoretical consequence of the mutations effects are to be described in a later chapter (Chapter 6, p.150).

Understanding the evolutionary process of viral genetic changes would allow us to develop ways to accelerate viral clearance by treatment with novel therapeutic vaccines and/or antivirals and hence to drive this virus to extinction.

The mutation discovered was associated with attempted clearance, which would indicate immune pressure on DHBV by the host. It is interesting to consider that in one duck, (W13), the mutation is associated with a fluctuating viraemia which would indicate several fundamental shifts in the balance between the effectiveness of the immune response and the capacity of the virus to avoid the response. The second occurrence of the mutation was associated with Duck W15 in which the mutation appears during the initial rise of viraemia only to be replaced by the wild-type just before clearance. The mutational changes in the DHBV also affect the polymerase protein, which may effect the replicative capacity of the virus. It would be possible to determine if this mutation, which was selected by the host response to the infection affects the ability of Duck Hepatitis B Virus to survive in the host and to spread from duck to duck.

Because the mutant was associated with attempted clearance and was also absent from the liver, the next experimental stage was to determine the replication competency of the mutant genome in relation to the wild-type genome, by *in vivo* passaging.

5. Study of the infectivity of DHBV variant by Serum Transmission, and Direct DNA Injection

5.1. Introduction

The serum of hepadnavirus infected hosts can consist of quasispecies, in which more than one type of virion, is being produced by the host, at the same time. The occurrence of quasi-species is usually associated with persistent infection in which small mutations are accumulated over time. Results from the previous chapter indicate that the serum of infected ducks can consist of DHBV quasispecies of both wild-type and mutant genomes, and the appearance of quasi-species occurs soon after inoculation. The replicative capacity of many mutant genomes has been shown to be lower than that of the wild-type, and is not preferentially selected, except when under immune pressure.

Three different methods of initiating studies of viral variation are in common use: direct serum transmission, cloning, and direct DNA injection. Direct serum transmission is perhaps the simplest and most effective at examining the overall *in vivo* difference. From such studies the interactions of the complex biological systems can be observed as a whole. While serum transmission would be a more natural infection, the serum used may contain many quasi-species which would affect the immunological and replication capacity of the infection. Cloning and expression of the mutant proteins allows the individual components to be investigated, such as the effect that the mutation would have on the polymerase protein, if it affects initiation, elongation, etc. A curious phenomenon that has been observed is that injecting DNA directly into cells can transform the cells, and they can start to produce the encoded protein/s, with transformation of bacterium being known for a long time (Griffith, 1928). This has been shown to function for several proteins at a time, and eventually a whole productive viral infection was achieved by direct DNA injection of a complete

hepadnavirus genome (Will *et al.*, 1982). Thus the directly injected DNA was able to transfect the hepatocytes, which produced all of the required viral proteins to form infectious virions.

Hepadnavirus patent infections have resulted from ligation of a full length genome to itself which forms a covalently closed circular genome (similar to the bacterial plasmid) (Will *et al.*, 1985), or ligation to another full length genome to produce a dimer (Will *et al.*, 1983), of which a head to tail dimer will contain at least one complete copy of every gene.

HBV infection from direct DNA injection has been achieved in chimpanzees. Both dimerised and closed circular DNA of three different serotypes was injected intravenously, directly into the liver, and intramuscularly into a single chimpanzee, producing typical, mild self-limited, acute hepatitis. Development of HBsAg, HBeAg, and HBcAg antibodies was detected with usual kinetics. HBV DNA was detected in both the liver and serum during the acute phase of infection, and found to have similar restriction digestion patterns to the mixture inoculated. The DNA extracted from the liver differed significantly, when compared by southern blot analysis, to that of the material injected, indicating selective replication (Will *et al.*, 1982).

Direct DNA injection has not only been shown to produce DHBV infection *in vitro* (Yang and Summers, 1998), but also *in vivo* and *in vivo* recombination (Sprengel *et al.*, 1987). Again both dimerised and closed circular DNA were used, and both produced active infection. Restriction analysis showed that the progeny virus had the same pattern as the injected head-to-tail cloned dimer, and as the naturally occurring DHBV on which the cloned material was produced. The infectivity of the virus was tested by injection of the serum of the transfected ducks into naive ducklings, which also became infected, proving that the clone produced replication competent progeny virus *in vivo*. Dot blot and southern blot were used to analyse the liver and showed that cloned DHBV DNA had initiated a normal replicative cycle. The morphology of the natural and cloned viruses was also indistinguishable (Sprengel *et al.*, 1984).

The molecular methods usually utilised for study of genomes require insertion of viral DNA into bacteria. This has many consequences: 1) firstly the DNA itself is slightly different from that found in eukaryotic cells in that it is methylated, which may change the physical shape of the DNA and thus affect regulatory properties, 2) the actual structure of the DNA is different because usually a linear strand of DNA is inserted into a plasmid, and this lacks many of the physical characteristics of virion encapsidated DHBV DNA, such as the

covalently linked terminal protein, and the nick-gap structure, and 3) it is devoid of associated proteins which may affect packaging.

Direct DNA injection provides a means in which a pure population of virus may be used to infect a host. In this study we used serum transmission and direct DNA injection to determine the relative replication and possibly immunologic efficiency of the wild-type and naturally occurring mutant versions of DHBV (Chapter 4, p.123), in baby ducks.

5.2. AIM

We hypothesise that the naturally occurring mutant is less able than the wild type to replicate *in vivo*, but that this does not preclude infectivity.

(1) To compare the transmissibility and kinetics of infection of the wild type virus with that of the naturally occurring mutant virus *in vivo*.

This would be achieved by:

(a) Passaging serum containing a mixture of the wild type and mutant virus and determine if this alters the outcome of infection.

(b) Producing an infectious PCR product of the wild type and mutant DHBV genome, which will allow the passage of the single species (wild type, or mutant) of virus to determine its replicative efficiency.

(c) Determining if mutations selected by the host response to infection affect the ability of DHBV to survive in the host and to spread from duck to duck.

5.3. MATERIALS AND METHODS

5.3.1. Production of a Full length infectious DHBV PCR Fragment

Full length PCR amplification was carried out as previous (2.2.2.1, p.69), using primers DHBV_C2fP, and DHBV_CrP, which were 5' phosphorylated to enable ligation. This PCR reaction produces a full length copy of the DHBV genome. When ligated to either itself or other fragments it produces circular monomers, dimers, or multimers; of which approximately half should be head to tail dimers that contain a complete Open Reading Frame of all DHBV proteins. The PCR was performed on Phenol / Cholorform extracted serum, which was either used neat or diluted between 1:10 and 1:1000, such that it produced a bright distinct band without excessive smearing. The wild type DHBV PCR fragment was

obtained from the DHBV051094 serum pool, while the mutant virus PCR fragment was obtained from duck W13 (Day 4 $2.8x10^4$ vge) serum sample of day 29 (see Figure 30, p.139).

Eight 25µL full length PCR reactions were set up (2.2.2.1, p.69). The reactions were pooled, divided into 4 tubes, and PEG precipitated (2.2.2.5, p.71) (Figure 29, p.138). Upon electrophoresis a 3kb fragment was produced as expected (Figure 29, p.138). Sequencing of this fragment was found to contain either pure wild type or mixture of mutant and wild type as originally seen (4.5.1, p.128). The pellets were then resuspended in 10µL of Klenow reaction mixture (Table 34, p.138), and incubated at 30°C for 15mins. The four tubes were re-pooled and split into 8 tubes of 5µL each, 5 µL of ligation reaction mixture added (Table 34, p.138), and incubated at 4°C or 15°C for 24hrs or 8hrs, respectively.

1x Klenow	Vol (µL)
10x Buffer	1
Klenow (5U/µL)	1
dH_2O	8

1x Ligase	Vol (µL)
10x Buffer	1
T4 DNA Ligase (400U/µL)	1
dH2O	3

Table 34. *Klenow and Ligase Reaction Mixture.*

The eight tubes were re-pooled, and several DNA species were seen following electrophoresis on an agarose gel (Figure 29, p.138). The original 3kb unligated fragments remain, while new 6, and 9kb fragments representing dimers, and trimers can been seen, as can a heavy smear near the well, indicating multimers. Also seen are smaller bands that may represent circular monomers and supercoiled circular monomers. The DNA concentration of the wild type and mutant multimer mixture was found to be 12.63mg/mL (1.01mg/80µL), and 11.50mg/mL (0.92mg/80µL), respectively, as determined by spectrometry (2.2.4, p.73).

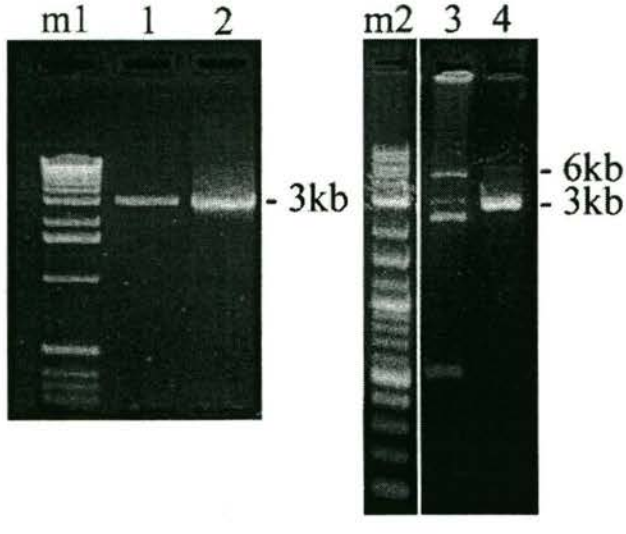


Figure 29. *Full length PCR product and Multimer mixture.*
m1: marker1, 1: Peg purified, 2: Full length PCR product, m2: marker2, 3: Multimer mixture, 4: Peg purified PCR product.

5.3.1.1. Injection of DHBV DNA

Fifty micrograms of dextran sulphate was added to 50μg of the multimer mixture and made up to 200μL with PBS. This was directly injected into three sites of the day old duckling liver, using a 1mL syringe with a 26G needle. This was equivalent to approximately $1x10^{13}$ vge.

5.4. EXPERIMENTAL PROTOCOL

5.4.1. Serum Passage Experiment

Serum from the Persistence/Clearance experiment - Chapter 3 (p.98) was directly passaged into ducklings. The wild type and mutant viruses were passaged directly by inoculation of ducklings with the serum containing both the wild-type and mutant genomes. Due to the limited amount of serum available; three samples from ducks W13, and W15 (Chapter 3, p.98), were selected: one wild type and two mutant. Serum from duck W13 (Day 4 $2.8x10^4$ vge) on day 20 (found to only contain wild type virus), and on day 34 (found to contain the mutant virus), and serum from duck W15 (Day 4 $2.8x10^4$ vge) on day 13 (mutant virus). The serum selected to be passaged relative to the viral kinetics of infection is highlighted (Figure 30, p.139).

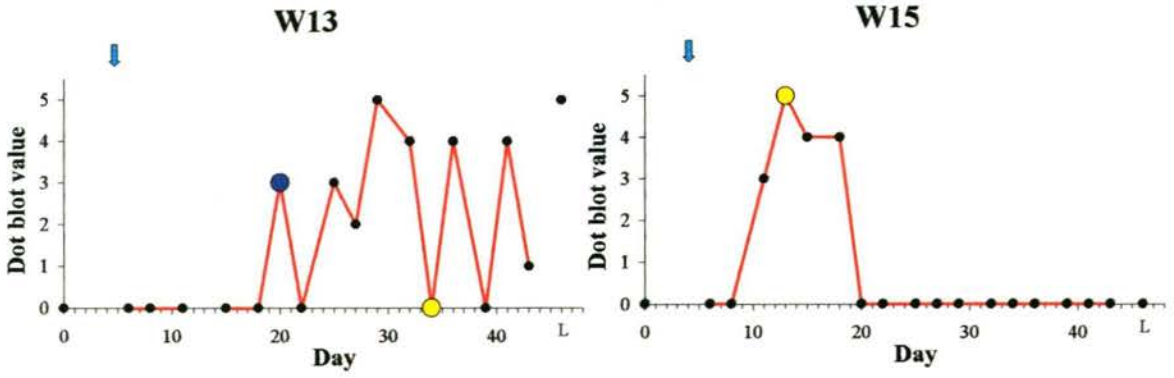


Figure 30. *Passaged serum samples relative to viral kinetics of infection.*
Blue= Wild type, Yellow= Mutant.

Two ducklings for each group were *intraperitoneally* injected on day 1 with 10μL of the original serum which was diluted with PBS to 200μL (Table 35, p.140). Serum, liver, and other organs were obtained on day 28, and were extracted for PCR analysis and sequencing.

The ducks of the Serum Passage experiment were kept for 1 month (28 days) and bled 9 times throughout this period (days 0, 4, 7, 11, 14, 18, 21, 25, and 28). Liver, spleen, pancreas, and kidney samples were obtained at euthanasia. Both serum and organ samples were subjected to dot blot hybridisation and PCR (both preS-S, and preC). Sequence data was also obtained from selected samples.

Original duck	Day	Type	vge	Ducks	Number
W13	20	wt	$2x10^6$	2	W81, W82
	34	mut	$<2x10^5$	2	B40, B47
W15	13	mut	$5x10^7$	2	G86/92, G94

Table 35. *Ducklings of the Serum Passage experiment.*

wt: wild type. mut: mutant virus. vge: viral genome equivalents injected into ducks. Both W13, and W15 were Day 4 $2.8x10^4$ vge ducks.

5.4.2. DirectDNA1 experiment

The directDNA1 experiment was performed on 4 ducks (2 wild-type, 2 mutant) (Table 36, p.140). The ducklings were injected with 50μg of DNA (as per 5.3.1.1, p.139), and euthanased 14 days later, when both serum and liver samples were obtained. The DNA from the serum and liver were extracted for dot blot hybridisation, PCR analysis, and sequencing.

Batch	Type	Ducks	Number
DirectDNA1	wild type	2	RH
			RB
	mutant	2	BH
			BB

Table 36. *Ducklings of the DirectDNA1 experiment.*

Note DirectDNA1 Transmission experiment involved the inoculation of serum from DirectDNA1 ducks (RH, RB, BH, and BB) into three 1 day old ducklings each (5.4.2.1, p.140).

5.4.2.1. Passage of Serum from DirectDNA1 (DirectDNA1 Transmission experiment)

The DirectDNA1 Transmission experiment involved the serum from the DirectDNA1 experiment ducks, which was passaged into naïve 1 day old ducks. For each of the four DirectDNA1 ducks (RH, RB, BH, and BB), three naïve ducks were *intraperitoneally* injected on day 1 with 100μL of serum from day 14 of the DirectDNA1 experiment (Table 37, p.140). Two positive control ducks were injected with pooled DHBV positive serum, and two negative ducks were injected with PBS). Serum and liver samples from these ducks were obtained at day 14, and subjected to dot blot hybridisation, and PCR (both preS-S, and preC). Sequence data was also obtained from selected samples.

Batch	Type	Serum	Ducks	DirectDNA1 Transmission
DirectDNA1	wild type	RH	3	RH1, RH2, RH3.
		RB	3	RB1, RB2, RB3.
	mutant	BH	3	BH1, BH2, BH3.
		BB	3	BB1, BB2, BB3.
	positive	-	2	pos1, pos2.
	negative	-	2	neg1, neg2.

Table 37. *Ducklings of the DirectDNA1 Transmission experiment.*

Note Serum transmission of DirectDNA1 ducks involved the inoculation of serum from DirectDNA1 ducks (RH, RB, BH, and BB) into three 1 day old ducklings each (5.4.2.1, p.140).

5.4.3. DirectDNA2 experiment

The directDNA2 experiment was performed on 14 ducks (10 wild-type, 4 mutant) (Table 38, p.141). Essentially this experiment was a repeat of the DirectDNA1 experiment with larger numbers of ducks. The ducklings were treated as per the DirectDNA1 experimental protocol (5.4.2, p.140), the same multimer mixture was used as previous, it was stored at –20°C, as the *in house* PCR protocols restricted storage of the mixture to the PCR room where a –70°C freezer was not available.

Batch	Type	Ducks	Number
DirectDNA2	wild type	10	dd2A, dd2B, dd2C, dd2D, dd2E, dd2F, dd2G, dd2H, dd2I, and dd2J.
	mutant	4	dd2O, dd2P, dd2Q, and dd2R.

Table 38. *Ducklings of the DirectDNA2 Transmission experiment.*

5.5. RESULTS

5.5.1. Passage of DHBV by serum

Of the six ducks in the Serum Passage experiment (Table 35, p.140), three died prematurely. Duck W82 died on day 3 of no definable cause and most likely a genetic defect. Ducks B40, and G94 died on day 18, also of no definable cause. Liver samples for each of these three ducks were still obtained. The dot blot and PCR data for the Serum Passage experiment have been graphed (Figure 31, p.142).

Several samples from ducks B47 (W13 mut), G86/92 and G94 (W15 mut) were sequenced (Figure 31, p.142), all were found to be wild type. Although W82 was found to be PCR positive in the liver, no sequence data could be obtained.

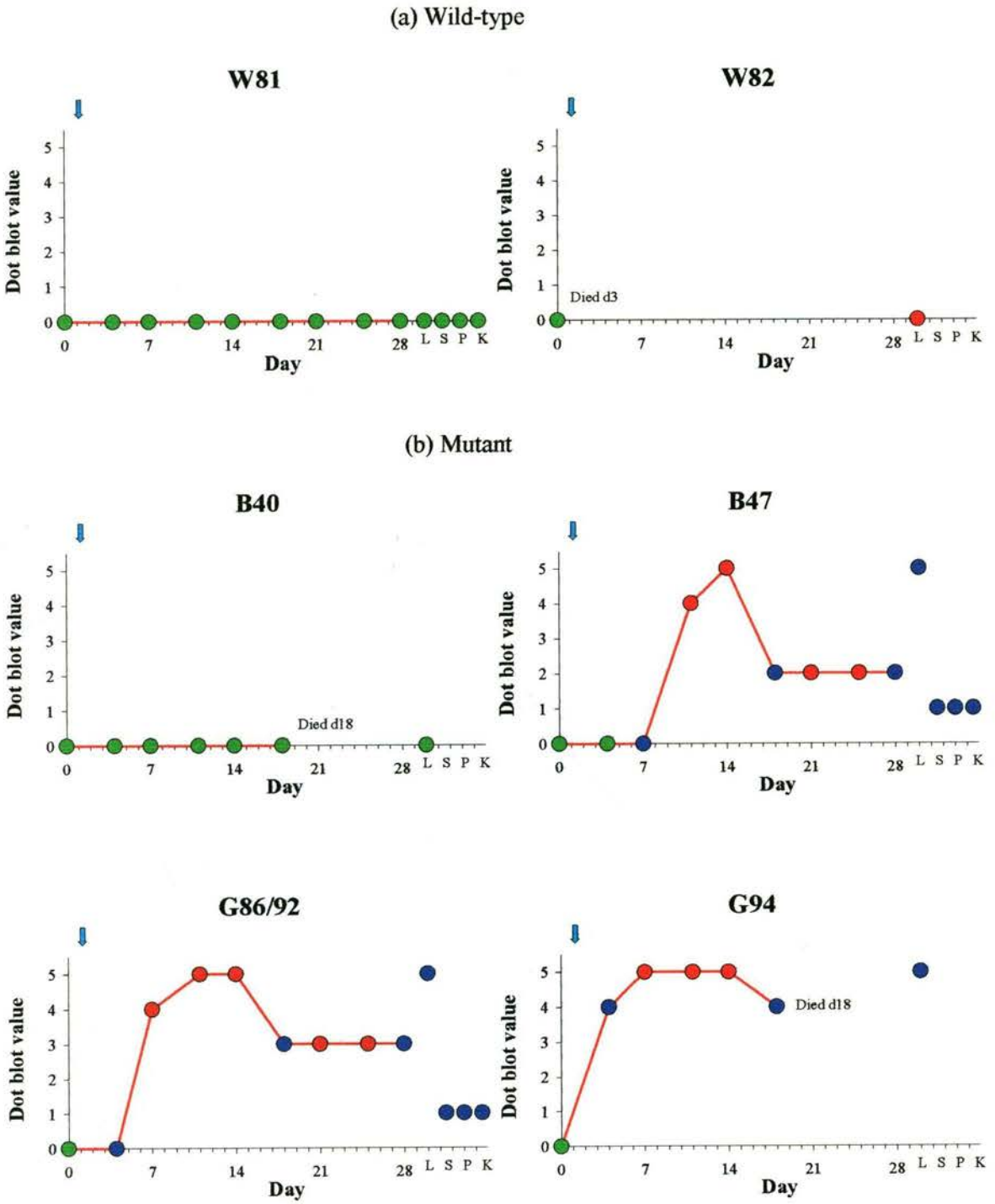


Figure 31. *Graphic results for the Serum Passage experiment ducks.*

Dot blot results are the plotted numerical value. PreS-S PCR results are indicated by data points: green= negative, red= positive. DNA Sequencing results are indicated by the Blue dots, all samples tested were wild-type. L= liver, S= spleen, P= pancreas, K= kidney. Ducks W81 and W82 were injected with wild type serum while ducks B40, B47, G86/92,and G94 were injected with mutant serum (Table 35, p.140).

5.5.2. DirectDNA1 experiment

Only one duck from the DirectDNA1 experiment was dot blot hybridisation positive: duck RH. The PCR results for the liver and serum of the DirectDNA1 batch are summarised (Table 39, p.143).

Original Duck	Duck	Dot blot		PreC PCR		PreS-S PCR	
		Serum	Liver	Serum	Liver	Serum	Liver
Wild type	RH	+	+	#	#	+	+
	RB	-	-	#	#	-	-
Mutant	BH	-	-	#	#	-	-
	BB	-	-	#	#	-	-

Table 39. *Summary of results for the DirectDNA1 experiment.*
PreC PCR produced multiple bands, which could not be interpreted.

Curiously, the PreC PCR produced multiple bands for all of the DirectDNA1 experiment ducks (Figure 32, p.143); bands of various sizes were observed (100-200, ~450-500, ~650-800, and ~1000bp). The positive control produced the expected clean band at approximately 304bp.

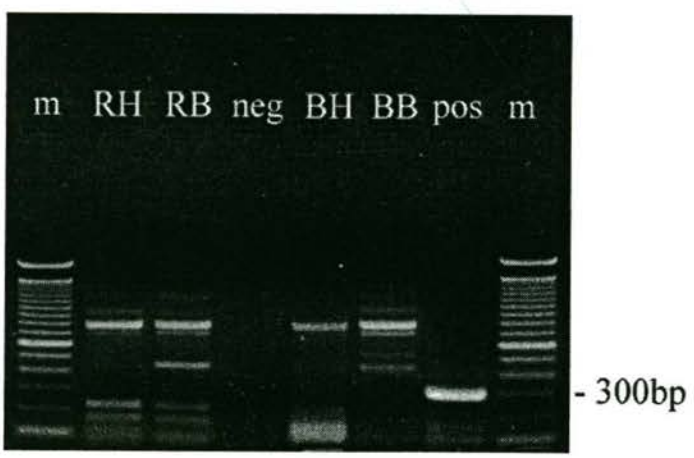


Figure 32. *Example of the multiple banding seen in the PreC PCR for the DirectDNA1 experiment.*
neg: DHBV negative duck serum. pos: DHBV positive duck serum producing a 304bp PCR product.

Sequence data from the PreS-S region was obtained from serum and liver of duck RH, and was shown to be the wild type virus. No sequence data were able to be obtained from the PreC PCR reactions, even though several bands were cut out of the gel.

5.5.2.1. DirectDNA1 transmission experiment

Serum from each of the four ducks of the DirectDNA1 experiment was injected intraperitoneally into three ducklings. The results for ducks used in the DirectDNA1 passage experiment are summarised (Table 40, p.144).

		Dot blot		PreC PCR		PreS-S PCR	
Original Duck	Duck	Serum	Liver	Serum	Liver	Serum	Liver
RH	RH1			-	-		
	RH2	-		-	-		
	RH3			-	-		
RB	RB1	-	-	-	-	-	
	RB2	-		-	-		
	RB3	-	-	-	-	-	-
BH	BH1	-	-	-	-	-	-
	BH2	-	-	-	-	-	-
	BH3	-	-	-	-	-	-
BB	BB1	-	-	-	-	-	-
	BB2	-	-	-	-	-	-
	BB3	-	-	-	-	-	-
-	neg1	-	-	-	-	-	-
	neg2	-	-	-	-	-	-
	pos1						
	pos2						

Table 40. *Summary of the DirectDNA1 passage experiment.*
PreS-S DNA sequencing data obtained.

All of the PreC PCR were negative, except for pos1, and pos2 ducks. There was no indication of multiple bands as found in the original DirectDNA1 experiment.

The sequence data for the DirectDNA1 passage experiment was shown to be only wild type DHBV. No sequence data was available for RB1 liver, and RB2 serum.

5.5.3. DirectDNA2 experiment

The DirectDNA1 experiment was repeated with a larger number of ducks (Table 36, p.140). The same multimer mixtures were used, they had been stored at –20°C as no lower temperature freezer was available. All ducks in the DirectDNA2 experiment were dot blot hybridisation negative for both serum and liver. Only one duck was found to be PCR positive (dd2R), and it was only positive for the PreS-S PCR. Unfortunately, no sequence data was able to be obtained from this sample.

5.6. DISCUSSION

Several examples of human hepatitis B virus strains with enhanced replication *in vitro* have been described, but whether this characteristic is a general phenomenon of the hepadnaviruses is unclear. In this study we compared the infection kinetics of a naturally occurring mutant with that of the wild type of the closely related duck hepatitis B virus. *In vivo* the variant was quickly outcompeted by the wild type even with the immature immune response.

The passage of DHBV by serum experiment included inoculating four ducks with serum that was known to contain a combination of wild type and mutant virus. Three of the four ducks developed a high-level viraemic infection, which when sequenced was found to only contain the wild-type form of the virus. The mutant form of DHBV was unable to e stablish an infection as a single dominant species, which would indicate that the wild-type has a much better complete package that is capable of establishing and maintaining a DHBV infection.

The stability of the DHBV genome is again evident from the passage of DHBV by serum study in which all of the sequence data obtained was again wild type. Other studies of hepadnaviruses have shown that reversion to more replicative efficient genomes happens quickly. An example of reversion can be seen in experiments involving the Direct Repeats, which produce aberrant replication when the 5' DR is eliminated (Loeb *et al.*, 1991). However, if the 3' DR is eliminated it was shown to rapidly convert to wild type (Condreay *et al.*, 1992). This apparently occurred as a consequence of conversion of newly synthesised Relaxed Circular to cccDNA, which might then serve as a template for the synthesis of wild type viral RNAs.

The preCore mutant hepatitis B virus often emerges from a mixed infection with combined wild type and preCore mutant viruses, but mutant does not seem to be an evolutionarily favoured strain. Competition between an e antigen-defective mutant and wild type DHBV found that the preCore mutant replication was less active than wild-type duck hepatitis B virus, and it could be overgrown by wild-type virus during the course of coinfection (Chuang *et al.*, 1994).

Study of a DHBV variant that had enhanced levels of cccDNA accumulation, was shown to be cytopathic *in vitro*, similar to a human HBV mutation species. *In vivo* liver damage caused by this variant (G133E) occurred only during the first 2 weeks *pi*, after which time cccDNA levels and liver histology returned to near normal despite continued virus replication (Lenhoff *et al.*, 1999). A shift from mutant to wild type infection has been seen in a mixed infection of ducklings with G133E and a small amount of wild-type virus, the

wild-type virus was detected as the predominant genotype after recovery of normal liver histology. Recovery from liver damage in G133E-infected ducklings was due to the emergence of spontaneous noncytopathic revertants rather than to host suppression of virus cytotoxicity (Lenhoff *et al.*, 1998). Acute liver injury may result from infection with a cytopathic hepadnavirus but that such viruses may be rapidly replaced by noncytopathic variants during persistent infection.

The frequency of revertants was found to by mixing the cytopathic virus with known amounts of a genetically marked wild-type virus, which was injected into ducklings. Virus outgrowth was accompanied by a co-selection of wild type and spontaneous revertants during recovery of the ducklings from the acute liver injury caused by death of the G133E-infected cells. The frequency of individual revertants in the selected noncytopathic virus population was estimated by determining the ratio of each revertant to the wild-type virus. Spontaneous revertants were found to be present at frequencies of 1 to 6 $x10^{-5}$ per G133E genome inoculated (Pult *et al.*, 2001a), and a mathematical model was used to estimate that the mutation rate was 0.8 to 4.5 $x10^{-5}$ per nucleotide per generation. If this data is accurate for all other forms of reversions then it is most likely that the majority of the outgrowth that we observed was due to the selection of the wild type virus.

The failure to consistently produce a productive infection by direct DNA infection is summed up by the dot blot positive infection that produced multiple bands for the PreCore PCR. There is no evidence that there was a problem with the PCR assay as the positive, and negative controls were as expected, and other samples run at the same time (data not shown) were also shown to either be negative or have a single tight band. The multiple bands of the PreCore PCR of the DirectDNA1 ducks can be accounted for by non-optimal priming by the forward primer at various sites of the DHBV genome (Figure 33, p.147).

When the ligated full length DNA mixture enters the hepatocyte, in theory the DNA starts to produce an infection. This infection should be similar to the natural and experimental infection produced by virions. But as has been seen for other DHBV research, DNA recombination does occur and may have produced some form of defective genome, which leads to ineffective infection. Yang and Summers have shown "illegitimate replication" in which linear hepadnavirus DNA in primary hepatocyte cultures efficiently participates in nonhomologous recombination at its ends (Yang and Summers, 1995). The products of this recombination are (a) monomeric covalently closed circular DNAs (cccDNAs) with deletions and insertions around the site of joining, and (b) oligomeric forms in which monomers are joined near the ends in random orientation. Further research, utilising linear DHBV DNA with engineered insertions, demonstrated that they could infect hepatocytes *in*

vivo, and that these hepatocytes proceeded to carry out illegitimate replication (Yang and Summers, 1998). The PreCore region of the DHBV has been shown not to be essential for viral replication (Chang *et al.*, 1987). If recombination altered one of the primer sites for the preCore PCR (by as little as a single nucleotide at the end of the primer), then it would explain the lack of the normal 304bp PCR fragment, and at the same time it is possible that non-optimal priming may occur producing the multiple bands seen in the PCR reaction (Figure 32, p.143). The difference in the multiple bands seen in the DirectDNA1 ducks may be due to random priming early in the reaction, which is multiplied by amplification during cycling. A small change to the nucleotide sequence may allow replication, producing a dot blot hybridisation positive infection, which is also preCore PCR negative, because the primers do not recognise the sequence.

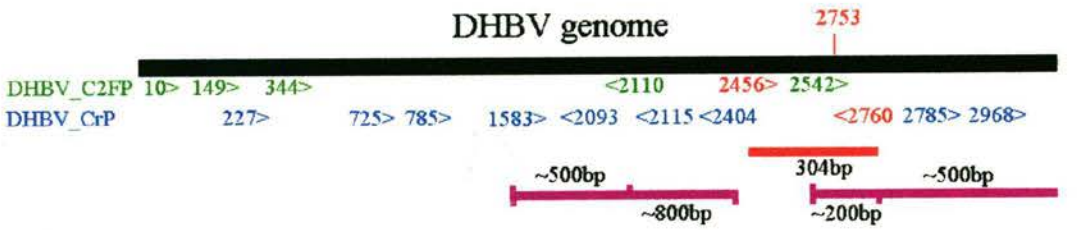


Figure 33. *Schematic of non-optimal PCR priming for the PreCore PCR assay.*
Sites listed are where the last 5 bases of the 3' end of the primer and the DHBV genome are exact. **Red**: normal priming and PCR fragment. **Magenta**: possible aberrant fragments (a tail to tail dimer would produce a ~1kb fragment from site 2542; other dimer forms would only produce fragments larger than observed). 2753: where the full length PCR starts and ends.

Duck RH, produced a dot blot positive infection that also had multiple bands for the PreCore PCR, and when serum from this duck was passaged, it again produced a dot blot positive infection in 3/3 ducks (RH1, RH2, and RH3). However in the passaged ducks the infection was unable to produce any PreCore PCR positive bands. This would indicate that some form of defective replication was being carried out. It may be as simple as a change in the PreCore PCR priming site, and this mutation may lead to inefficient replication or a virus that is not able to infect new hepatocytes as well as the wild type. PreCore deficient mutants of DHBV have been produced and are replication competent, albeit at a reduced rate, and the current results appears to add evidence that a wild type preC region is not required for replication.

In the next chapter we investigate the theoretical implications of the mutation on replication, and the Surface protein, which would play a large role in the immune response to the mutant virus.

5.7. SECTION I OVERVIEW

When neonatal ducks are injected at 1, 4, and 7 days of age, five patterns of viraemia are evident: classic persistence, self-limiting acute, biphasic, fluctuating, and non-viraemic.

The variable outcomes may be due to the balance of the immune response and viral replication. Non-specific immunity is the main contributing host response in the first few days of infection. After about a week, specific immune mechanisms should be actively contributing to the immune response.

The biphasic pattern was only seen in ducks when injected at 4, or 7 days of age, and was associated with an unsuccessful attempt at clearance of the virus. The biphasic pattern consisted of an initial spike of viraemia, in which viral DNA was only present for a few days, followed by a period of low level viraemia (which was only PCR detectable), lasting for about a week, after which viraemia rebounded to previous high levels. The biphasic pattern is associated with reduction and subsequent rebound of viral DNA in the serum of several orders of magnitude, within a few days.

The rebound of viral DNA in the biphasic pattern is in the presence of the specific host immune response.

Key effectors of clearance may be to specific epitopes of DHBV.

The DHBV genome is highly conserved, with almost no change in the sequence throughout the course of infection. However, a double T⇒A substitution mutation at nt 731 and 732 was found to be associated with two ducks that either cleared or were attempting to clear the DHBV infection. This mutation affects both the surface ORF and the polymerase protein.

The unsuccessful clearance attempt, in which the mutation was observed, consisted of several episodes. In each episode, the level of viral DNA in the serum increased, and subsequently decreased by several $\log_{10}$ within a few days. Indicating several shifts in the balance of the immune response, and viral replication. The second observation of the mutation, was seen in a self-limiting acute infection, in which the mutation was present during the initial viraemia, but absent just before clearance from the serum.

Attempts to transmit this mutation to baby ducks either by inoculation of serum, or by direct DNA injection were unsuccessful.

Injection of mixtures of the wild-type and mutant virions, produce an infection of purely wild type virions, suggesting that the mutant genome is not as replication efficient as the wild type.

The lack of a detectable preCore region in the directDNA experiments confirms previous evidence that the preC region is not essential for replication.

Section II

6. THEORETICAL MODELLING OF THE DHBSAG

6.1. AIMS

(1) To identify putative antigenic epitopes on the Surface ORF gene of DHBV and select the optimal fragments (peptides) for use in a lymphoblastogenesis assay.

(2) To model the difference between the wild type and the mutant virus described in Chapter 4.

(3) To compare the putative DHBV epitopes with those described for other hepadnaviruses

(4) To examine the similarity of the selected peptides to known proteins.

(5) To examine the possible effect of the mutation on the replicative capacity of the mutant virus.

6.2. EXPERIMENTAL DESIGN

The nucleotide sequence of the Australian DHBV strain was used for the Surface ORF gene and the Polymerase gene. Two forms of the genes were translated into their respective proteins; the wild type and the mutant form (T⇒A double substitution mutation at nt 731 and 732), described in Chapter4 (p.123) were used for modelling purposes.

Several computer programs were used to determine models of the Surface ORF protein in terms of the Antigenic Index, Hydrophilicity, and Surface Probability. Similar models have been utilised in the study of HBV (Lambert *et al.*, 1990; Berting *et al.*, 1995). From these models the Surface ORF protein was divided into smaller peptides of 15-20 amino acids, for use in the lymphoblastogenesis assay.

The same parameters were also used to analyse possible effects of the amino acid substitution on the sAg of the mutant protein.

The peptides were then placed into several sequence similarity matching programs to seek any sequence homology with all other known proteins.

The mutation was also mapped onto the Polymerase gene to determine what, if any, effect the mutation might have on its function.

6.3. MATERIALS AND METHODS

6.3.1. Sequence Source

The nucleotide source sequence was obtained from the NCBI GenBank (accession number AJ006350) (Triyatni *et al.*, 2001). A second sequence was produced from the original wild type by changing nucleotides 731 and 732 from T to A to produce a mutant genome. The location of the proteins and the mutation can be seen in Figure 34 (p.151).

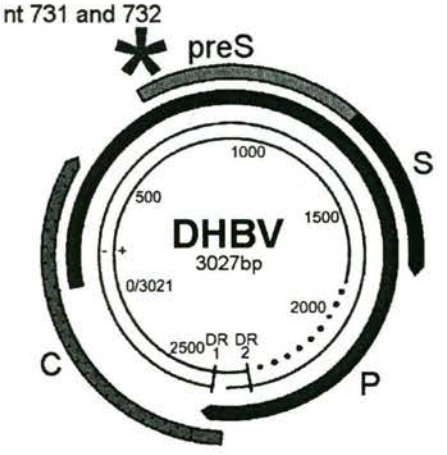


Figure 34. *DHBV genome showing location of the proteins and the mutation.*

The translated proteins were obtained by use of the computer program Flip ORFs (ANGIS), which translates locates ORFs by finding regions that code for at least 20 amino acids in a row. The DNA sequence was translated into a protein sequence, by the computer program Translate (GCG), which uses a codon translation table to convert the three nucleotide codon sequence into the protein sequence. The Surface ORF protein was translated for both the wild type and mutant form of DHBV.

6.3.2. Theoretical Modelling

To assess the secondary structure of both the wild type and mutant forms of the Surface ORF proteins, several algorithms were used. PeptideStructure (GCG), uses the original Chou-Fasman method to predict helices, sheets, and turns (Chou and Fasman, 1978). It resolves overlapping regions of alpha-helices and beta-sheets with the overall probability procedure introduced by Nishikawa (Nishikawa, 1983). This same procedure also locates turns that are not in conflict with other secondary structures. The Chou-Fasman rules are slightly modified

as follows: Sheet: a minimum length of five residues is required. Secondary structure was also predicted according to a slightly modified method of Robson-Garnier, in which the minimum length of an alpha-helix was six and of a beta-sheet, four (Garnier *et al.*, 1978). Regions without adequate predictions are replaced by the conformational state of the next best probability.

6.3.2.1. Hydrophilicity

Hydrophilicity values for individual amino acids were calculated using the well-established algorithm (Kyte and Doolittle, 1982). The algorithm was used to assess the hydrophilic character of individual amino acids from the target sequence with a method that utilises predetermined hydrophilicity values for individual amino acids based upon water-vapour transfer free energies. It also uses empirical data based on the partitioning of individual amino acids to the exterior of the proteins with known structures. The aggregation of non-polar side chains in the interior of a protein is favoured by the increase in entropy of the water molecules that would otherwise form ordered "cages" around the hydrophobic groups. The greater the hydrophilicity of a side chain, the more likely it is to occupy the exterior of a protein and vice versa. A window of 7 residues was used to lower the noise without smoothing out significant peaks. This effect is the major determinant of native protein structure.

Two computer programs PeptideStructure (GCG) and Grease (Pearson and Lipman, 1988) were used to obtain the hydrophilicity results, and the results averaged.

6.3.2.2. Surface Probability

The propensity of amino acids to reside exposed on the surface of the protein was modelled using the Emini algorithm (Emini *et al.*, 1985), which was developed to assess surface probability. Predictions are based on values for individual amino acids that have in turn been derived from experimentally determined side-chain solvent accessibility values (Janin and Wodak, 1978).

6.3.2.3. Antigenicity

The antigenic index (AI) is a measure of the probability that a region is antigenic. Antigenicity is related to peptide surface features that are hydrophilic and have a high degree of exposure to the surrounding aqueous fluid. These regions have a high number of turns. It combines weighted measures of several predictions of secondary structure: hydrophilicity, surface probability, flexibility, Chou-Fasman values (Chou and Fasman, 1978), and Robson-Garnier values (Garnier *et al.*, 1978). The output of the algorithm is the result of a linear antigenic surface contour of the protein (Jameson and Wolf, 1988).

6.3.2.4. Sequence Similarity Searching

Several computer programs were used for searching sequence databases for similar sequences. BlastP (Altschul *et al.*, 1997), was used to search a protein sequence database with a protein query sequence, while PSI-Blast (Altschul *et al.*, 1997) was used to search for distant protein homologs in a sequence database by iterated profile search. The FastA (Pep) computer program (Pearson and Lipman, 1988) scans a protein or nucleotide sequence database for sequences similar to the input sequence. Ssearch (Pearson and Lipman, 1988) searched a sequence database with a query sequence.

6.4. RESULTS

6.4.1. Determining Regions of Theoretical Antigenicity and selection of peptides

Graphs of antigenicity, hydrophilicity and surface probability were produced (Figure 35, p.154). The peaks of antigenicity, hydrophilicity, and surface probability from the computer modelling output were correlated to estimate regions of high immunogenicity, and used to divided the Surface ORF into smaller peptides of 15 or 20aa.

The region of approximately 110-180aa demonstrates high values and peaks in all models, and therefore has the highest likelihood of inducing a helper immunogenic response. Subsequently shorter 15 aa peptides with 5 aa overlaps with both the previous and subsequent peptide (Table 41 p.155) were then derived for this stretch of sequence. Although most CTL epitopes are between 8 and 12 amino acids, the use of peptides of 15 amino acids long is based on antigen presentation in which peptides of up to 15 aa are processed and incorporated into the MHC complex (Niedermann *et al.*, 1996).

The very start of the Surface ORF gene contained the T to A double substitution mutation (nt 731 and 732), which would encode a single amino acid change of Tryptophan (W) to Arginine (R) (aa 14). Two peptides were produced for this region, a wild type peptide, 7-14W-27 (ISGYLNI**W**LHSKASLIIGNFN) and a mutant peptide, 7-14R-27 (ISGYLNI**R**LHSKASLIIGNFN).

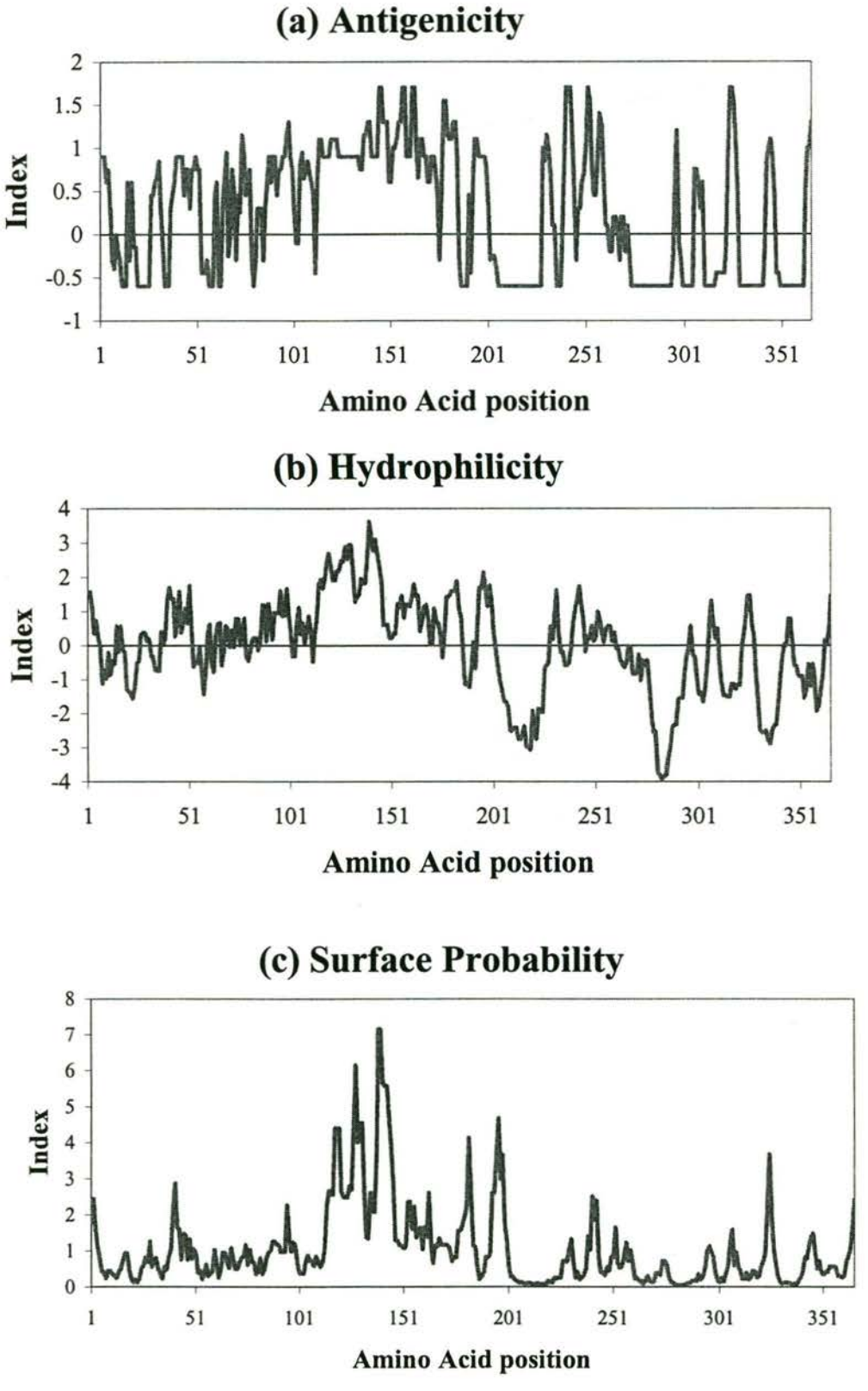


Figure 35. *Computer Modelling of the DHBV Surface gene ORF.*

(a) Antigenicity: (Jameson and Wolf algorithm)

(b) Hydrophilicity: (Kyte and Doolittle algorithm)

(c) Surface Probability: (Emini algorithm)

Peptide	Size	Position	Peptide Sequence
1-15	15	1-15	MKQESFISGYLNIWL
7-14W-27	21	7-27	ISGYLNI**W**LHSKASLIIGNFN
7-14R-27	21	7-27	ISGYLNI**R**LHSKASLIIGNFN
22-41	20	22-41	IIGNFNTLSSNIKFLMGQQP
37-56	20	37-56	MGQQPAKSMDVRRIEGGELL
54-73	20	54-73	ELLLNQLAGRMIPKGTVTWS
71-90	20	71-90	TWSGKFPTIDHLLDHVQTME
87-106	20	87-106	QTMEEVNTLQQQGAWPAGAG
101-120	20	101-120	WPAGAGRRLGLTNPAPQEPP
116-130	15	116-130	PQEPPQPQWTPEEDQ
126-140	15	126-140	PEEDQKAREAFRRYQ
136-150	15	136-150	FRRYQEERPPETTTI
146-160	15	146-160	ETTTIPPTSPTPWKL
156-170	15	156-170	TPWKLQPGDDPLLEN
166-180	15	166-180	PLLENKSLLETHPLY
176-195	20	176-195	THPLYQNPEPAVPVIKTPPL
191-210	20	191-210	KTPPLKKKKMAGTFGGILAG
210-229	20	210-229	GLIGLLVGFFLLIKILEILR
229-248	20	229-248	RRLDWWWISLSSPKGKMQCA
248-267	20	248-267	AFQDTGAQISPHYAGFCPWG
267-286	20	267-286	GCPGFLWTYLRLFIIFLLIL
287-306	20	287-306	LVTAGLLYLTDNMSIILGKL
307-326	20	307-326	QWESVSALFSSISSLLPSDQ
327-346	20	327-346	KSLVALMFGLLLIWMTSSSA
347-366	20	347-366	TQTLVTLTQLATLSALFYKN

Table 41. *Surface ORF gene peptides.*

Peptide 7-27 has a wild type and mutant version called 7-14W-27 and 7-14R-27 respectively. The difference is indicated in **bold** (W to R substitution). Overlap with previous peptide is indicated in light font. Size and position are indicated as amino acids.

6.4.2. Comparison of Wild type and Mutant Surface ORF gene

When the output from the computer modelling predictions of both the wild type and mutant Surface ORF gene are overlaid onto the same graph only a slight difference is apparent (Figure 36 p.156).

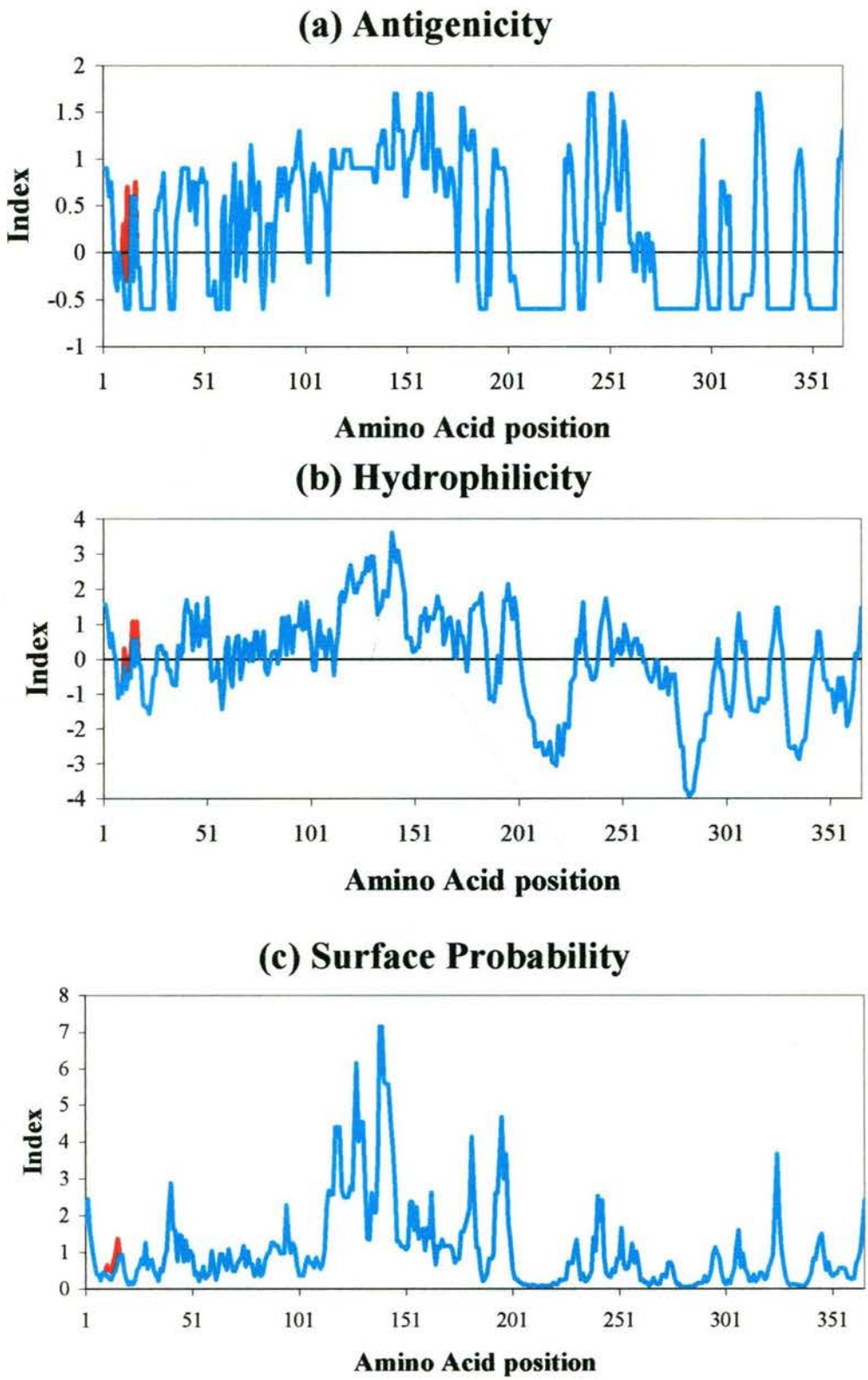


Figure 36. *Differences in the Computer Modelling of the wild type and mutant DHBV Surface ORF gene.*

(a) Antigenicity: (Jameson and Wolf algorithm)

(b) Hydrophilicity: (Kyte and Doolittle algorithm)

(c) Surface Probability: (Emini algorithm)

The **red** line indicates the modelling difference of the mutant.

6.4.3. Sequence Similarity Searching

All of the peptides of the Surface ORF protein were submitted to the various computer programs and compared with the sequences in the databases. All of the peptides were found to be similar to other DHBV species. Most were then found to be decreasing related to Snow Goose, Crane, Heron, and Stork *hepadnaviruses*, respectively.

6.4.3.1. Hepadnavirus relationships

Peptides 1-15, 7-14W-27, 7-14R-27, 22-41, and 166-180, were only found to be related to DHBV. Peptides 210-229, 229-248, and 267-286, were found to be slightly related to the human HBV envelope protein.

Peptide	Duck	Snow Goose	Crane	Heron	Stork	Human	Woodchuck	AGS	GS	Miscellaneous
1-15	1									
7-14W-27	1									
7-14R-27	1									
22-41	1									
37-56	1	3	2	4	4					
54-73	1	2	3	5	4					
71-90	1	2	2	3	4					
87-106	1	2	3	5	4					
101-120	1	3	2	4	3					
116-130	1	2	3							
126-140	1	1	2	3						
136-150	1	1	2							
146-160	1	2								
156-170	1	2								
166-180	1									
176-195	1	3	2							TcR
191-210	1	2	3	4	5					
210-229	1	3	2	4	4	5				*S. agalactiae*
229-248	1	2	4		3	8	5	6	7	
248-267	1	2	3	4	5					
267-286	1	1	2	3	3	4				
287-306	1	2	3		4					
307-326	1	1	2	4	3					

Table 42. *Sequence similarity of the peptides from the Surface ORF gene.*

NB: The numbers indicate the ranking of similarity. (1 the greatest similarity, 2 less, and so on, equal numbers indicate an equal similarity). AGS: Arctic Ground Squirrel. GS: Ground Squirrel.

6.4.3.2. Other relationships

Peptide 176-195 was found to have similarity to a rearranged T-cell Receptor (TcR) of a murine cytotoxic T lymphocyte (Chien *et al.*, 1984; Saito *et al.*, 1984b) (SwissProt TCA_MOUSE P01849) and a human cytotoxic T lymphocyte (Schneider *et al.*, 1977) (SwissProt TCA_HUMAN P01848). Other rearrangements of the murine TcR were

previously described (Saito *et al.*, 1984a), the TcR was sequenced from the alloreactive CTL clone 2C, of BALB.B origin and specific for products of the D end of BALB/c H-2 complex (d haplotype) (Kranz *et al.*, 1984). The human TcR was isolated from the human leukaemic T-cell line Jurkat. In both the human and murine TcR the similarity occurred in the beginning of the C region of the TcR (Figure 37, p.158), the human was further characterised into the alpha subunit (Yanagi *et al.*, 1985).

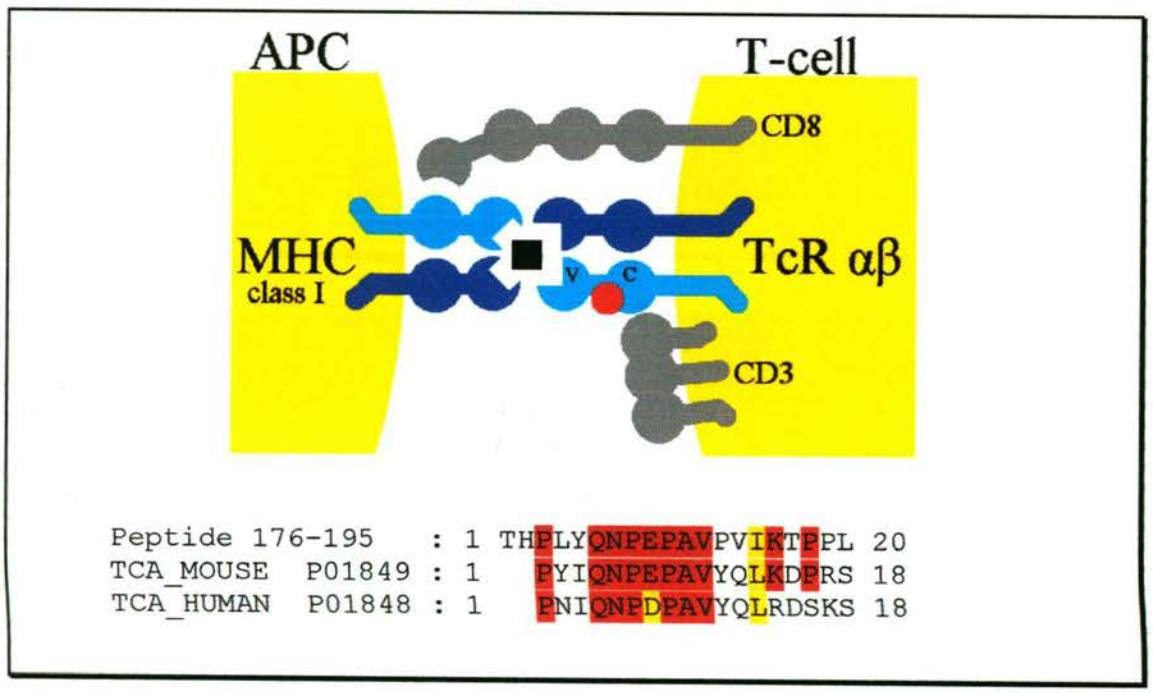


Figure 37. *Sequence similarity of peptide 176-195 with a Human and Murine TcR (Sequence and Position).*

The location of the sequence similarity of the central amino acids of peptide 176-195 on the murine TcR is indicated by the red dot in the schematic diagram of an Antigen Presenting Cell (APC) and a T-cell. It is located at the start of the Constant (C) region; V: the variable region. Black square: Peptide. red: Identical amino acids. Yellow: Similar amino acids.

Peptide 210-229 was found to have similarity to a peptide of the bacterium *Streptococcus agalactiae* serotype III (GenBank Q8E3S), and V (GenBank Q8DY59). Peptide 210-229 overlaps a region in human HBV that contains both a CD 4 and CD8 epitope (Figure 48, p.167). The similarity is demonstrated diagrammatically (Figure 38, p.158).

```
Peptide 210-229 :   1 GLIGLLVGFFLLIKILEILR 20
                      ::.::..:::
Q8E3S5 & Q8DY59 : 84 GLLGLMIGFFAKKLAIQLSG 103
                       :: :  :::
Human HBV       :184 PLLVLQAGFFLLTKILEILR 204
```

Figure 38. *Sequence similarity of peptide 210-229, Streptococcus agalactiae and human HBV.*

Red: Identical amino acids. Yellow: Similar amino acids.

6.4.4. Surface Sequence alignment for the Hepadnaviruses

The sequence of the PreSurface protein was obtained from Embank for several hepadnaviruses and aligned with ClustalW, and PileUp (11.6.1, p.A42). From the alignment it is obvious that there are differences in the PreSurface region (Figure 39, p.159). The PreS region is considered to provide the specificity of the viral attachment factor (Chouteau *et al.*, 2001).

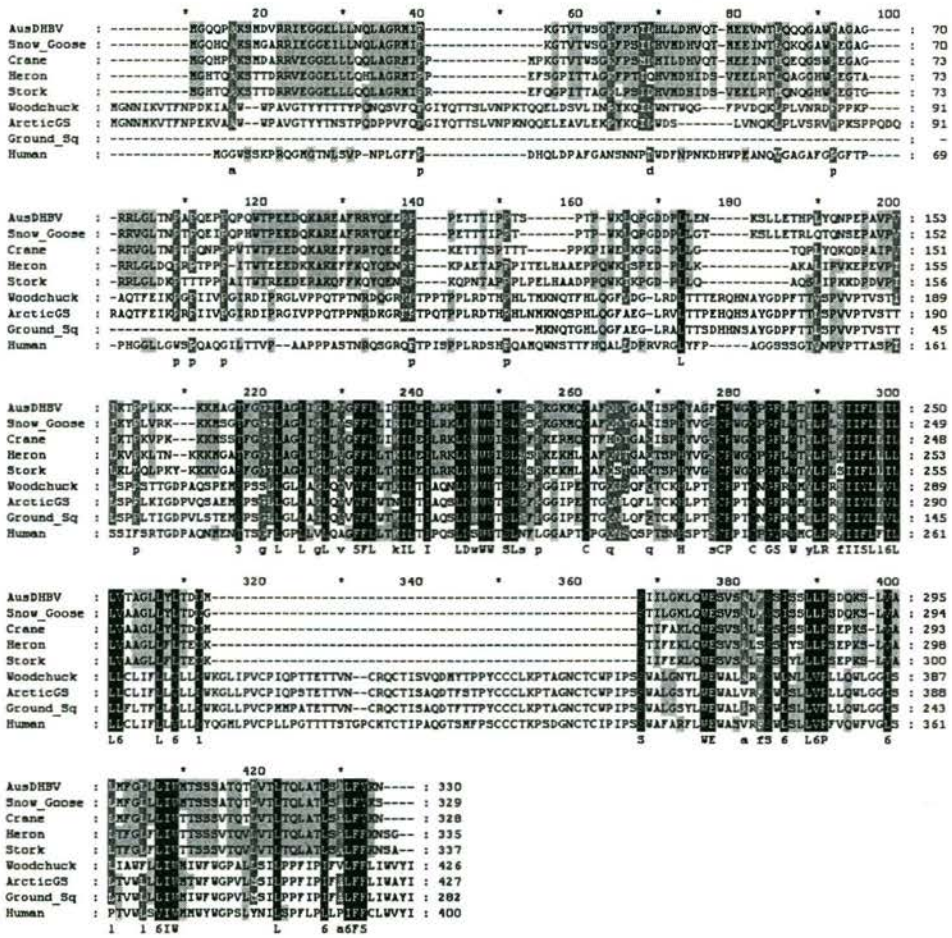


Figure 39. *Sequence alignment of the PreSurface protein of several Hepadnaviruses.* Note **Black boxes** indicate peptides that are conserved in all hepadnaviruses. **Grey boxes** indicate conservation in most of the hepadnaviruses.

From the sequence alignment a phylogenetic tree can be produced for the surface protein (Figure 40, p.160), which is closely related to trees produced using the polymerase protein, and complete genomes (data not shown).

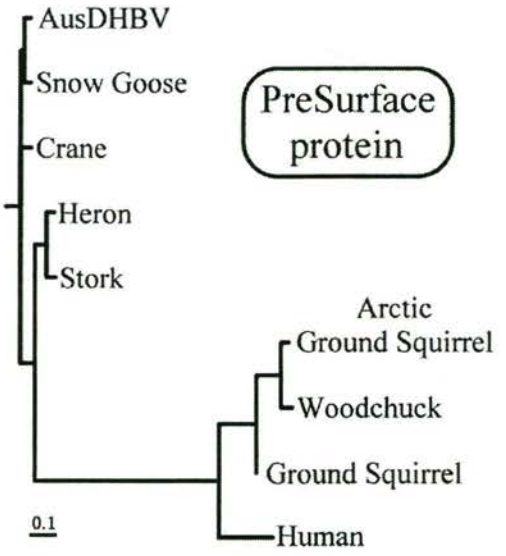


Figure 40. *Phylogenetic tree of the PreSurface protein of several Hepadnaviruses.*

6.4.5. Polymerase Sequence alignment for the Hepadnaviruses

The sequence of the Polymerase protein was obtained from Embank for several hepadnaviruses and aligned with ClustalW, and PileUp (11.6.1, p.A42) (Figure 39, p.159). The PreSurface protein overlaps the Polymerase protein from approximately aa 175 to 541 for the DHBV genome.

6.4.6. Mapping the mutation to the DHBV genome

The double T⇒A substitution at nt 731 and 732 would encode a silent nucleotide change at amino acid 13 (ATT⇒ATA), and a Tryptophan (W) to Arginine (R) substitution at amino acid 14 (TGG⇒AGG) of the Surface protein. Due to the overlapping reading frame this sequence change also affects the Polymerase protein in which a single substitution of Leucine (L) to Lysine (K) would occur at aa 188 (TTG⇒AAG). The location of the mutation can be seen on the DHBV genome (Figure 42, p.162).

Other non-coding sequences that serve as attachment sites for various enzymes and proteins, are not found in the region of DHBV between nucleotides 730 and 735, which would indicate that replication should not necessarily be affected. It is interesting to note that there is a TATA box (nucleotide sequence TTTATA) approximately one hundred nucleotides before the predicted start of the DHBV Surface protein, which is upstream of the start of the Surface ORF. The TATA box is associated with the start of translation, but this does not however exclude the full ORF from being translated into a protein.

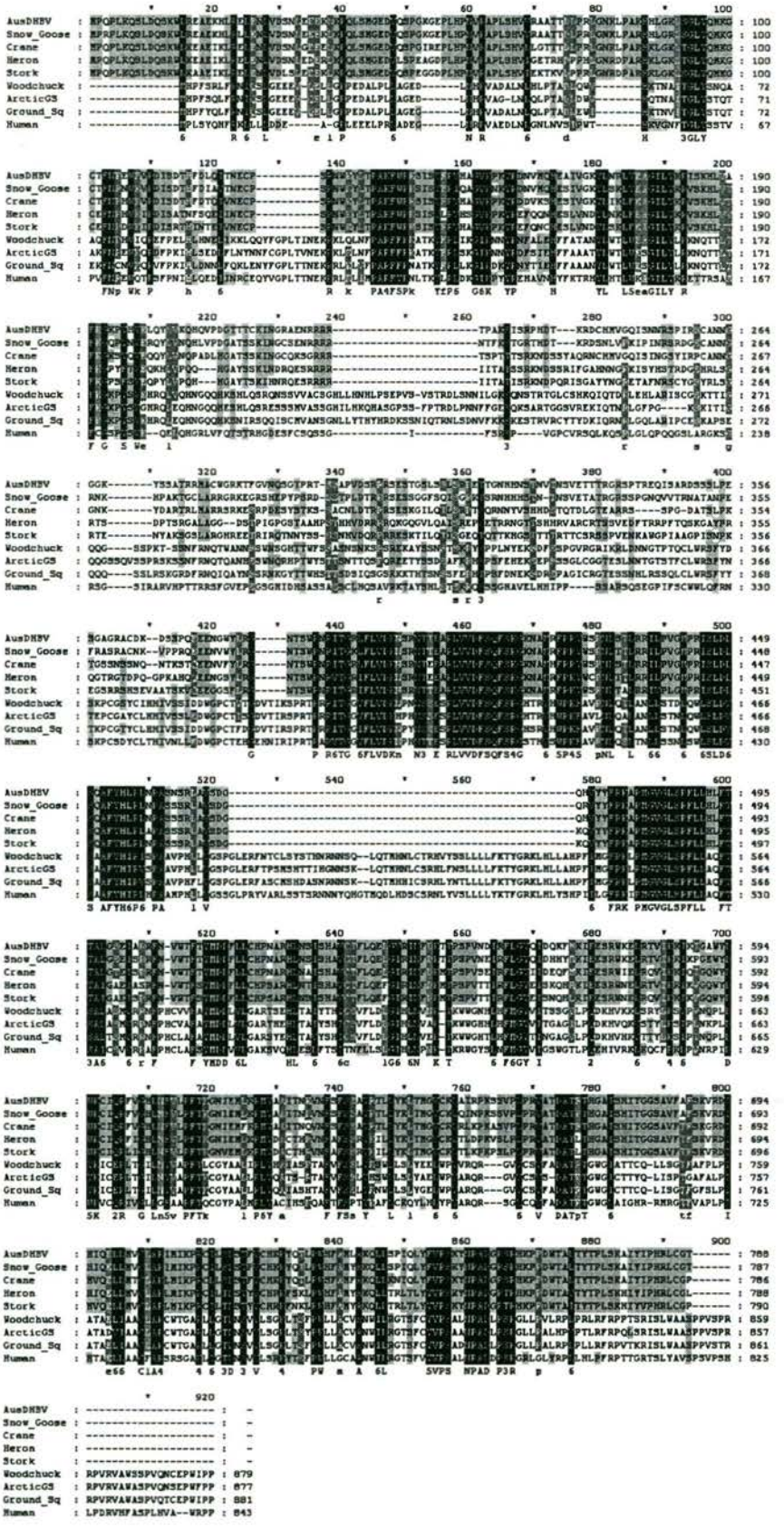


Figure 41. *Sequence alignment of the Polymerase protein of several Hepadnaviruses.*
Note **Black boxes** indicate peptides that are conserved in all hepadnaviruses. **Grey boxes** indicate conservation in most of the hepadnaviruses. PreSurface protein overlaps the Polymerase protein from approximately aa 175 to 541 for DHBV.

```
                  660         *       680         *       700
AusDHBV     : TGCAGCATGAGGCAATAGTAGGTAAATATTTAAACAGGCTCTATGAAGCA :  700
Surface     : ----------------------------------------M--K--Q :    3
Polymerase  : M--Q--H--E--A--I--V--G--K--Y--L--N--R--L--Y--E--A- :  177

                      *       720         *       740         *
AusDHBV     : GGAATCCTTTATAAGCGGATATCTAAACATTTGGTTGCATTCAAAGGCAA :  750
DHBV mut    : GGAATCCTTTATAAGCGGATATCTAAACATAAGGTTGCATTCAAAGGCAA :  750
Surface     : --E--S--F--I--S--G--Y--L--N--I--W--L--H--S--K--A-- :   19
Surf mut    : --E--S--F--I--S--G--Y--L--N--I--R--L--H--S--K--A-- :   19
Polymerase  : -G--I--L--Y--K--R--I--S--K--H--L--V--A--F--K--G--K :  194
Pol mut     : -G--I--L--Y--K--R--I--S--K--H--K--V--A--F--K--G--K :  194

                  760         *       780         *       800
AusDHBV     : GCCTTATCATTGGGAACTTCAATACCTTGTCAAGCAACATCAAGTTCCTG :  800
Surface     : S--L--I--I--G--N--F--N--T--L--S--S--N--I--K--F--L- :   36
Polymerase  : --P--Y--H--W--E--L--Q--Y--L--V--K--Q--H--Q--V--P-- :  210

                      *       820         *       840         *
AusDHBV     : ATGGGACAACAACCTGCAAAATCAATGGACGTGCGGAGAATCGAAGGAGG :  850
Surface     : -M--G--Q--Q--P--A--K--S--M--D--V--R--R--I--E--G--G :   53
Polymerase  : D--G--T--T--T--C--K--I--N--G--R--A--E--N--R--R--R- :  227
```

Figure 42. *Mapping of the mutation to the DHBV genome.*
Turquoise: start of the Surface ORF gene. Yellow: wild type. Red: mutant. Green: Predicted start of translation of the Surface protein.

It is interesting to note that DHBV does not make use of the usual non-coding regions that are associated with transcription in vertebrates in general. The lack of a well established Kozak sequences at the start of any of the ORF demonstrates this very clearly. The Kozak sequence is the nucleotide sequence that is from –6 of the ATG to +4 and is usually GCCACCatgG (Kozak, 1981; Kozak, 1987). Although this is generally considered to be required for transcription, the sequence is not absolutely rigidly required, as it has been shown that the +4 nucleotide may be substituted (but the substituted nucleotides are not as efficient as the G) (Kozak, 1997). The original Kozak sequences were associated with proteins that were expressed in abundance and thus required extremely efficient transcription, however many proteins that are being discovered are more tightly regulated and/or do not need to be transcribe as efficiently (Kozak, 1996). As such, care must be taken when interpreting theoretical modelling of proteins and their expression, as there are no simple absolute rules governing the processes.

6.4.7. Polymerase protein in relation to the Surface protein

The Polymerase gene overlaps the entire Surface gene. The Polymerase protein (Kaplan *et al.*, 1973; Sprengel *et al.*, 1985), consists of several regions of specific function (terminal protein, spacer reverse transcription, and RNaseH) (Fourel *et al.*, 1987). The mutation is found in the spacer region of the Polymerase (Figure 43, p.163). The spacer region does not appear to have any function; as large insertions into this area do not effect replication (Chang

et al., 1990), and the only other point of interest is that it contains a protease cleavage site, which has yet to be shown to be physiologically important (Lin *et al.*, 1995).

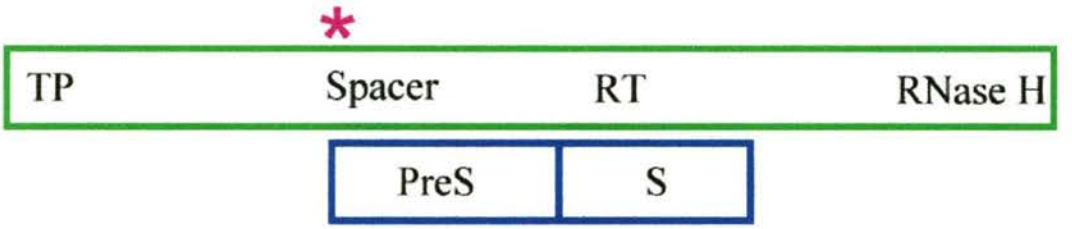


Figure 43. *Location of the mutation in relation to the Polymerase protein.*
green: Polymerase protein. Blue: Surface protein. (*) location of the mutation. TP: Terminal Protein region. RT: Reverse Transcriptase region. S: Surface region – note that the PreS protein includes both the PreS and S regions.

There are several functionally essential regions of the Reverse Transcriptase section of the Polymerase protein that are conserved in many hepadnaviruses and overlap with the end of the Surface gene (Figure 44, p.163).

```
                    *       1620          *       1640          *
AusDHBV     : AGGAAAGCTCCAATGGGAGTCGGTCTCAGCCCTTTTCTCCTCCATCTCTT : 1650
Surface     : --G--K--L--Q--W--E--S--V--S--A--L--F--S--S--I--S-- :  319
Polymerase  : -R--K--A--P--M--G--V--G--L--S--P--F--L--L--H--L--F :  494

                  1660          *       1680          *       1700
AusDHBV     : CACTACTGCCCTCGGATCAGAAATCGCTCGTCGCTTTAATGTTTGGACTT : 1700
Surface     : S--L--L--P--S--D--Q--K--S--L--V--A--L--M--F--G--L- :  336
Polymerase  : --T--T--A--L--G--S--E--I--A--R--R--F--N--V--W--T-- :  510

                    *       1720          *       1740          *
AusDHBV     : TTACTTATATGGATGACTTCCTCCTCTGCCACCCAAACGCTCGTCACCTT : 1750
Surface     : -L--L--I--W--M--T--S--S--S--A--T--Q--T--L--V--T--L :  353
Polymerase  : F--T--Y--M--D--D--F--L--L--C--H--P--N--A--R--H--L- :  527

                  1760          *       1780          *       1800
AusDHBV     : AACTCAATTAGCCACGCTGTCTGCACTTTTCTACAAGAATTAGGAGTGCG : 1800
Surface     : --T--Q--L--A--T--L--S--A--L--F--Y--K--N--*-------- :  366
Polymerase  : -N--S--I--S--H--A--V--C--T--F--L--Q--E--L--G--V--R :  544
```

Figure 44. *Conserved regions of the Polymerase protein and their relation to the end of the Surface protein.*
Yellow: conserved regions of the Polymerase protein (Chang *et al.*, 1990). Green: peptide 287-306. Red: peptide 307-326.

6.4.8. Mapping of Antibody Responses to the Surface gene.

The surface proteins of the Hepadnaviruses tend to have distinct PreS regions, while more conservation is observed in the S region. The known antibody epitopes for several hepadnaviruses are shown on a sequence alignment (Figure 45, p.164) and in relation to the computer models of antigenicity, hydrophilicity, surface probability (Figure 46, p.165).

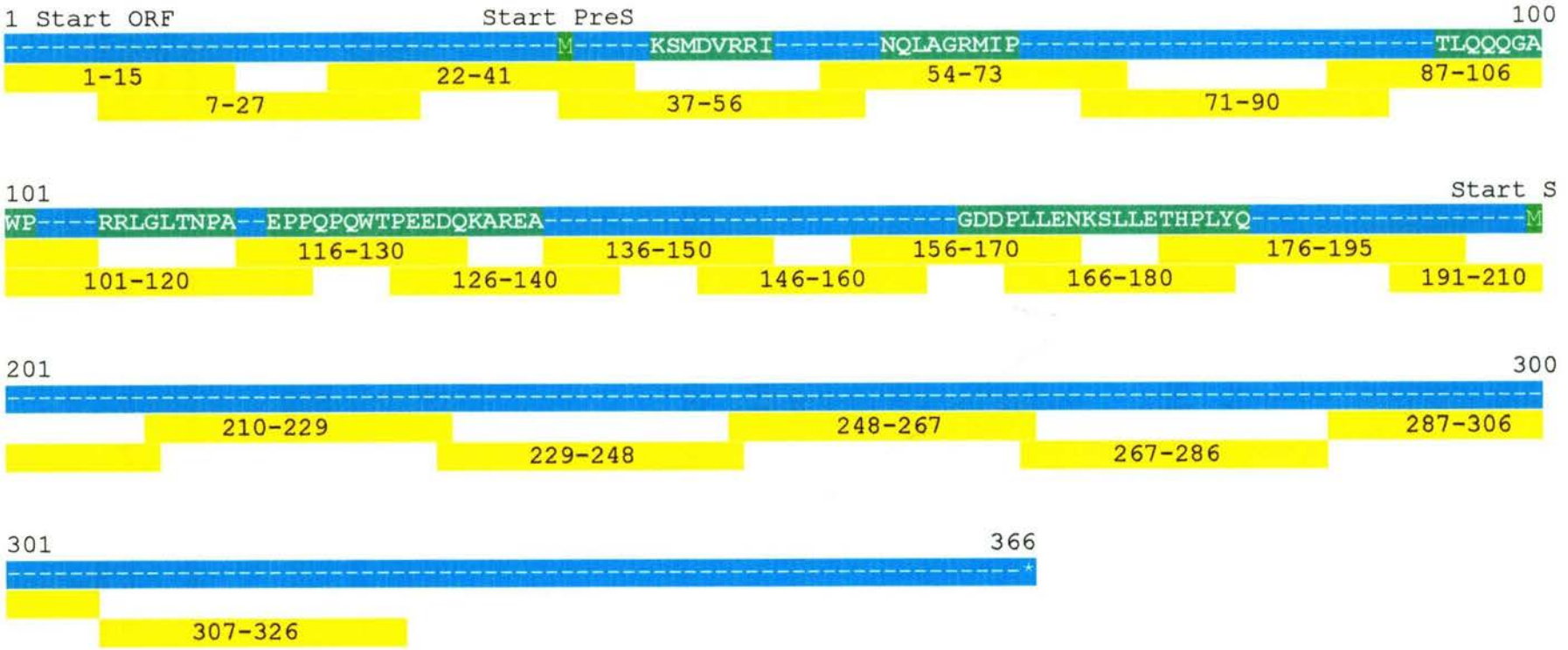


Figure 45. *Known Antibody epitopes in the Surface protein of DHBV.*

Light Blue: Surface ORF protein. **Dark green**: Known DHBV Antibody Epitopes – both naturally occurring (Chassot *et al.*, 1994), and Neutralising MAb epitopes (Yuasa *et al.*, 1991; Chassot *et al.*, 1993). **Yellow**: Position of peptides selected for this study. **M**: Predicted start of translation of the PreS protein, and S respectively.

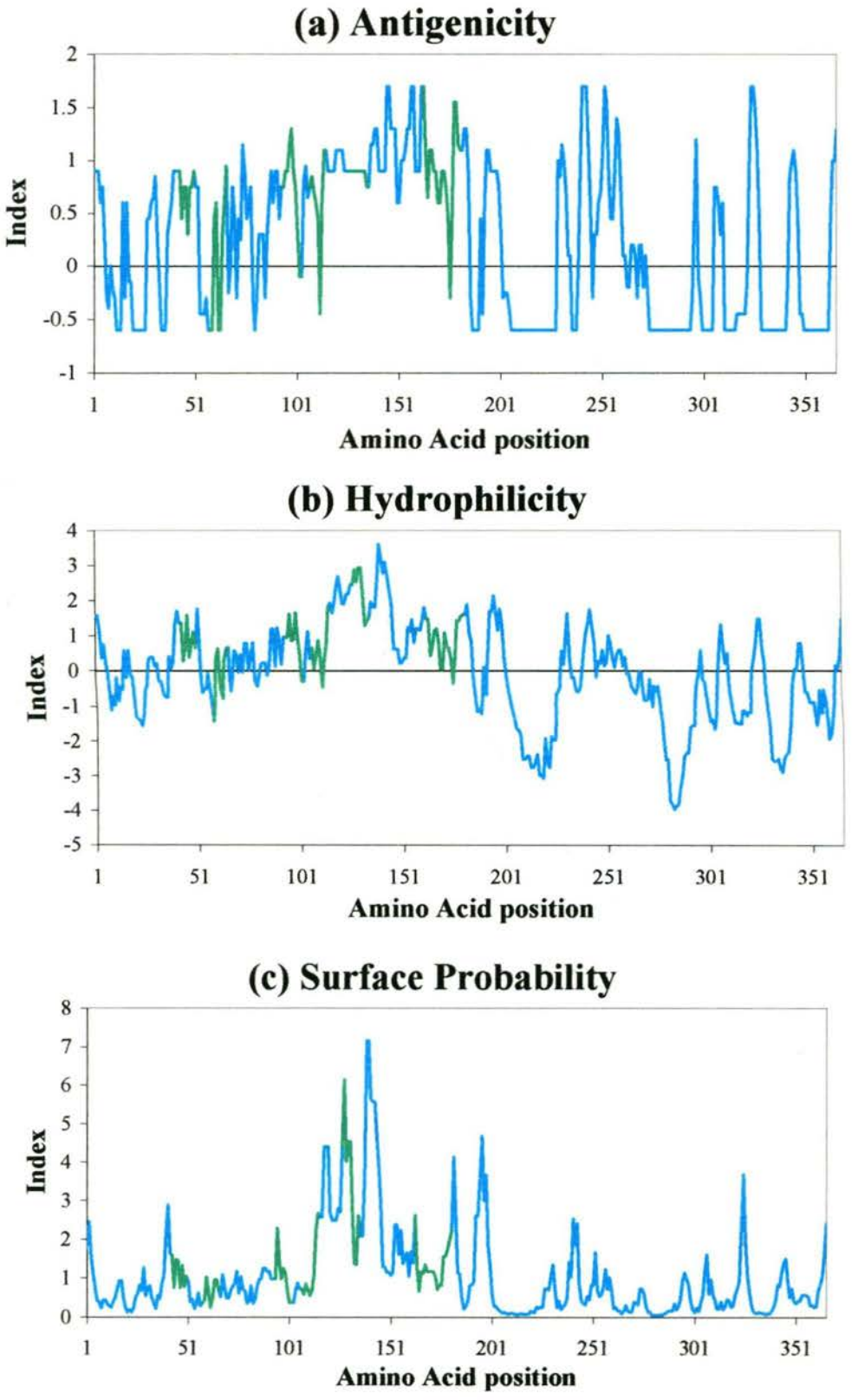


Figure 46. *Known Antibody epitopes in relation to Computer Modelling of the DHBV Surface ORF gene.*

(a) Antigenicity: (Jameson and Wolf algorithm), (b) Hydrophilicity: (Kyte and Doolittle algorithm), (c) Surface Probability: (Emini algorithm). The **Dark green** line indicates known DHBV antibody epitopes.

6.4.9. Mapping of CMI Responses to the Surface gene.

The surface proteins of the Hepadnaviruses tend to have distinct PreS regions, while more conservation is observed in the S region (Figure 39, p.159). The known CMI epitopes for human HBV are shown on a Surface protein sequence alignment with DHBV (Figure 47, p.166), and in relation to the computer models of antigenicity, hydrophilicity, surface probability (Figure 48, p.167).

```
AusDHBV_S : MKQESFISGYLNIWLHSKASLIIGNFNTLSSNIKFLMG---QQPAKSMDV :  47
HBV_env   : ------------------------------------MGGWSSKPRQGMGT :  14
                                                   MG     P   M

AusDHBV_S : RR-IEG--GELLLNQLAGRMIPKGTVT-WSGKFPTIDH--LLDHVQT-ME :  90
HBV_env   : NLSVPNPLGFFPDHQLDPAFGANSNNPDWD-FNPNKDHWPEANQVGAGAF :  63
              PLGFFPDHQL
                G     QL            W    P  DH      V

AusDHBV_S : EVNT--LQQQGAWPAGAGRRLGLTNPAPQEPPQPQWTPEEDQKAREAFRR : 138
HBV_env   : GPGFTPPHGGLLGWSPQAQGILTTVPAAP-PPAST------NRQSGRQPT : 106
                                  T PA   PP

AusDHBV_S : YQEERPPETTTIPPTSPTPWK----LQPGDDPLLENKSLLE--THPLYQN : 182
HBV_env   : PISPPLRDSHPQA--MQWNSTTFHQALLDPRVRGLYFPAGGSSSGTVNPV : 154
                          MQWNSTTFHQALLDP

AusDHBV_S : PEPAVPV--IKTPP-LKKKKMAGTFGGILAGLIGLLVGFFLLIKILEILR : 229
HBV_env   : PTTASPISSIFSRTGDPAQNMENTTSGFLGPLLVLQAGFFLLTKILTIPQ : 204
                                                 FLLTKILTIPQ
            P  A P   I          M  T  G L  L  L  GFFLL KIL I

AusDHBV_S : RLDWWWISLSSPKGKMQCAFQDTGAQISPHYAGFCPWGCPGFLWTYLRLF : 279
HBV_env   : SLDSWWTSLNFLGGAPTCPGQNSQSPTSNHSPTSCPPICPGYRWMCLRRF : 254
             LD WW SL    G   C  Q      S H    CP  CPG  W  LR F

AusDHBV_S : IIFLLILLVTAG-LLYLTD--NMSIILGKL-------------------- : 306
HBV_env   : IIFLFILLLCLIFLLVLLDYQGMLPVCPLLPGTTTTSTGPCKTCTIPAQG : 304
            IIFL ILL     LL L D   M      L

AusDHBV_S : ------------------------------QWESVSALFSSISSLLPSD : 325
HBV_env   : TSMFPSCCCTKPSDGNCTCIPIPSSWAFARFLWEWASVRFSWLSLLVPFV : 354
                                        WE  S  FS  S L P

AusDHBV_S : QKSL-VALMFGLLLIWMTSSSATQTLVTLTQLATLSALFYKN---- : 366
HBV_env   : QWFVGLSPTVWLSVIWMMWYWGPSLYNILSPFLPLLPIFFCLWVYI : 400
            Q          L  IWM          L     L   F
```

Figure 47. *Aligned Surface protein sequences showing known HBV CMI epitopes.*
Red: MHC-II, CD4 epitopes. **Blue**: MHC-I, CD8 epitopes. **Black**: 'a' determinant of HBV. **Yellow**: Conserved regions in the Polymerase protein (Figure 44, p.163). Letters under the sequences indicate conserved amino acids. **M**: Predicted start of translation of the PreS protein, PreS2, and S respectively.

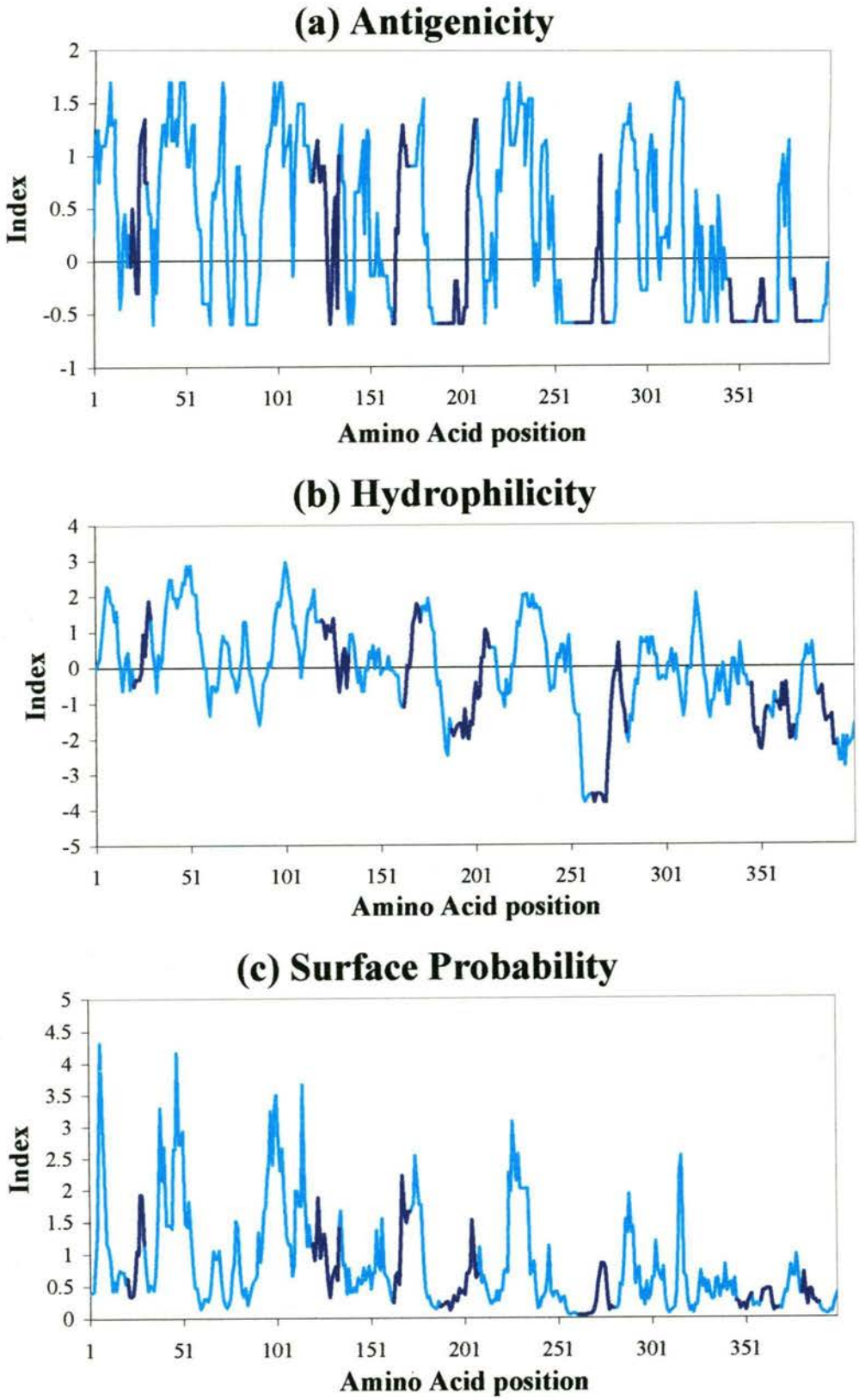


Figure 48. *Known CMI epitopes in relation to Computer Modelling of the HBV Surface gene.*

(a) Antigenicity: (Jameson and Wolf algorithm). (b) Hydrophilicity: (Kyte and Doolittle algorithm). (c) Surface Probability: (Emini algorithm). The **Dark Blue** line indicates the CMI epitopes. Neutralising antibodies are directed against the 'a' determinant located between aa 121 and 150. Note the similarity of the overall pattern of the HBV computer model in relation to that for the DHBV Surface ORF protein (Figure 35, p.154).

6.5. DISCUSSION

Overall, the S region of the large DHBV surface protein sequence resembles that of HBV and the other hepadnaviruses, however DHBV does not contain a homologue of the 'a' determinant region (between HBsAg aa 284-336 Figure 47, p.166), which is the immunodominant area of the surface protein of the mammalian hepadnaviruses. The preS regions differ, as would be expected because the region is considered to contain the virus receptor, providing the high-level tissue and species specificity. Although sequence differences are apparent, it is likely that the overall conformation of the large DHBV surface protein resembles that of HBV and the other hepadnaviruses. This reflects their sharing the same functions in the virion structure.

Several immunodominant epitopes are shared by the two arms of the CMI response in human HBV infection (Figure 48, p.167), it is unknown whether this overlap exists in other hepadnavirus systems. These epitopes have been associated with both the MHC-I (CD4) and MHC-II (CD8) presentation pathways. It is however, possible that antibody responses associated with an event such as clearance, may in fact be surrogate markers of the T-cell response, which is actually producing the effect.

Phylogenetic analysis of the amino acid sequences of the surface (Figure 40, p.160), and polymerase proteins, show similar relationships amongst the hepadnaviruses, to that derived form the nucleotide sequences of the entire genome.

The two peptides that were shown to contain homology with non-hepadnavirus proteins are of interest (176-195, and 210-229). Peptide 176-195 has sequence homology with the TcR of a human and murine cytotoxic T lymphocyte, and this could provide an immune evasion mechanism for the virus. The significance of this similarity is currently unknown but could well be a mechanism in which DHBV is able to subvert and modulate the immune response directed against it. Peptide 210-229 has sequence similarity to a protein produced by *S. agalactiae*, and this bacterium may be present in such things as the feed that was given to the ducklings. This homology may be advantageous because it mimics an antigen widely available in the environment. It is interesting that this DHBV region closely resembles that of human HBV, and may well have similar functional significance in the human infection.

The mutant virus does not appear to create a large conformational difference, in antigenicity, hydrophilicity, and surface probability, as the differences apparent in Figure 36 (p.156), are slight and unlikely to affect antibody production. Its effect may be more pronounced for the CMI response, which cannot be effectively modelled.

The mutant is unlikely to have a direct effect on replicative capacity because the mutation is in the spacer region of the DHBV polymerase. However it may affect regulation of replication as the region of the genome near the mutation was shown to be necessary for efficient replication. Template switching, which is required for synthesis of plus-stranded DNA, has been shown to require the region of nt 723-833 (Havert and Loeb, 1997).

6.6. CONCLUSION

These theoretical predictions need to be tested – by the ability of putative peptides of interest to induce a measurable CMI, and by the association with biological events.

7. Cell Mediated Immune Response to DHBV

7.1. Introduction

The recovery from hepadnavirus infection with its massive hepatocellular involvement (Jilbert *et al.*, 1992) is usually attributed to the cellular arm of the immune response (Rehermann *et al.*, 1996b; Tang *et al.*, 2001). The effector response can involve either cytotoxic (Grandits *et al.*, 1991), or helper T-lymphocytes (Hellstrom *et al.*, 1985). The specificity of these responses is still being elucidated. Most work has so far concentrated on the cytotoxic response to the Core protein, as seen in humans (Mondelli *et al.*, 1982), woodchucks (Menne *et al.*, 1997), and by a lymphoblastic CMI response in ducks (Vickery *et al.*, 1999a). CMI responses to HBsAg have been found in humans, associated with previous exposure , but the significance of DHBV surface CMI responses is unknown. Earlier work has shown that there is a good temporal relation between the appearance of anti-DHBS antibody and S-specific CMI response (Vickery *et al.*, 1989; Vickery *et al.*, 1999b).

The humoral arm of the immune response, in contrast, is responsible for immunity from infection. It has been shown that antibodies to the Surface protein provide effective immunity from duck hepatitis B infection (Vickery *et al.*, 1989), although the role of anti-DHB core antibody in the pathogenesis of infection remains unknown.

The persistence of HBV has been attributed to weak or negligible CMI in patients (Bertoletti *et al.*, 1991; Missale *et al.*, 1993; Nayersina *et al.*, 1993; Rehermann *et al.*, 1995). There is also evidence that suppresser T-lymphocytes can be found in patients with persistent infection (Barnaba *et al.*, 1985), these suppressor cells have been found to inhibit the responsiveness other HBV specific lymphocytes.

Immunosuppressive drugs used in transplantations, have profound effects on hepadnavirus infection (Samuel and Kimmoun, 2003). Reactivation of hepadnavirus infection is a well

documented complication of cytotoxic or immunosuppressive therapy in asymptomatic HBV carriers (Vento *et al.*, 2002), even in patients who are HBsAg negative (Nagington, 1977; Nagington *et al.*, 1984). Its clinical manifestation include fulminant hepatitis (Kumagai *et al.*, 1993) but generally a high level of viraemia coexists with little liver damage in these patients.

The striking effect of age on the pathogenesis of hepadnavirus infection is presumed to relate to the progressive increase in the effectiveness of CMI responses after birth.

Anti-DHBS antibody appears in conjunction with the S-specific CMI response, and the current study has revealed a mutation in the S gene which might be attributable to immune pressure. It was decided to extend these findings by dissecting the specificity of the lymphoproliferative response to DHBs.

The lymphoblastogenesis assay involves incubating mononuclear cells (which are generally T-cells), with a peptide. Incorporation of labelled thymidine is used to measure proliferation of cells which recognise the peptide. This technique has been previously used to determine the CMI response of vaccinated ducks to the core, and surface protein as a whole (Vickery *et al.*, 1997; Vickery *et al.*, 1999a). This assay is capable of determining the Th immune response, but not the Tc response.

It is interesting to note that in both humans and murine models, it has been found that several T-cell immunogenic epitopes are both CTL and T helper. Human T-cells from HBV vaccine recipients that expressed a short peptide from the amino terminus of HBsAg induced both a proliferative and cytotoxic response in hepatitis B-specific T-cells (Celis *et al.*, 1988). In euthymic mice, HBcAg efficiently induces IgM and IgG antibodies, in spite of the absence of T-cells in nude mice, and also stimulates T-cell proliferation *in vitro* and helper T-cell function *in vivo* (Milich and McLachlan, 1986).

In this experiment synthetic peptides which had been selected on the basis of the theoretical modelling process, described in the previous chapter, were used to stimulate duck SMC purified from DHBV naïve, infected and immune ducks in a lymphoblastogenesis assay to determine the specific parts of the S ORF gene that are important in immunity to DHBV.

7.2. AIMS

(1) To determine the sAg immunodominant epitopes in immune ducks challenged with DHBV.

(2) To show whether chronically infected ducks have specific defects in their CMI response repertoire to sAg peptides.

(3) To determine if the T-cell immune response to the peptide from the mutant sAg is different from that of the corresponding wild type peptide.

7.3. MATERIALS AND METHODS

7.3.1. Animals

The CMI response to DHBsAg peptides was tested in three types of ducks: naïve uninfected controls, protein vaccinated DHBV immune, and DHBV inoculated positive controls ducks. The animals used in this experiment are summarised (Table 43, p.172).

Group	Ducks	Number
Negative	24	P24P53, V2T, V2U, 1A, 1B, 1C, 1D, 1E, 1F, 1G, 1H, 1I, 1J, 1K, 1L, 2A, 2B, 2C, 2D, 2E, 2F, 2G, 2H, 2I
Vaccinated	15	G51, G53, G63, G99, P63, W45, V2J, V2K, V2L, V2M, V2N, V2O, V2P, V2Q, V2S
Positive	12	G531, G58, P631, G631, G72, G89, P72W48, V2R, W105, W106, W107, W111

Table 43. *Ducks used to determine the CMI response to DHBV.*

At euthanasia ducks were bled to purify PBMC (7.3.2.1, p.174), for whole blood counts (2.2.9.2, p.76) and DHBV DNA analysis from serum. Liver samples from all ducks were obtained at euthanasia and tested for DHBV DNA by dot blot and PCR. Small sections of spleen, liver, pancreas, and kidney were placed into 10% formalin and treated (Methods and Materials, 2.2.10, p.77) for later histological analysis.

7.3.1.1. Naïve uninfected Negative control ducks

Twenty one ducks (1A, 1B, 1C, 1D, 1E, 1F, 1G, 1H, 1I, 1J, 1K, 1L, 2A, 2B, 2C, 2D, 2E, 2F, 2G, 2H, 2I) were obtained from a DHBV negative flock at 6 to 8 weeks of age. Blood and tissue samples were obtained at euthanasia, which was within a week of arrival.

Three ducks were obtained as day-old ducklings and were maintained separately from other ducks until euthanasia at day 44 (V2T, and V2U) or day 70 (P24P53).

7.3.1.2. DHBV Immune ducks

Fifteen Ducks were immunised with an inactivated protein vaccine as described in Methods and Materials (2.2.11, p.77). These ducks were challenged with 100µL DHBV200197 ($2.0x10^9$ vge) 2 to 6 weeks prior to euthanasia and harvesting of lymphocytes to maximise the chance of detecting the short lived duck CMI responses (Vickery *et al.*, 1999b; Higgins *et al.*, 2000; Tang *et al.*, 2001).

The vaccine was prepared as described previously (Vickery *et al.*, 1989). It contained complete native DHBsAg, and thus all the protein sequences used in the test plates.

Two vaccination regimes were used:

a) Six ducks (G51, G53, G63, G99, P63, and W45), were inoculated on days 10, 17, and 24, with 15µg of protein vaccine in TitreMax adjuvant *im* in two sites each time. They were bled twice per week post challenge, until euthanasia on day 70 when tissue samples were taken.

b) Nine ducks (V2J, V2K, V2L, V2M, V2N, V2O, V2P, V2Q, and V2S) were inoculated on days 7, 14, and 21, initially with 10µg of protein vaccine in PBS *ip*, while the second and third boosters were 20µg of protein vaccine in TitreMax adjuvant *im* in two sites each time. They were bled at challenge, and prior to euthanasia on day 43-44 when tissue samples were also taken.

7.3.1.3. DHBV inoculated Positive control ducks

Twenty five ducks were infected with DHBV at 4 weeks of age. Twelve ducks were used for the positive control group for the CMI response (G531, G58, G631, G72, G89, P72W48, P631, V2R, W105, W106, W107, and W111). The other ducks were used for histology, and cell counts (G86, G511, G991, P17, P54, P57, P531, W34, W43, W48, W103, W139, and W451), and are described in more detail in Chapter 9 (p.226). Most ducks were inoculated with $2.0x10^9$ vge of DHBV from serum pool DHBV200197 (equivalent to $1ID_{50}$), while ducks G631, G72, G89, were inoculated with $2.0x10^{10}$ vge of DHBV from serum pool DHBV200197.

7.3.2. Lymphoblastogenesis assay

The lymphoblastogenesis assay was used to measure the lymphocyte response to mitogens and antigens and is schematically depicted in Figure 49 (p.174).

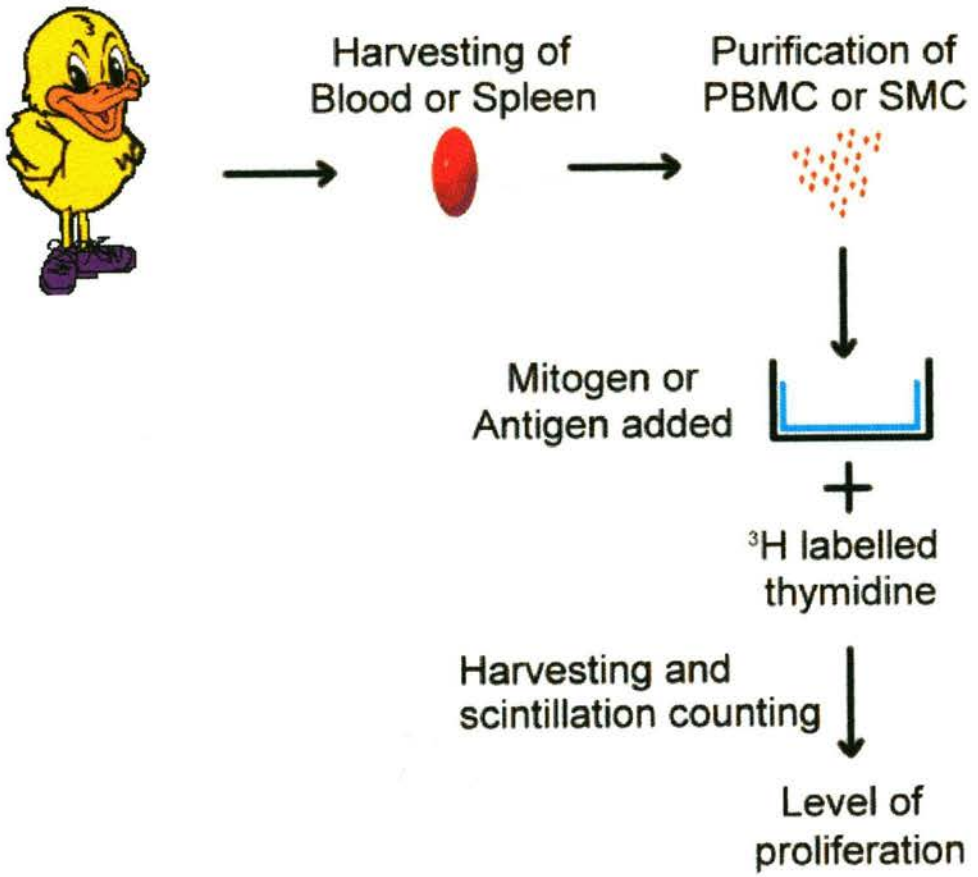


Figure 49. *Schematic diagram of the Lymphoblastogenesis assay.*

7.3.2.1. Purification of Peripheral Blood Mononuclear Cells (PBMC)

Blood (10mL) was collected from the jugular vein into an equal volume of 10 IU/mL Heparin in PBS. The syringe was inverted several times to facilitate mixing and prevent localised clotting of the blood.

The blood/heparin mixture was placed into a sterile plastic Petri dish and aliquots of approximately 7mL were layered onto 3mL of Ficoll-Paque (Pharmacia, Uppsala, Sweden) and centrifuged at 1200rpm for 25min in a Super Minor centrifuge (MSE, England). The interface layer, containing the mononuclear cells was harvested, while the pellet, containing red blood cells, was discarded. The cells were then washed 3 times: each wash consisted of resuspending the cells in 10mL media, followed by centrifugation at 1200rpm for 10min. After each wash the cells were re-suspended in approx. half the number of tubes, so that by the third wash there was a single pellet of cells. After the final spin the cells were resuspended in exactly 10mL of media, counted and viability tested by exclusion of Trypan blue dye (2.2.9.1, p.76).

The cells were diluted to a concentration of $4.0x10^6$ cells/mL, and 200µL of cell suspension (i.e. $2.0x10^5$ cells) was pipetted into the prepared tissue culture plates.

7.3.2.2. Purification of Spleen Mononuclear Cells (SMC)

After blood was taken for PBMCs, Valabarb (Jurox, Silverwater, Australia) was injected, using the same needle, until euthanasia. The ducks ventral abdomen was soaked in 70% ethanol, to reduce airborne feathers and down, prior to being plucked. The abdomen was again washed in 70% ethanol, opened and the spleen removed aseptically using a fresh set of sterile instruments. The spleen was sliced and briefly washed in medium (7.3.2.3, p.175) to remove some of the red blood cells still present in the spleen.

The spleen was then diced with scissors and gently passed through a 120-mesh stainless steel sieve into approximately 50mL of medium. Aliquots of 7mL were layered onto 3mL of Ficoll-Paque (Pharmacia, Uppsala, Sweden) and centrifuged at 1200rpm for 25min in a Super Minor centrifuge (MSE, England). The interface layer, containing the mononuclear cells, was harvested, while the pellet, containing red blood cells, was discarded. The cells were then washed, counted, and viability tested in the same manner as for PBMCs (7.3.2.1, p.174).

The cells were diluted to a concentration of $2.5x10^6$ cells/mL. 200µL of cell suspension (ie. $5.0x10^5$ cells/well) was pipetted into the prepared tissue culture plates.

7.3.2.3. Tissue Culture Conditions

RMPI 1640 (Sigma, St. Louis, USA) was buffered with 2g/L $NaHCO_3$, and contained 100 IU/ml benzyl-penicillin, 100mg/ml di-hydrostreptomycin sulphate (both Sigma, St. Louis, USA) and was supplemented with 10% PDS (Pooled negative Duck Serum, 2.2.8, p.75) and 5% FCS (CSL, Melbourne, Australia) (Vickery *et al.*, 1997). A pool of DHBV negative duck serum (Methods and Materials, 2.2.8, p.75) was produced and used throughout the experiments, as was the same single batch of FCS.

Solutions of antigens or mitogens at 11 times the required concentrations were made up with media (7.3.2.3, p.175). 20µL of the antigen or mitogen solution, (or 20µL of media in the case of controls) were added to 6 wells of a 96 well flat-bottomed microculture plates (Nunc, Denmark). The trays could then be frozen for storage, for a maximum of two weeks, thawed and warmed up to RT before use. Freezing the trays prior to use did not influence the effectiveness of the antigens or mitogens prior to the addition of 200µL of cell suspension, but allowed them to be prepared prior to cell harvesting.

The cells were incubated at 40°C (near duck body temperature), in an atmosphere containing 5% CO_2, and a relative humidity of 95%. The mitogenic and antigenic responses were measured after 3 and 6 days incubation respectively.

7.3.2.4. Mitogens

Used as a control of cell viability for the antigen-specific assay to determine if the harvested cells were capable of producing a response.

Lipopolysacharride (LPS, *E.coli* serotype 011:B4) (Sigma, L2630, St. Louis, USA) and Red mung bean Phytohaemagglutinin (PHA, *Phaseolus vulgaris*) (Sigma, L9132, St. Louis, USA), were dissolved in sterile dH_2O to a concentration of 5mg/mL. Concentrations of 1, 5, and 10μg/mL where used for PHA, while concentrations of 1, 5, 10, 20, and 40μg/mL were used for LPS. Six replicates were used for each concentration of mitogen.

7.3.2.5. DHBV Surface Antigens

Computer modelling techniques were used to analyse several parameters: Hydophilicity, Antigenicity, and Surface probability. These parameters were used to divide the sAg into smaller segments of between 15-20 aa peptides that would be used in a lymphoblastogenesis assay. The Surface ORF gene was divided into 24 segments (Chapter 6, p.150). Twenty-three peptides were synthetically produced, the final two segments (327-346 and 347-366) were not tested because there was difficulty in producing such hydrophobic peptides, and segment 7-27, was synthesised in two forms: a wild type (7-14W-27), and a mutant form (7-14R-27) (Chapter 4, 123).

The peptides (Auspep, Parkville, Australia), were dissolved in sterile dH_2O to a concentration of 1mg/mL.

7.3.2.6. Radiolabelling and Harvesting of Cells

Cell were radiolabelled by the addition of 20μL of media containing 0.5μCi of methyl-^{3}H labelled thymidine (Amersham, Buckinghamshire, England) to each well. The cells were then incubated for 6h before being harvested onto GF/C glass-fibre mats (Whatman, Maidstone, USA) using a semi-automated harvester (Skatron, Lierbyen, Norway).

The mats were air dried at RT overnight, the individual discs placed into 3ml of Biodegradable Counting Scintillant (Amersham, NBCS104, Buckinghamshire, England) in plastic vials. The vials were then read in a 1214 Rakbeta Counter (LKB Wallac, Stockholm, Sweden), using the parameters detailed in Appendix 11.3 (p.A3).

The response was measured by ^{3}H-labelled tritium uptake, measured in cpm. All cultures included unstimulated unlabelled and unstimulated labelled controls.

7.3.3. Response to Mitogens and Peptides

The response to mitogens and the peptides was determined in two ways, initially a simple method was used, while later a more powerful method was utilised. The latter significant P/N method, was also used for all CMI responses detailed in further chapters.

7.3.3.1. Initial analysis (>5000 cpm)

A specific lymphoblastogenesis response to a peptide occurred when the mean cpm of stimulated labelled wells was >5000 cpm above the mean of the unstimulated labelled controls.

7.3.3.2. Final analysis (sig P/N)

A specific lymphoblastogenesis response to a peptide occurred when the mean cpm of stimulated labelled wells was >1000 cpm above the mean of the unstimulated labelled controls and these means were shown to be significantly different by the Students t-test (2 tailed, 2 sample). This more powerful analysis of the data removes mathematically significant, but biologically insignificant responses.

7.3.3.3. Statistical Analysis

The Fisher's exact test was used to compare the number of vaccinated and negative control ducks that respond to each peptide. The difference in response to a peptide was considered to be significant if the P value was less than 0.05.

7.4. RESULTS

7.4.1. DHBV DNA Analysis

7.4.1.1. Naïve uninfected Negative control group

All ducks (24/24) were dot blot hybridisation and PCR negative throughout.

7.4.1.2. DHBV Immune group

The protein vaccine was well tolerated with no apparent side effects or sequelae.

All ducks (15/15) were immune to challenge with $2.0x10^{9}$ vge of DHBV on day 29, or 30 (5-9 days after the third vaccine inoculation). All ducks were dot blot hybridisation and PCR negative throughout; as such this group was successfully immunised against DHBV, using the protein vaccine.

7.4.1.3. DHBV infected control group

All but one (G531), of the twelve infected ducks tested for CMI response were viraemic at some point in the experiment. This duck and two others, (P72W48, and W106) were dot blot hybridisation negative in the liver at euthanasia. The other ducks (G58, G631, G72, G89, P631, V2R, W105, W107, and W111), were all viraemic and found to be DHBV positive in the liver at euthanasia, day 45 (V2R) or day 70 (G531, G58, G631, G72, G89, P72W48, P631, W105, W106, W107, and W111). The dot blot hybridisation and PCR results for the positive control group are tabulated (Table 44, p.178).

Duck	Days post inoculation												
	0-1	4	7-8	10-11	13-14	16-19	20-23	27	29-31	34	37-38	40-43	L
G58	0	0	0	0	0	0	0		0			0	5
G531	0	0	0	0	0	0	0		0			0	0
P631	0	0	0	0	0	0	0		0			2	5
G72	0		3		5		0		1				5
G89	0		0		0		0		3				5
G631	0		0		0		0		5				5
P72W48	0	0			0	0	0	0	0	0	0		0
V2R	0					0			0				4
W105		0			0	0	0	0	0	0	0	0	5
W106		0			0	0	0	0	0	0	0	0	0
W107		0			0	0	0	0	0	0	0	0	5
W111		0			0	0	0	1	2	1	1	2	5

Table 44. *Tabulated dot blot hybridisation and PCR results for the Positive control ducks.*

Dot blot results are the numerical value (0=not detected ($\leq 1x10^6$vge/mL), $1=1x10^7$vge/mL, $2=1x10^8$vge/mL, $3=1x10^9$vge/mL, $4=1x10^{10}$vge/mL, $5>2x10^{10}$vge/mL, +=positive$>1x10^7$vge/mL). Shaded blocks indicate DHBV PCR results: positive ($>2x10^3$ vge/mL), negative ($<2x10^3$ vge/mL), clear = not tested. L=Liver.

7.4.2. Lymphoblastogenesis Assay

Ten millilitres of blood normally yielded between $1x10^7$ and $5x10^8$ viable PBMCs. On the trypan blue exclusion test the proportion of dead cells varied between 5 and 10% but was usually around 5% depending on the time required for processing.

The spleen yielded between $1x10^7$ and $2x10^9$ viable SMC. On the trypan blue exclusion test the proportion of dead cells varied between 5 and 20% but was usually around 10% depending on the time required for processing.

The full results for each individual duck are in the Appendix (11.9, p.A43).

7.4.2.1. Mitogen Results

Both SMC and the PBMC from all the negative control and immune ducks responded well to PHA stimulation *in vitro* demonstrating the viability of the purified cells. However, not all the cells that were able to respond to PHA were able to respond to LPS (Table 45, p.179).

All but one (duck P631) of the 12 DHBV infected ducks PBMC responded to PHA, but the response of SMC to PHA was depressed in 5 ducks (G531, G58, G631, P631, and W105). Despite the SMC poor response to PHA, the cells from these ducks (all but W105) were able to respond to antigenic stimulus demonstrating their viability. The SMC PHA depressed ducks showed several infection patterns; no detectable viraemia and liver negative (G531), PCR only viraemia and liver positive (G58, and W105), and both viraemia and liver positive (G631, and P631).

The response to LPS was even poorer, with only two ducks SMC (ducks P72W48, and W111), and 2 different ducks PBMC (ducks W105, and W106) responding. LPS is apparently less effective than PHA, because it is only a really potent inducer of lymphoblastic responses in mice.

Overall, the naïve and vaccinated groups responded significantly better than the infected positive control group in both SMC PHA ($p=0.002$, $p=0.010$, respectively), and SMC LPS ($p<0.001$, $p=0.006$, respectively). There was no significant difference between the PBMC results for either of the three groups.

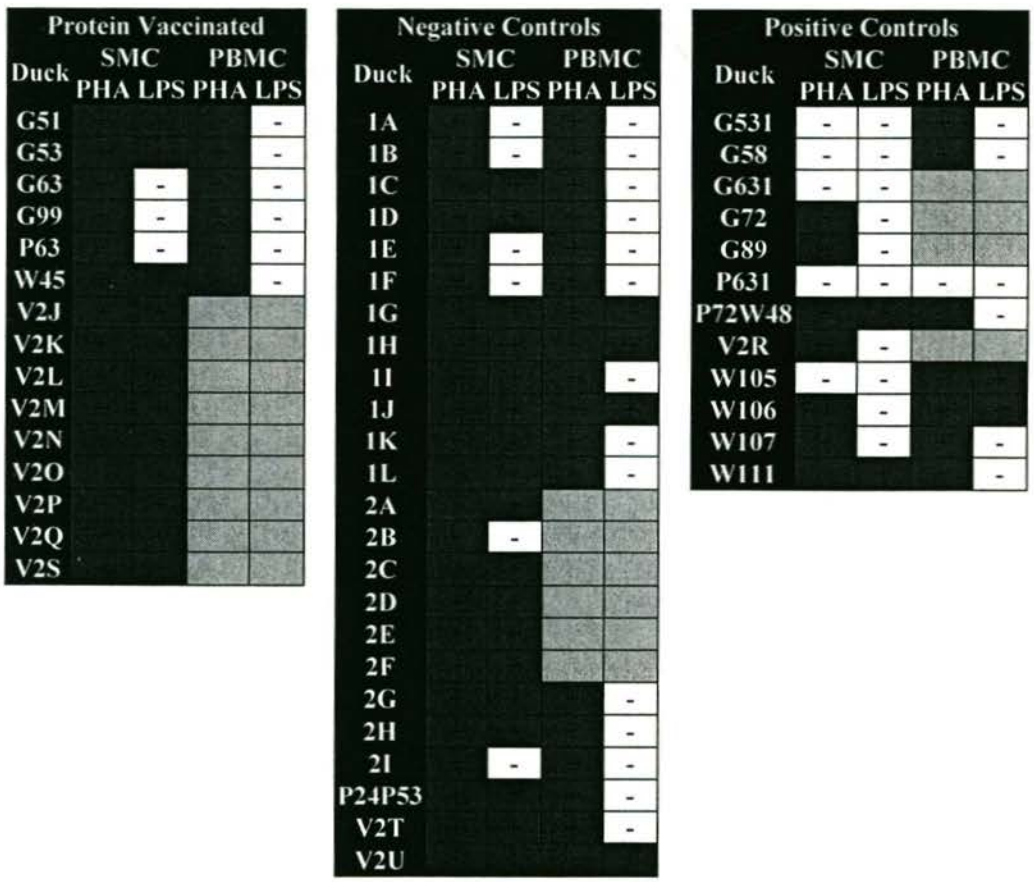


Table 45. *Summary of CMI response of Ducks to Mitogens (significant P/N).*
■ Positive response. - negative response. Empty shaded box (▒) not tested.

7.4.2.2. Antigen Response

7.4.2.2.1. Initial method of analysis

The results from the protein vaccinated and negative control ducks for the greater than 5000cpm change have been summarised, Table 46 (p.181), and Table 47 (p.182), and the statistical analysis of the greater than 5 000cpm increase has been summarised (Table 48, p.183).

From the initial interpretation of the lymphoblastogenesis assay, both the wild type and mutant form of peptide 7-27 (7-14W-27, and 7-14R-27), as well as peptides 37-56, 71-90, 101-120, 229-248 and 307-326, were found to be significant in ducks immune response to DHBV. Peptide 267-286, although not significant ($P<0.09$) in this experiment, might also be important.

From this initial analysis of the results seven peptides were selected to be incorporated into a DNA vaccine: 1-15, 7-14W-27, 71-90, 101-120, 229-248, 267-286, and 307-326. Peptide 1-15 was included because it added only an extra 6 amino acids to the sequence (the end overlaps with peptide 7-14W-27), and was intended to be a spacer region for the DNA vaccine (explained in Chapter 8, p.200).

Note that peptide 37-56 was not included in the DNA vaccine because at the time of the initial interpretation of the results, the statistical data for this peptide was lacking.

Peptide	Increase of >5000 cpm															Peptide	Resp	nonR
	G51	G53	G63	G99	P63	W45	V2J	V2K	V2L	V2M	V2N	V2O	V2P	V2Q	V2S			
1-15										+	+					1-15	2	13
7-14W-27	+			+		+	+	+	+	+	+	+	+			7-14W-27	10	5
7-14R-27	+			+	+	+		+	+		+		+			7-14R-27	8	7
22-41		+						+								22-41	2	13
37-56				+				+					+			37-56	3	12
54-73				+												54-73	1	14
71-90	+							+	+							71-90	3	12
87-106				+					+							87-106	2	13
101-120				+						+	+	+	+			101-120	5	10
116-130	+			+												116-130	2	13
126-140													+			126-140	1	14
136-150							+									136-150	1	14
146-160				+												146-160	1	14
156-170																156-170	0	15
166-180				+												166-180	1	14
176-195				+												176-195	1	14
191-210																191-210	0	15
210-229	+					+		+			+		+	+		210-229	6	9
229-248	+			+		+			+	+			+	+		229-248	7	8
248-267	+								+				+			248-267	3	12
267-286				+					+				+	+		267-286	4	11
287-306														+		287-306	1	14
307-326	+			+	+					+				+		307-326	5	10
SMC PHA	+	+	+	+		+	+	+	+	+	+	+	+	+	+	SMC PHA	15	0
SMC LPS	+						+	+	+		+		+	+		SMC LPS	7	8
PBMC PHA	+	+	+	+		+										PBMC PHA	5	1
PBMC LPS																PBMC LPS	0	6

Table 46. *Summary of CMI response of Challenged Immune ducks to Surface ORF peptides (>5000cpm increase).*

Resp: Number of ducks that responded (increase of >5000 cpm over background) (+). **NonR**: Non-responders (blank box). Empty shaded box ( ): not tested.

Peptide	Increase of >5000 cpm																								Peptide	Resp	nonR
	1A	1B	1C	1D	1E	1F	1G	1H	1I	1J	1K	1L	2A	2B	2C	2D	2E	2F	2G	2H	2I	P24P53	V2T	V2U			
1-15																									1-15	0	24
7-14W-27													+											+	7-14W-27	2	22
7-14R-27																								+	7-14R-27	1	23
22-41		+	+																						22-41	2	22
37-56																									37-56	0	24
54-73		+																							54-73	1	23
71-90																									71-90	0	24
87-106																									87-106	0	24
101-120																								+	101-120	1	23
116-130																									116-130	0	24
126-140																									126-140	0	24
136-150																									136-150	0	24
146-160																								+	146-160	1	23
156-170																								+	156-170	1	23
166-180																									166-180	0	24
176-195																									176-195	0	24
191-210																									191-210	0	24
210-229								+													+		+	+	210-229	4	20
229-248																				+				+	229-248	2	22
248-267																								+	248-267	1	23
267-286			+																						267-286	1	23
287-306																								+	287-306	1	23
307-326		+																							307-326	1	23
SMC PHA		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	SMC PHA	24	0
SMC LPS			+	+														+	+	+				+	SMC LPS	6	18
PBMC PHA		+	+		+	+	+		+	+	+	+							+	+	+		+	+	PBMC PHA	14	4
PBMC LPS																									PBMC LPS	0	18

Table 47. *Summary of CMI response of Negative control ducks to Surface ORF peptides (greater than 5000cpm increase).*

Resp: Number of ducks that responded (increase of >5000cpm over background) (+). NonR: Non-responders (blank box). Empty shaded box ( ): not tested.

	Protein vaccinated group		Negative Control group			Fisher Exact	
	Resp	NonR	Resp	NonR		P	< 0.05
1-15	2	13	0	24	1-15	0.142	
7-14W-27	10	5	2	22	7-14W-27	0.001	*
7-14R-27	8	7	1	23	7-14R-27	0.001	*
22-41	2	13	2	22	22-41	0.631	
37-56	3	12	0	24	37-56	0.050	*
54-73	1	14	1	23	54-73	1.000	
71-90	3	12	0	24	71-90	0.050	*
87-106	2	13	0	24	87-106	0.142	
101-120	5	10	1	23	101-120	0.024	*
116-130	2	13	0	24	116-130	0.142	
126-140	1	14	0	24	126-140	0.385	
136-150	1	14	0	24	136-150	0.385	
146-160	1	14	1	23	146-160	1.000	
156-170	0	15	1	23	156-170	1.000	
166-180	1	14	0	24	166-180	0.385	
176-195	1	14	0	24	176-195	0.385	
191-210	0	15	0	24	191-210	ns	
210-229	6	9	4	20	210-229	0.141	
229-248	7	8	2	22	229-248	0.015	*
248-267	3	12	1	23	248-267	0.279	
267-286	4	11	1	23	267-286	0.062	
287-306	1	14	1	23	287-306	1.000	
307-326	5	10	1	23	307-326	0.024	*
SMC PHA	15	0	24	0	SMC PHA	ns	
SMC LPS	7	8	6	18	SMC LPS	0.185	
PBMC PHA	5	1	14	4	PBMC PHA	1.000	
PBMC LPS	0	6	0	18	PBMC LPS	ns	

Table 48. *Summary of the Statistical analysis of the Protein vaccination response (greater than 5000cpm increase).*

The asterisk indicates a significant difference ($P<0.05$) while the shade indicates a possible trend ($P<0.10$). ns: non significant.

7.4.2.2.2. Final analysis using sig P/N method of assessment

The results from the negative, challenged immune, and infected positive control ducks, for the significant P/N analysis have been summarised, Table 49 (p.184), Table 50 (p.185), and Table 51 (p.186), and the statistical analysis of the significant P/N values has been summarised (Table 52, p.187).

None of the DHBV positive infected ducks responded significantly to any of the peptides (see individual results in Appendix 11.9, p.A43).

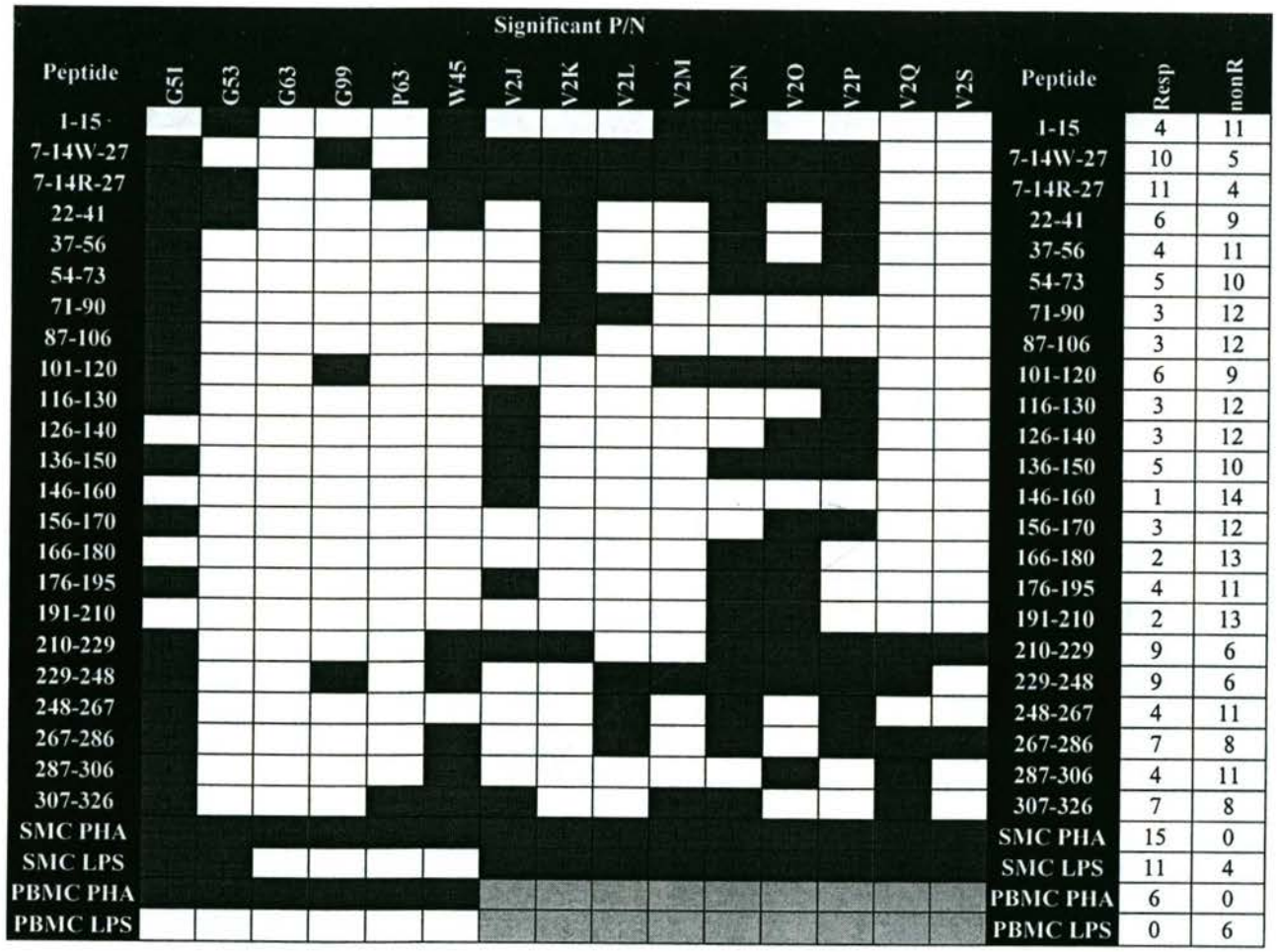


Table 49. *Summary of CMI response of Challenged Immune ducks to Surface ORF peptides (significant P/N).*

Resp: Number of ducks that responded (significant P/N) (■). NonR: Non-responders (blank box). Empty shaded box (▒): not tested.

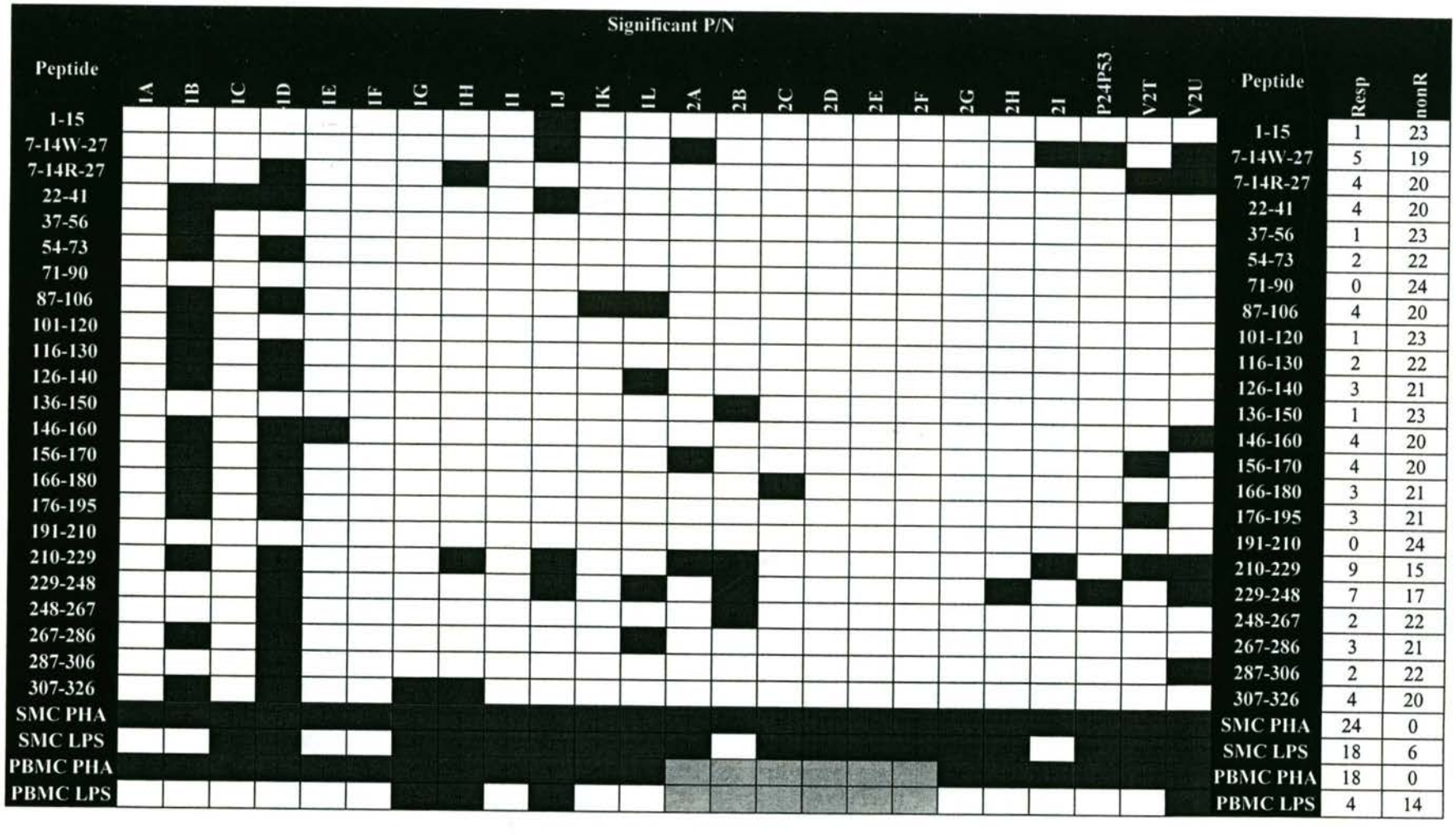


Table 50. *Summary of CMI response of Negative control ducks to Surface ORF peptides (significant P/N).*

Resp: Number of ducks that responded (significant P/N) (■). NonR: Non-responders (blank box). Empty shaded box (▒): not tested.

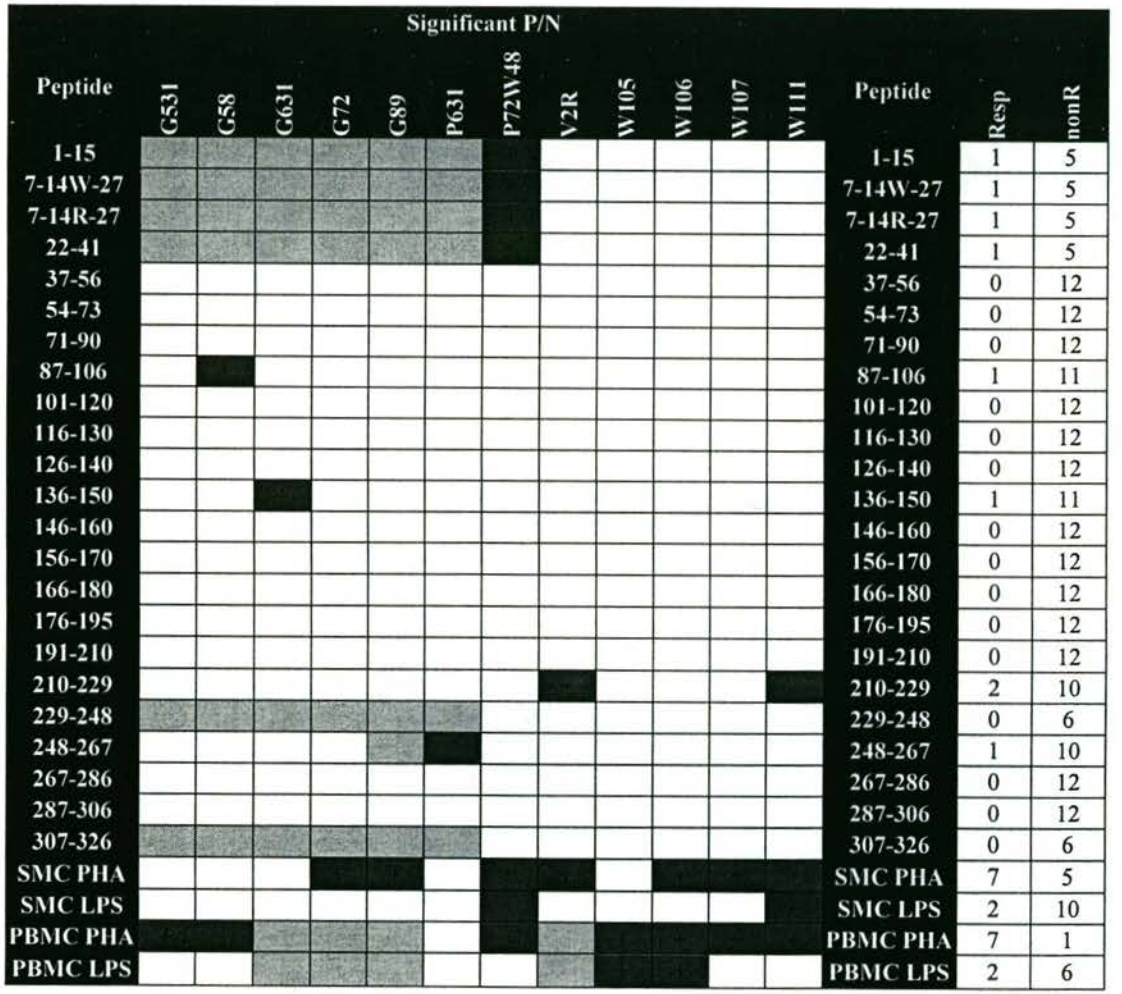


Table 51. *Summary of CMI response of Positive control ducks to Surface ORF peptides (significant P/N).*

Resp: Number of ducks that responded (significant P/N) (■). NonR: Non-responders (blank box). Empty shaded box (▒): not tested.

	Protein vaccinated group		Negative Control group			Fisher Exact	
	Resp	NonR	Resp	NonR		P	< 0.05
1-15	4	11	1	23	1-15	0.062	
7-14W-27	10	5	5	19	7-14W-27	0.007	
7-14R-27	11	4	4	20	7-14R-27	0.001	
22-41	6	9	4	20	22-41	0.141	
37-56	4	11	1	23	37-56	0.062	
54-73	5	10	2	22	54-73	0.085	
71-90	3	12	0	24	71-90	0.050	
87-106	3	12	4	20	87-106	1.000	
101-120	6	9	1	23	101-120	0.008	
116-130	3	12	2	22	116-130	0.354	
126-140	3	12	3	21	126-140	0.658	
136-150	5	10	1	23	136-150	0.024	
146-160	1	14	4	20	146-160	0.631	
156-170	3	12	4	20	156-170	1.000	
166-180	2	13	3	21	166-180	1.000	
176-195	4	11	3	21	176-195	0.396	
191-210	2	13	0	24	191-210	0.142	
210-229	9	6	9	15	210-229	0.203	
229-248	9	6	7	17	229-248	0.094	
248-267	4	11	2	22	248-267	0.180	
267-286	7	8	3	21	267-286	0.027	
287-306	4	11	2	22	287-306	0.180	
307-326	7	8	4	20	307-326	0.068	
SMC PHA	15	0	24	0	SMC PHA	ns	
SMC LPS	11	4	18	6	SMC LPS	1.000	
PBMC PHA	6	0	18	0	PBMC PHA	ns	
PBMC LPS	0	6	4	14	PBMC LPS	0.539	

Table 52. *Summary of the Statistical analysis of the Challenged Immune group to that of the Negative control group (significant P/N).*

The asterisk indicates a significant difference (P<0.05) while the shade indicates a possible trend (P<0.10). ns: non significant.

For the naïve and immunised groups there was good correlation between the >5000cpm analysis results and the significant P/N analysis. After significant P/N analysis of the challenged immune compared to the negative control ducks, both the wild type and mutant form of peptide 7-27 (7-14W-27, and 7-14R-27), as well as peptides 71-90, 101-120, 136-150, and 267-286 were found to be significant (P<0.05) in ducks immune to DHBV. Peptides 1-15, 37-56, 54-73, 229-248, and 307-326 were found to possibly be important (P<0.10). Four of the peptides that were placed into the DNA vaccine on the basis of the >5000 count analysis (7-14W-27, 71-90, 101-120, and 267-286), were again shown to be significant (P<0.05), while the other three (1-15, 71-90, and 101-120), were at least important (P<0.10).

The statistical analysis of the significant P/N values between the challenged immune and the infected control groups has been summarised (Table 53, p.188). These results very much mirror the comparison of the challenged immune and negative control groups.

	Protein vaccinated group		Positive Control group			Fisher Exact	
	Resp	NonR	Resp	NonR		P	< 0.05
1-15	4	11	1	5	1-15	1.000	
7-14W-27	10	5	1	5	7-14W-27	0.063	
7-14R-27	11	4	1	5	7-14R-27	0.046	
22-41	6	9	1	5	22-41	0.613	
37-56	4	11	0	12	37-56	0.106	
54-73	5	10	0	12	54-73	0.047	
71-90	3	12	0	12	71-90	0.231	
87-106	3	12	1	11	87-106	0.605	
101-120	6	9	0	12	101-120	0.020	
116-130	3	12	0	12	116-130	0.231	
126-140	3	12	0	12	126-140	0.231	
136-150	5	10	1	11	136-150	0.182	
146-160	1	14	0	12	146-160	1.000	
156-170	3	12	0	12	156-170	0.231	
166-180	2	13	0	12	166-180	0.487	
176-195	4	11	0	12	176-195	0.106	
191-210	2	13	0	12	191-210	0.487	
210-229	9	6	2	10	210-229	0.047	
229-248	9	6	0	6	229-248	0.019	
248-267	4	11	1	10	248-267	0.356	
267-286	7	8	0	12	267-286	0.008	
287-306	4	11	0	12	287-306	0.106	
307-326	7	8	0	6	307-326	0.061	
SMC PHA	15	0	7	5	SMC PHA	0.010	
SMC LPS	11	4	2	10	SMC LPS	0.006	
PBMC PHA	6	0	7	1	PBMC PHA	1.000	
PBMC LPS	0	6	2	6	PBMC LPS	0.473	

Table 53. *Summary of the Statistical analysis of the Challenged Immune group to that of the Positive control group (significant P/N).*

The asterisk indicates a significant difference (P<0.05) while the shade indicates a possible trend (P<0.10). ns: non significant.

The only difference between the infected ducks and negative control groups was that the negative control group responded significantly better in both SMC PHA and SMC LPS.

	Positive Control group		Negative Control group			Fisher Exact	
	Resp	NonR	Resp	NonR		P	< 0.05
1-15	1	5	1	23	1-15	0.366	
7-14W-27	1	5	5	19	7-14W-27	1.000	
7-14R-27	1	5	4	20	7-14R-27	1.000	
22-41	1	5	4	20	22-41	1.000	
37-56	0	12	1	23	37-56	1.000	
54-73	0	12	2	22	54-73	0.543	
71-90	0	12	0	24	71-90	ns	
87-106	1	11	4	20	87-106	0.646	
101-120	0	12	1	23	101-120	1.000	
116-130	0	12	2	22	116-130	0.543	
126-140	0	12	3	21	126-140	0.536	
136-150	1	11	1	23	136-150	1.000	
146-160	0	12	4	20	146-160	0.278	
156-170	0	12	4	20	156-170	0.278	
166-180	0	12	3	21	166-180	0.536	
176-195	0	12	3	21	176-195	0.536	
191-210	0	12	0	24	191-210	ns	
210-229	2	10	9	15	210-229	0.268	
229-248	0	6	7	17	229-248	0.290	
248-267	1	10	2	22	248-267	1.000	
267-286	0	12	3	21	267-286	0.536	
287-306	0	12	2	22	287-306	0.543	
307-326	0	6	4	20	307-326	0.557	
SMC PHA	7	5	24	0	SMC PHA	0.002	
SMC LPS	2	10	18	6	SMC LPS	0.001	
PBMC PHA	7	1	18	0	PBMC PHA	0.308	
PBMC LPS	2	6	4	14	PBMC LPS	1.000	

Table 54. *Summary of the Statistical analysis of the Positive and Negative control groups (significant P/N).*

The asterisk indicates a significant difference (P<0.05) while the shade indicates a possible trend (P<0.10). ns: non significant.

When the percentage of ducks from the challenged immune and negative control groups that responded to the various peptides is plotted in relation to where that response is in the Surface ORF gene the significant peptides can be seen, as can a correlation in which the overall pattern of the negative ducks follows that of the protein vaccinated ducks (Figure 50, p.190).

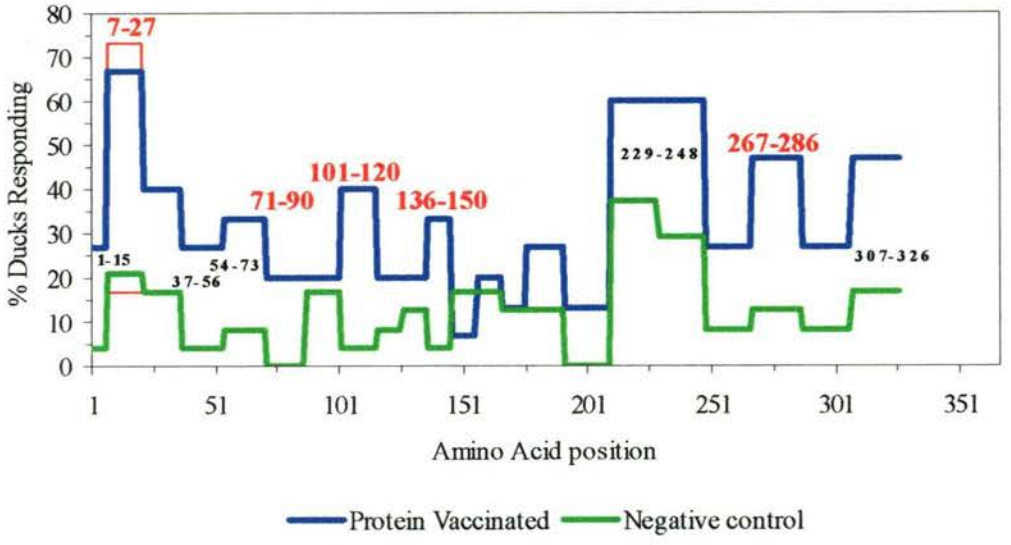


Figure 50. *Plot of response of ducks to peptides in relation to their position in the Surface ORF gene peptide (significant P/N).*

Peptides 7-27, 71-90, 101-120, 136-150 and 267-286 are significantly different ($P<0.05$) (**large font**). Peptides 1-15, 54-73, and 307-326 may be important ($P<0.10$) (**small font**). The thin line indicates the response to the mutant version of peptide 7-27 (7-14R-27).

When the response of the ducks to the peptides (Figure 50, p.190) are compared to the computer modelling predictions of Antigenicity, Hydrophilicity, and Surface probability (Chapter 6, p.150), there is not much similarity. An interesting difference between the computer modelling predictions and the experimental determined values is seen in the case of peptide 210-229, in which the experimental values are much higher than the modelling values.

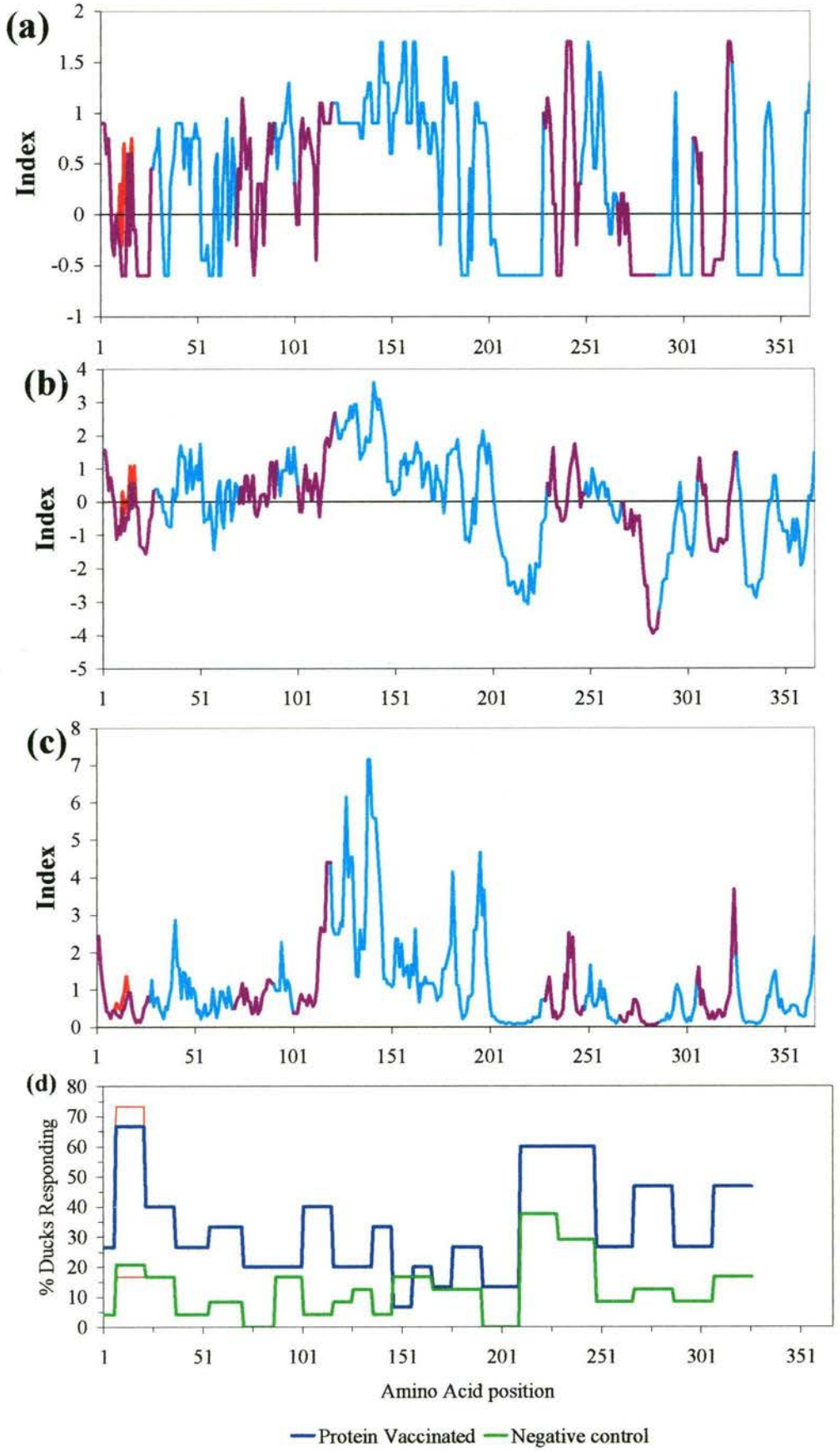


Figure 51. *CMI epitopes in relation to Computer Modelling of the DHBV Surface gene.*

(a) Antigenicity: (Jameson and Wolf algorithm). (b) Hydrophilicity: (Kyte and Doolittle algorithm). (c) Surface Probability: (Emini algorithm). **Dark Blue** line indicates the CMI epitopes selected for DNA vaccine. **Red** line is the difference of the mutation. (d) Peptide response **Blue** line vaccinated. **Green** negative controls. **Red** mutant (7-14R-27).

7.5. DISCUSSION

The results of this study emphasise the inability of hepadnavirus infected individuals to respond to any of the surface antigen-derived peptides, which are significant in the CMI induced by S protein immunisation. The aim of a therapeutic vaccine would be to overcome this unresponsiveness, by using a different mechanism of antigen presentation to the immune system.

Clearance of hepadnaviruses during acute hepatitis is associated with a strong, polyclonal, multi-specific cytotoxic T lymphocyte (CTL) response to the viral envelope, nucleocapsid and polymerase proteins that persists for decades after clinical recovery. It has been demonstrated that chronically infected patients who experience a spontaneous or interferon-induced remission develop a CTL response to HBV that is similar in strength and specificity to patients who have recovered from acute hepatitis (Rehermann *et al.*, 1996b). This suggests that specific immunotherapeutic enhancement of the CTL response to hepadnaviruses should be possible in chronically infected patients, and that it could lead to viral clearance in these individuals with resolution of chronic liver disease.

DNA vaccines have been known to produce effective immune responses in other persistent infections. For instance, healthy adult volunteers were enrolled in a Phase I safety and tolerability clinical study of a DNA vaccine encoding a malaria antigen. The study determined that there were no severe or serious adverse events, and that excellent CTL responses were induced by intramuscular injection of the DNA vaccine (Le *et al.*, 2000). The DNA vaccine technique has also been used for prophylaxis of HBV, but the very small doses used appeared to act only as a booster (Tacket *et al.*, 1999). In the tree shrew model, good antibody responses that reduced experimental transmission, were obtained (Zhou *et al.*, 2003), while both humoral and cellular immunity were strongly stimulated in the mouse model (Du *et al.*, 2003).

In the present experiment the use of a DHBV challenge on the protein vaccinated ducks was two-fold: To show that the vaccination was indeed protective, and to re-stimulate the CMI response, which is known to be transient. The T-cell response in ducks has been shown to decrease rapidly after resolution of DHBV infection (Vickery *et al.*, 1999b; Tang *et al.*, 2001), and vaccination to *Riemerella anatipestifer* (Higgins *et al.*, 2000). In all of these studies the CMI response was reduced almost to undetectable levels after approximately 4-5 weeks. In humans it has been shown that a CMI response is detectable much longer, for 2 to 13 years after clinical resolution of disease (Penna *et al.*, 1996). But the long lasting response in humans may be due to incomplete clearance of HBV from the host (Rehermann *et al.*, 1996a). This low level persistence may be a constant stimulus, which maintains the

activity of the T-cell response. This low level persistence suggests that sterilising immunity to HBV frequently fails to occur after recovery from acute hepatitis and that traces of virus can maintain the CTL response for decades following clinical recovery, apparently creating a negative feedback loop that keeps the virus under control, perhaps for life. In the current experiment the challenge inoculum contained sufficient antigenic mass to serve as a booster dose in its own right.

The use of two methods in analysing the protein vaccinated ducks and the negative controls (>5000cpm, and significant P/N) produced similar results (Table 55, p.194). Four of the six epitopes selected for the DHBV DNA vaccine on the basis of the >5000 counts method were significant ($P<0.05$) by the P/N analysis and the other two were important ($P<0.10$). It was observed that a large difference between SI and P/N values was seen when the background (unstimulated unlabelled) and the controls (unstimulated labelled) had similar values, but these large SI values were completely non-physiological. The original >5000cpm was chosen to be a physiological size response in the assay, based on the average of the negative control ducks. Due to time constraints this less complicated analysis was used as the basis for determining the peptides for use in the DHBV DNA vaccine. Later deliberation and research suggested a more mathematically significant method in which the peptide results were compared with the unstimulated labelled controls using a Student's t-test (2 tailed, 2 sample), this was further limited by only including samples in which a greater than 1000cpm increase over the unstimulated labelled controls was obtained. This 1000cpm limitation was used to remove mathematically significant differences that were not considered to be physiologically relevant (most of the discarded results had a P/N of less than 2.1). These results were then analysed using a Fisher's exact test. The combination of these statistical tests provided greater confidence in assigning biological significance to the results.

The CMI response to the Surface peptide in challenged immune ducks was polyclonal with 6 epitopes (7-14W-27, 7-14R-27, 71-90, 101-120, 136-150, and 267-286), having significantly better responses than the negative controls by the significant P/N analysis ($P<0.05$), and another 4 (1-15, 37-56, 54-73, and 307-326), that show importance ($P<0.10$). All six of the peptides that were selected by the >5000cpm method were significant (4/6), or at least important (2/6). It is interesting to note that many ducks in all groups responded to the 210-229 peptide which was modelled to have similarity to the bacterium *Streptococcus agalactiae* serotype III and V, which could have been present in the stock feed given to the ducks.

	>5000cpm			Sig P/N	
	P	< 0.05		P	< 0.05
1-15	0.142		1-15	0.062	
7-14W-27	0.001	*	7-14W-27	0.007	
7-14R-27	0.001	*	7-14R-27	0.001	
22-41	0.631		22-41	0.141	
37-56	0.050	*	37-56	0.062	
54-73	1.000		54-73	0.085	
71-90	0.050	*	71-90	0.050	
87-106	0.142		87-106	1.000	
101-120	0.024	*	101-120	0.008	
116-130	0.142		116-130	0.354	
126-140	0.385		126-140	0.658	
136-150	0.385		136-150	0.024	
146-160	1.000		146-160	0.631	
156-170	1.000		156-170	1.000	
166-180	0.385		166-180	1.000	
176-195	0.385		176-195	0.396	
191-210	ns		191-210	0.142	
210-229	0.141		210-229	0.203	
229-248	0.015	*	229-248	0.094	
248-267	0.279		248-267	0.180	
267-286	0.062		267-286	0.027	
287-306	1.000		287-306	0.180	
307-326	0.024	*	307-326	0.068	

Table 55. *Comparison of the Statistical analysis for the Challenged Immune group compared to the Negative control group (>5000cpm and significant P/N).*

The asterisk indicates a significant difference (P<0.05) while the shade indicates a possible trend (P<0.10). ns: non significant. The peptides selected for the DHBV DNA vaccine are in black text with light blue background.

The present study has found overlap of CMI and antibody epitopes. The Surface protein is highly antigenic in all hepadnaviruses, and when injected as a protein, most vaccinees produce high levels of antibody. Some of these antibodies are neutralising, and in humans this is the basis of the HBV vaccine; these neutralising antibodies and have been mapped to various regions of the Surface protein (Figure 52, p.196). One of the epitopes of the human antibody response is the hepatocyte attachment region (aa 32-47) (Petit *et al.*, 1991). This hepatocyte attachment region has also been found to overlap with both CD4 and CD8 epitopes (Jin *et al.*, 1988; Ferrari *et al.*, 1992). The present study has found that the epitopes 101-120, and 136-150 overlap with previously determined antibody epitopes (Figure 52, p.196).

The SMC of the positive controls responded significantly less to PHA and LPS than the naïve or vaccinated groups. It is possible that the DHBV infection, is able to induce tolerance by down regulating the immune response in a general way, and thus we observe a significant reduction in response of SMC to PHA and LPS. There is a lack of human SMC

experimental data, but PBMCs of human chronic carriers have been shown to become insensitive to PHA (Scudeletti *et al.*, 1986; Nouri-Aria *et al.*, 1988), while others have demonstrated that lymphocyte transformation by PHA was normal in patients with Hepatitis B, chronic active hepatitis, asymptomatic carriers, and patients with chronic persistent hepatitis (Wicks *et al.*, 1975). CMI suppression, implicating defective T-cells, or accessory inhibitory cells or pathways, may be associated with ducks exhibiting evidence of prolonged liver infection.

The immunogenicity of the mutant peptide is approximately equal to the wild-type form in immune challenged ducks (10 of 15 ducks responded to the wild-type, while 11 of 15 responded to the mutant). This indicates that the lymphoblastogenesis assay is unable to determine any difference between the immunogenicity of the mutant and wild-type forms, but does not exclude the possibility that the mutant has some other immunomodulating effect that we have not been able to determine. The difference in the response of the negative controls to the wild-type and mutant forms was also negligible (5 of 24 verses 4 of 24, respectively). The number of responders from the negative controls for each form was approximately average for all of the peptides (which ranged from 0 to 9 responders). Although the number of responders is significantly lower for the negative controls compared to the immune challenged group, it is interesting to note that there were responders to most of the peptides, indicating that the immune repertoire present in the ducks is capable to responding to several epitopes quite quickly.

Overall the positive control ducks responded to very few epitopes, this may be due in part to the assay technique which uses cells from the spleen. If, during persistent infection, the majority of the cells that are able to respond to DHBV, leave the spleen and travel to the main site of infection (liver), then a low response from the spleen would be expected, and a better response would be obtained from T-cells obtained from the liver. It has been observed that in persistent HBV infections, that higher than normal number of CD8+ cells are found in the liver (Tang *et al.*, 2003), and that they may be recruited from their normal locations (such as the spleen). It has long been known that the absolute number and the percentage of T lymphocytes are significantly decreased in persistently infected patients (Thomas, 1981; Thomas *et al.*, 1982), and in patients with active liver disease (Del Vecchio-Blanco *et al.*, 1980). The distribution of specific immune cells may be modulated by Lamivudine treatment, which in chronic hepatitis B patients, leads to the reconstitution of virus-specific T-cells in the circulation, which may originate from precursor cells within lymph nodes (Malacarne *et al.*, 2003).

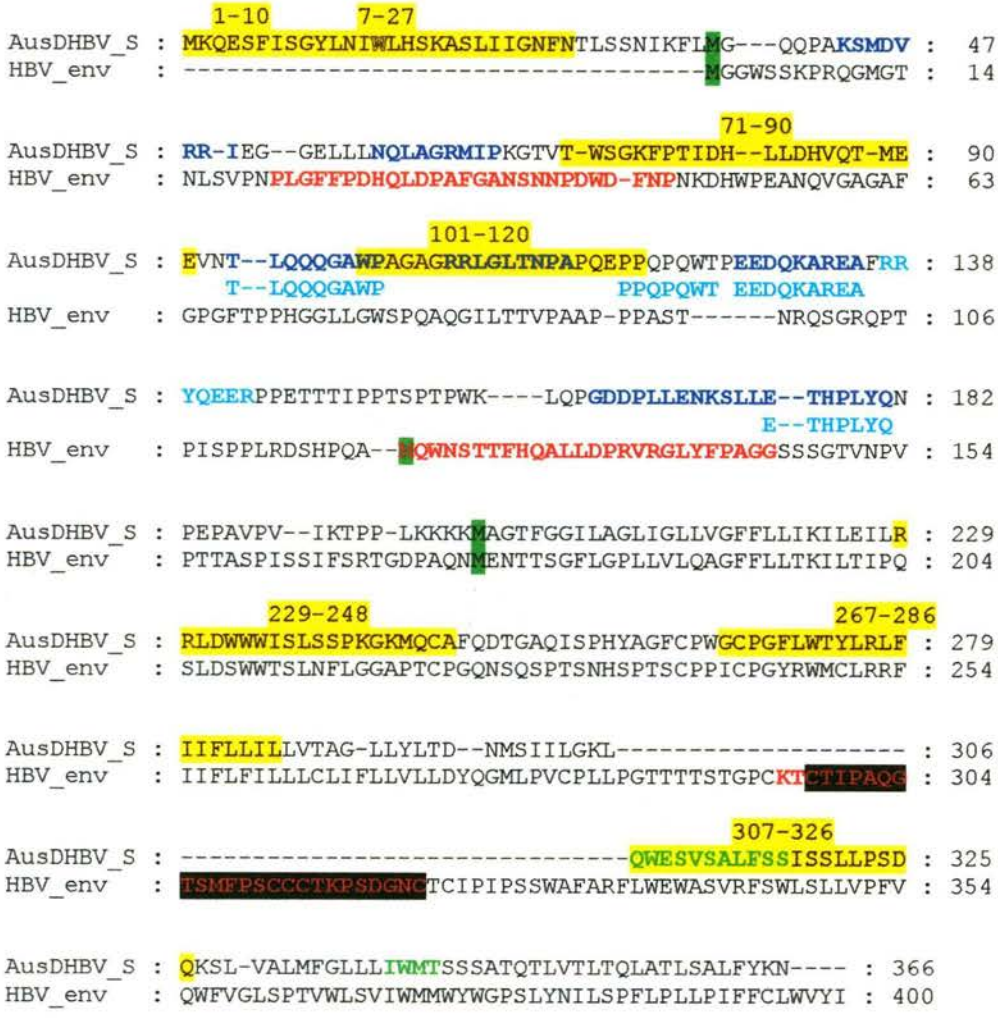


Figure 52. *Known Antibody epitopes in the Surface protein of Hepadnaviruses.*

Dark Blue: Naturally occurring DHBV Ab epitopes (Chassot *et al.*, 1994). **Light Blue**: DHBV Neutralising MAb epitopes (Yuasa *et al.*, 1991; Chassot *et al.*, 1993). **Red**: HBV Ab epitopes (Neurath *et al.*, 1986a; Neurath *et al.*, 1986b; Neurath *et al.*, 1986c; Petit *et al.*, 1991). **Black**: 'a' determinant of HBV. **Green**: Conserved regions in the Polymerase protein (6.4.5, p.160). **Yellow**: the position of selected peptides. **M**: Predicted start of translation of the PreS protein, PreS2, and S respectively.

When the known CMI epitopes of HBV are compared with the determined CMI epitopes of DHBV, there is very little overlap (Figure 53, p.197). The only complete overlap was with peptide 307-326 and a MHC-I restricted CD8 epitope (Nayersina *et al.*, 1993). Peptide 229-248 overlaps with the end of a MHC-II restricted, CD4 epitope (Barnaba *et al.*, 1994).

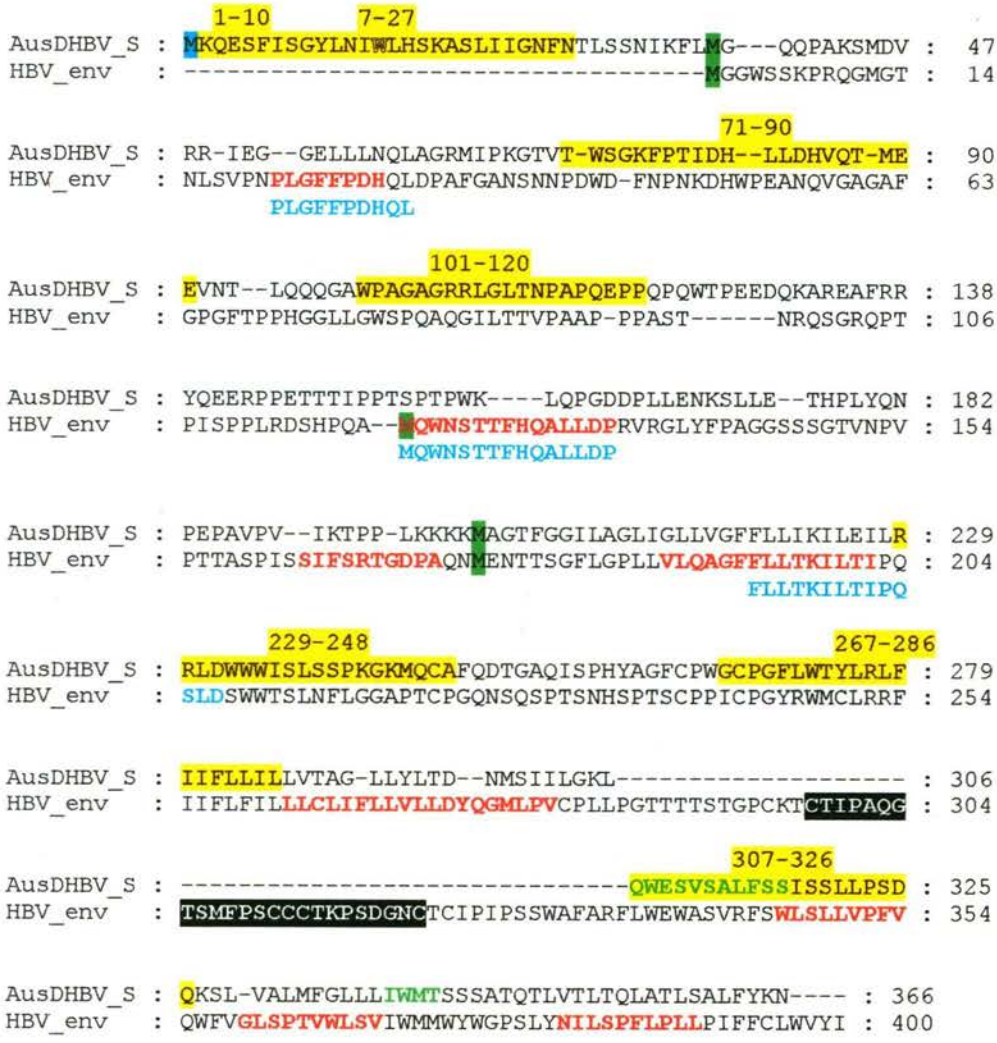


Figure 53. *Aligned Surface protein sequences showing known HBV CMI epitopes.*
Red: MHC-I, CD8 epitopes. **Blue**: MHC-II, CD4 epitopes. **Black**: 'a' determinant of HBV. **Green**: Conserved regions in the Polymerase protein (6.4.5, p.160). **Yellow**: the position of CMI epitopes that were included in the DHBV DNA vaccine. **M**: Predicted start of translation of the PreS protein, PreS2, and S respectively.

Existing computer modelling techniques of proteins cannot be used for the prediction of T-cell epitopes because of the way in which the epitopes are processed for recognition, and so experimental evidence must be produced. However, when the CMI epitopes of both Human and Duck HBV are compared in relation to the computer modelling of the Surface protein an interesting feature is evident. It appears that the CMI epitopes are peptide sequences that seem to have vastly different modelling characteristics over the length of the epitope ie. they have a hydrophilic end and are hydrophobic at the other. This concurs with studies using overlapping peptides of sperm whale myoglobin which have shown a direct correlation

between MHC class II and T-cell receptor binding of epitopes and secondary structure conformation (Berzofsky *et al.*, 1986). The results suggested that MHC class II restricted T-cell epitopes are usually amphipathic structures perhaps so that MHC anchor residues are hydrophobic while the hydrophilic side may interact with the T-cell receptor.

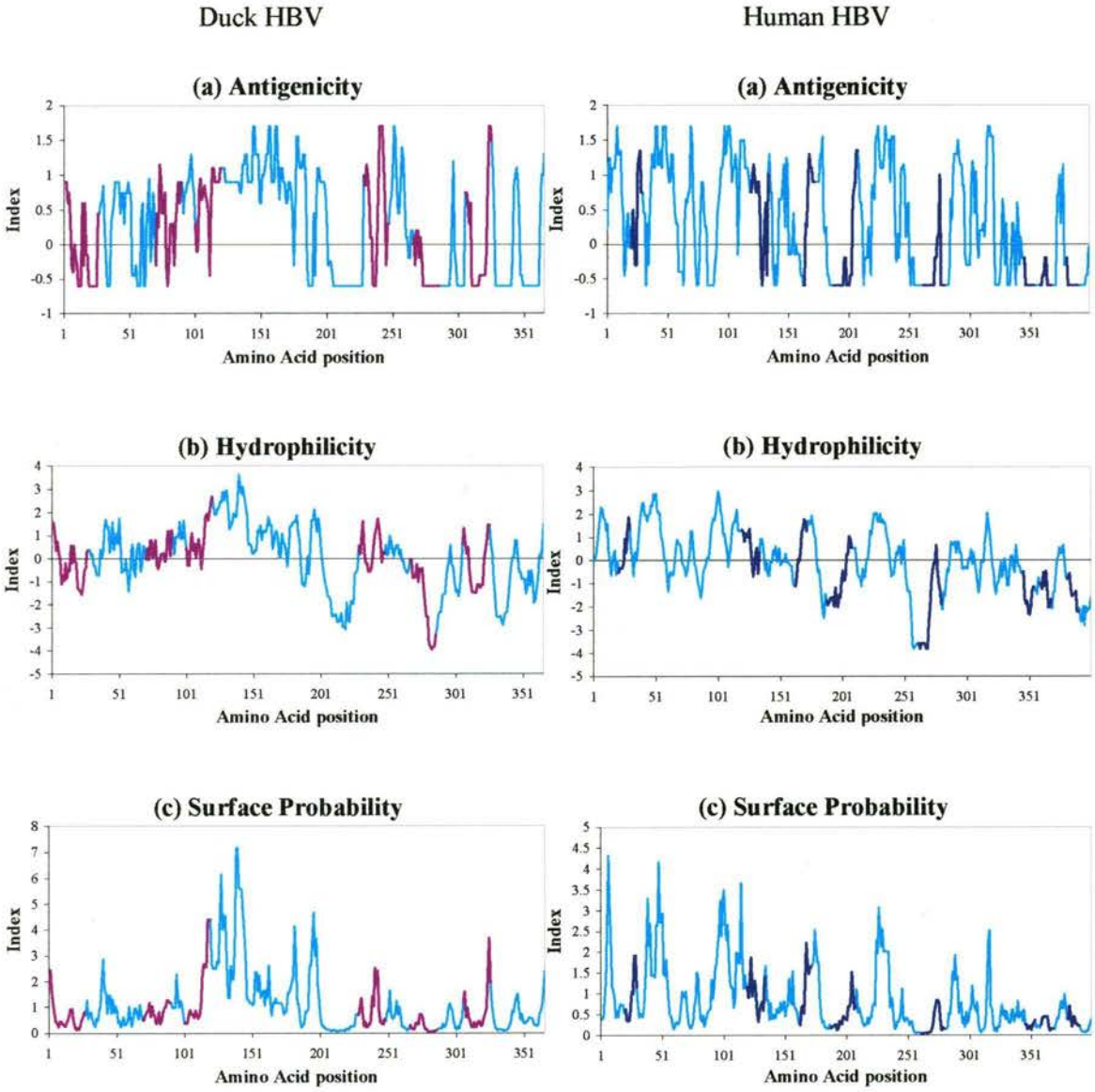


Figure 54. *Comparison of the CMI epitopes for DHBV and HBV in relation to the Computer Modelling.*

DHBV models (CMI epitopes selected for the DHBV DNA vaccine) are on the Left, while HBV models are on the Right. (a) Antigenicity: (Jameson and Wolf algorithm). (b) Hydrophilicity: (Kyte and Doolittle algorithm). (c) Surface Probability: (Emini algorithm). The Dark line indicates the CMI epitopes.

The possibility that the some of the DHBsAg peptides may in fact inhibit the proliferation of the lymphocytes was also investigated using the response data. Several methods were utilised to determine if any down regulation occurred. For the most powerful test used, inhibition was determined to be when the mean cpm of stimulated labelled wells was

Section III

8. DNA VACCINATION

8.1. INTRODUCTION

Increased knowledge of the roles of different T-cell subsets in protection against infectious diseases and in the pathology associated with allergic responses has allowed a rational approach to the development of novel preventive and therapeutic vaccines. It is now possible to design vaccination strategies capable of selectively stimulating different classes of immune responses to specific antigenic epitopes by varying the mode of presentation of antigens and the use of only some of the antigenic repertoire of the infecting agent.

DNA vaccination has been demonstrated to have many functional characteristics. A humoral immune response can be induced to a specific encoded antigen (Tang *et al.*, 1992). An immune response can be generated that is large enough to protect against a lethal influenza challenge (Fynan *et al.*, 1993; Ulmer *et al.*, 1993), or HIV-1 antigen-expressing targets (Wang *et al.*, 1994). It has since been demonstrated that DNA vaccines induce strong immune responses against proteins from infectious agents such as malaria (Wang *et al.*, 1998), tuberculosis (TB) (Lowrie *et al.*, 1997), rabies virus (Xiang *et al.*, 1994), HSV (Kriesel *et al.*, 1996), Ebola virus (Xu *et al.*, 1998), HIV (Boyer *et al.*, 1999), and hepatitis B virus (Davis *et al.*, 1994; Tacket *et al.*, 1999).

The method of administration of a DNA vaccine is very important in determining efficacy. Several methods have been utilised: needle injection into muscle or skin, "gene-gun" bombardment, or topical application to skin or mucosa. Each one of these methods of delivery introduces vaccine to distinct areas of immune surveillance and therefore primes the immune system in distinct ways. Forms of delivery targeting the skin, including *id* injection, gene-gun bombardment, and topical application, have been shown to elicit a humoral response primarily, characterised by a rapid progression to a Th2-type response, associated with the production of an IgA and IgG1 antibody isotype (Boyle *et al.*, 1997). Conversely, injection into muscle results in the induction of a strong cellular-mediated response, or Th1 type, that primes antigen-specific CTLs and is associated with the production of IgG2a antibody (Sin *et al.*, 1999a).

The plasmid pDNAVACC, has been tested while encoding a polytope protein, which contained multiple contiguous minimal murine CTL epitopes (Thomson *et al.*, 1998b). Mice vaccinated with this plasmid made MHC-restricted CTL responses to each of the epitopes, and protective CTLs were demonstrated in recombinant vaccinia virus, influenza virus, and tumour challenge models. CTL responses generated by polytope DNA plasmid vaccination lasted for 1 year (Thomson *et al.*, 1998b). A refinement to pDNAVACC was the incorporation of an Endoplasmic Reticulum signal to enhance the efficiency of class II-restricted endogenous presentation of minimal class II-restricted CTL epitopes by specifically targeting a polyepitope protein to class II processing compartments through the endosomal and/or lysosomal pathway. A significantly enhanced stimulation of virus-specific CD4+ T-cell clones by antigen-presenting cells (APC) expressing the recombinant polyepitope protein targeted to the endocytic/secretory pathway was readily demonstrated in cytotoxicity assays (Thomson *et al.*, 1998a). Such vaccines may even be able to break the immunologic 'tolerance' which characterises many persistent infections including hepatitis B.

Recently, several articles have been published, in which the DNA vaccination approach was used with hepadnaviruses, producing varying results. The DHBV model was used to evaluate the efficacy of combination therapy; adefovir and DNA-immunisation (using the entire preSurface gene) were compared with respective monotherapies. Eight weeks after the third DNA boost, viraemia within the combination therapy group tended to be lower than that of the other groups. An additive effect was also observed since there was a 51% decrease of DHBV DNA in liver at autopsy, while only 38 and 14% during pCI-preS/S or adefovir monotherapies, respectively. This effect was sustained for 12 weeks after the end of therapy (Le Guerhier *et al.*, 2003). Another study was designed to test the efficacy of antiviral treatment with entecavir in combination with DNA vaccines expressing DHBV antigens (preSurface, Surface, preCore, and Core) as a therapy for persistent DHBV infection in ducks. Intramuscular administration of five doses of a DNA vaccine, both alone and concurrently with ETV treatment, did not result in any significant effect on viral markers (Foster *et al.*, 2003). A murine model was used to examine the functionality of a DNA vaccine expressing the HBV surface antigen, in combination with various cytokines. It was determined that the HBV DNA vaccine was strongly antigenic for both humoral and cellular immunity, which can be promoted by a plasmid expressing IL-2 or IL-12. It was also elucidated that it was the CD8+ cells that executed the CTL activities (Du *et al.*, 2003).

We therefore undertook production of a DNA vaccine that contained the T-cell epitopes, which we had shown to be important in the response to DHBV (Chapter 7, p.170). The immunogenicity of the DNA vaccine was determined in naïve ducks, by measuring the T-

cell response in a lymphoblastogenesis assay. Its protective efficacy was determined by challenge and its mechanism further investigated by determining whether neutralising antibodies were induced. The therapeutic efficacy was determined by observing the effect of vaccination on viraemia in persistently infected ducks.

8.2. AIMS

(1) To characterise the response of naïve ducks to DNA vaccination by measuring the production of a CMI response to the epitopes used in the DNA vaccine, protection from challenge, and production of neutralising antibodies.

(2) To determine the effect of the DNA vaccine when used therapeutically in persistent carrier ducks.

8.3. EXPERIMENTAL DESIGN

8.3.1. Vaccine production

A DNA vaccine was designed incorporating the seven antigenically important T-cell epitopes identified in the previous chapter. Published methods (Thomson *et al.*, 1995) were used and Dr. Scott Thomson very kindly helped in the design of the DNA vaccine, and provided the DNA vaccine plasmid pDVERA2.

A Duck Poly (DP) DNA fragment encoding the T-cell epitopes was produced, and then cloned into a bacterial vector. The DP was then subcloned into the DNA vaccine vector pDVERA2.

8.3.2. Immunisation protocol: DNAvacc1 - Immunogenicity and protective efficacy.

Fourteen ducks were divided into two equal groups. One group was vaccinated with the DNA vaccine once a week for three weeks, while the second was treated in the same manner but with PBS. In the fourth week, the CMI response of the ducks to the peptides incorporated into the DNA vaccine was determined, and then the ducks were challenged with $2.5x10^{10}$ vge of DHBV (this was equivalent to approximately $10ID_{50}$, Dr. Karen Vickery, personal communication). The ducks were then bled twice a week for another four weeks before the CMI response was again determined and samples taken from serum and the liver.

8.3.2.1. Mechanism of protection

The serum from two protected, and one unprotected duck was then used to determine the presence of neutralising antibodies by inoculating day old ducklings with the mixtures of serum and virus after a one hour incubation at room temperature *in vitro*.

8.3.3. Immunisation protocol: DNAvacc2 - Therapeutic vaccination.

The therapeutic potential of the DNA vaccine was determined by vaccination of six persistently infected ducks and comparing the outcome with another five unvaccinated persistently infected ducks.

8.4. MATERIALS AND METHODS

8.4.1. Preparation of DNA Vaccine

The seven antigenically important epitopes identified in the previous experiment were peptides 1-15, 7-14W-27, 101-120, 136-150, 229-248, 267-286, and 307-326 (Table 56, p.203).

Peptide	Size (aa)	Protein Sequence	nt
1-15	15	MKQESF**ISGYLNIWL**	693-737
7-14W-27	21	**ISGYLNIWL**HSKASLIIGNFN	711-773
101-120	20	TWSGKFPTIDHLLDHVQTME	903-961
136-150	20	WPAGAGRRLGLTNPAPQEPP	992-1051
229-248	20	RRLDWWWISLSSPKGKMQCA	1376-1435
267-286	20	GCPGFLWTYLRLFIIFLLIL	1490-1549
307-326	20	QWESVSALFSSISSLLPSDQ	1610-1669

Overlap of RW1 and RW2 is indicated by bold lettering

Table 56. *Antigenically Important Peptides.*

8.4.1.1. Artificial Duck Polytope

The protein sequences of these epitopes were lined up to form a single chain of amino acids, the Duck Polytope (DP). The overlap of peptide 1-15 and 7-14W-27 (Table 56, p.203) was removed, producing a single peptide of 127 aa.

8.4.1.1.1. Design of the Duck Polytope

Normal T-cell epitopes vary between 8-12 aa, and require spacer regions between them to allow enhanced processing and presentation, however, because our epitopes were non-minimal CD8 epitopes (ie. between 15-21 aa), it was considered that no spacer regions were required between the epitopes. The large size of our epitopes are an advantage in this case, as they eliminate the need for unnatural flanking regions which may have unforeseeable effects on processing of the DP peptide and the immune response to it.

When the DNA is translated into mRNA it must have a sequence that specifies ribosome binding upstream of or overlapping the initiation, AUG codon. In eukaryotes, there is a consensus sequence called the Kozak sequence (CCACC). This sequence was placed immediately before the ATG codon, on the DuckPolytope.

A requirement of an artificial polytope is that the peptide must begin with the normal start site of translation, an ATG (Methionine, Met, or M), again, our DP already had a start codon, and as such, one did not have to be added. Translation must also be stopped, and this was achieved by placing a stop codon (TGA) at the end of the DP.

Because certain codons for the individual amino acids are preferentially found in nature, the aa sequence for the DP was reverse transcribed to produce a DNA sequence that contained the highest frequency codons for vertebrates. This technique should allow for the most efficient translation of the DP, and was achieved using the program BackTranslate (GCG).

After reverse translation the DNA sequence was checked for restriction enzyme sites using Map and MapPlot (GCG). Any common sites are removed to allow the use of cheap, common restriction enzymes in the cloning process (Figure 55, p.205). The sequence of the DP did not have to be altered.

Restriction enzyme (RE) sites were added to the DNA sequence to allow it to be cut out of the cloned vector and then subcloned into the DNA vaccine vector pDVERA2. The RE chosen were NotI and XbaI for the DP, and NotI and AvrII for the DNA vaccine vector pDVERA2. XbaI (TCTAGA) and AvrII (CCTAGG) are related by having the same cohesive overlap (CTAG) so that when the two pieces of DNA, which are cut with an enzyme each, are joined, the resulting sequence is not recognised by either RE (TCTAGG). The RE map of the DP was double checked to make sure that it did not contain any sequences that would be recognised by the RE.

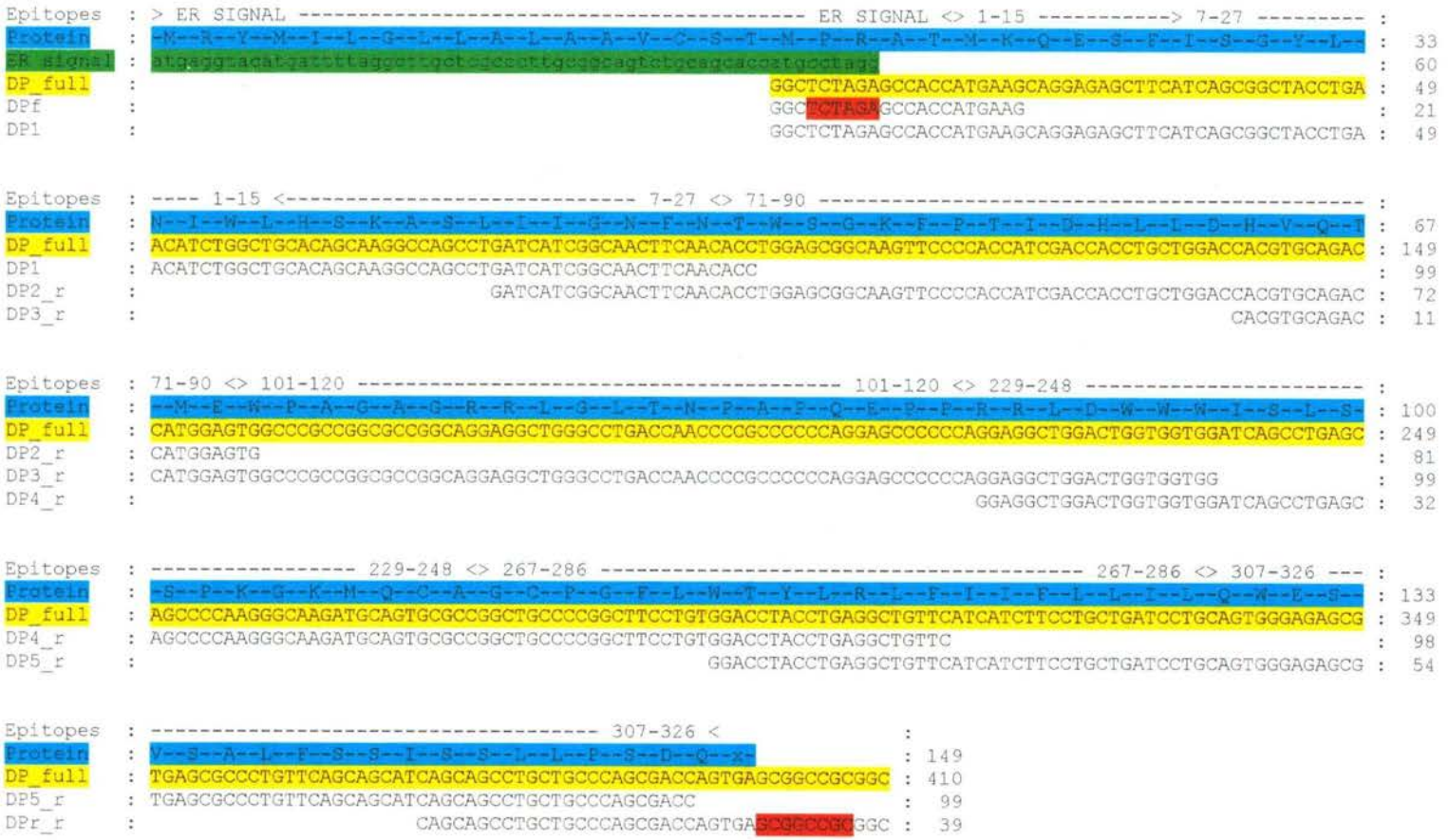


Figure 55. *DNA sequence of DuckPoly aligned with protein sequence and DP oligonucleotides.*

Blue: Protein sequence of DP. Yellow: DNA sequence of DP (optimised codons – not necessarily the sequence of the original DHBV). Green: The ER signal sequence (which is part of pDVERA2 plasmid). pDVERA2 and DP were joined by ligating AvrII-cut and XbaI-cut fragments respectively, and at the other end by ligation of NotI-cut fragments. DPx_r (where x=2-5) indicates the reverse complement of the oligonucleotide to more easily see the alignment. DPf is the forward primer. DPr is the reverse primer. The red indicate the restriction enzyme sites XbaI (TCTAGA), and NotI (GCGGCCGC).

8.4.1.1.2. Production of the DP gene

After the DP was designed and optimised it was 410 nt long. It is currently not technically possible to artificially produce such a long strand of DNA, so the sequence was then divided into approximately equal length oligonucleotides (approx. 100 nt each), each overlapping the next by 10-20 nt.

These oligonucleotides were then synthetically produced (SigmaGenosys, www.sigmagenosys.com.au) at the 0.02μM scale, in the same manner that normal PCR primers are produced. However, there is a high error rate when such long fragments are produced and the number of shorter fragments is very high, and the fragment also tends to branch producing other artefacts. Purification is required before further manipulations are attempted.

8.4.1.1.3. Purification of the DP oligonucleotides

The DP oligonucleotides were purified by running on a polyacrylamide gel and then cut out. The lyophilised DP oligonucleotides were resuspended in 200μL of TE (1mM EDTA, 10mM Tris, pH 8.0). A 5% polyacrylamide gel was produced (2.3.4.1.2, p.89), incorporating 1mg/mL Ethidium bromide. The DP oligonucleotides were diluted 1:10 with dH_2O, and 5μL was mixed with 5μL 2x PCR loading buffer, and loaded onto the gel. The gel was run at 90-100V, 250mA, for 50-90mins. The gel was photographed and the DP oligonucleotide bands cut out.

The fragment of gel that was removed was placed into an Eppendorf and homogenised with the plunger of a 1mL syringe. The eppendorf was spun in a benchtop centrifuge at 15000rpm for 15mins. The supernatant was used to produce the full length DP by PCR.

The synthesised DP oligonucleotides were found to contain many smaller, and even larger fragments, which are branched forms of DNA, all of these are artefacts of the synthetic production technique (Sigma-GenoSys, personal communication). Without purification there is little likelihood of successfully producing the full DP by PCR because only a smear would result (Figure 56, p.207).

8.4.1.1.4. Production of the full length DP by PCR

The full length DP is produced by stepwise asymmetric PCR (Sandhu *et al.*, 1992). Basically, all the overlapping primers are placed into a single PCR reaction, and eventually a full-length fragment is produced.

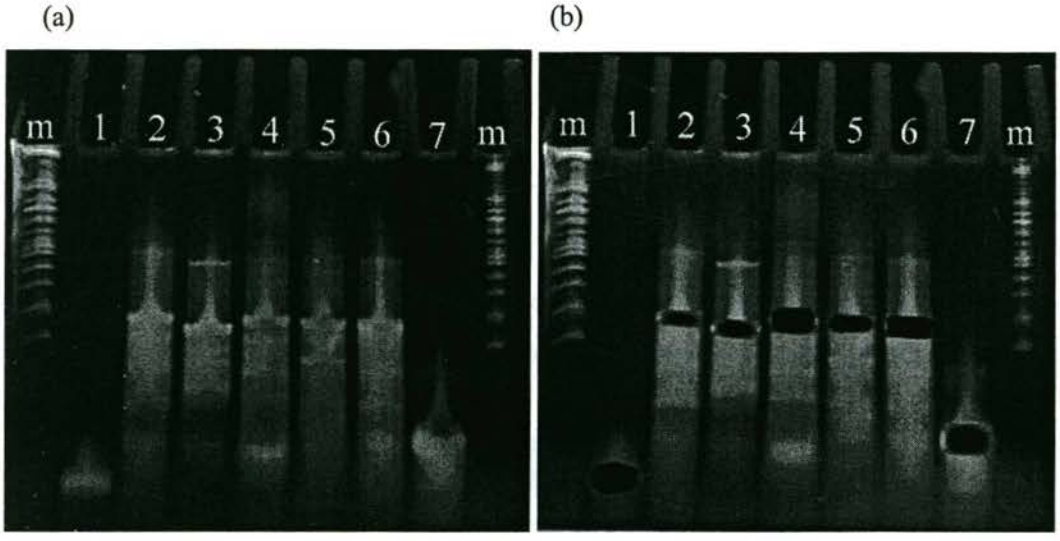


Figure 56. *Photographs of the Polyacrylamide gel used to purify the DP oligonucleotides.*

(a) Gel after run. (b) Gel after DP oligonucleotides cut out.

1-7: DP oligonucleotides - DPf, DP1, DP2, DP3, DP4, DP5, and DPr, respectively.

The quantity of the internal primers is highly limited, and the resultant reaction causes an asymmetric single-stranded amplification of the total sequence due to an excess of the two flanking primers. In subsequent PCR cycles, the asymmetrically amplified fragments, which overlap each other, yield a double-stranded, full-length product. A PCR cocktail was produced (Table 57, p.207), that contained all of the purified oligonucleotides. HiFi polymerase mixture (Boehringer Mannheim, Mannheim, Germany, or Roche, Mannheim, Germany), was utilised to limit the amount of incorrectly incorporated nucleotides. The fidelity of the HiFi mixture is quoted as an error rate of 8.5×10^{-6}, which is approximately a third of that for Taq (2.6×10^{-5}) (Boehringer Mannheim, Mannheim, Germany). Cycling conditions consisted of an initial denaturation at 95°C for 5min, thence 30s, annealing at 55°C for 1min, extension at 72°C for 1min, with a final extension at 72°C for 10min after 30 cycles.

Reagent	DP PCR	DP ReAmp
10xBuffer	1x	1x
$MgCl_2$	2.5mM	2.5mM
dNTP	200nM	200nM
Primer (each)	4µL DP1-DP5	0.4µM DPf+DPr
Polymerase	2U /25µL	2U /25µL
dH_2O	to 25µL	to 25µL

Table 57. *DP PCR cocktail contents.*

The DP PCR was re-amplified using the DP ReAmp cocktail (Table 57, p.207), and the same cycling conditions as per DP PCR. The DP ReAmp reaction consisted of only the outer two primers (DPf, and DPr) (Figure 55, p.205), which should only amplify a full-length copy of

the DP gene. The DP and ReAmp PCR fragments were visualised on a 2% agarose gel as per normal PCR (2.2.2.4, p.71). The DP ReAmp product was PEG purified as per Sequencing (2.2.2.5, p.71).

The DP PCR and DP ReAmp PCR had to be optimised to obtain a cocktail and cycling combination that was adequate to produce amplification of the required DNA fragment: the optimised reaction is given in Table 11 (p.69). When both of the reactions were run on a gel, a band of ~400bp was found as expected in the ReAmp reaction (Figure 57, p.208).

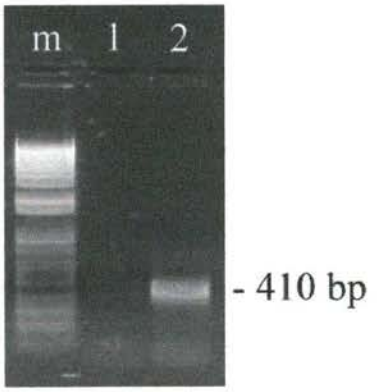


Figure 57. *DP PCR products.*
m: marker, (1) DP PCR, (2) DP ReAmp.

When the DP ReAmp PCR product was sequenced it was found to have the correct sequence, but was not perfectly clean (Figure 58, p.208). Because of the use of the high fidelity polymerase mixture, it is unlikely that the errors were due to the PCR reaction, but rather the synthesis of the oligonucleotides.

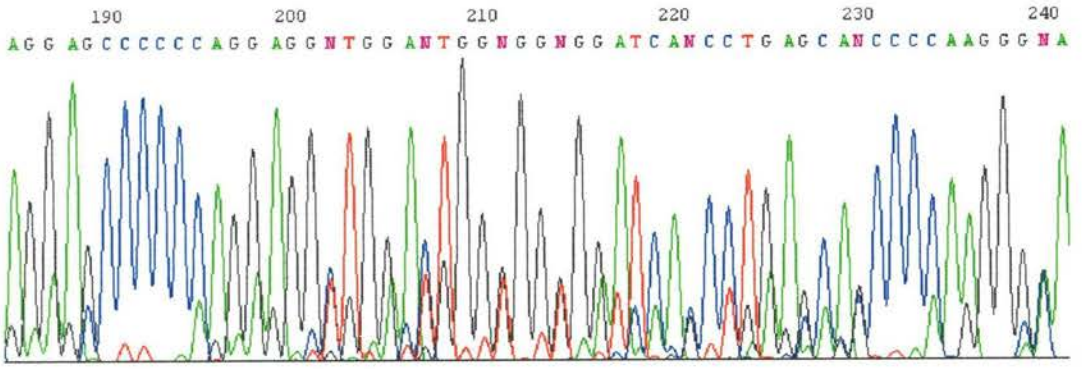


Figure 58. *Short section of the PCR sequence data for the full DP PCR product.*
Note: Many peaks can be seen which contain more than 1 type of nucleotide.

8.4.1.2. Cloning of the DP

The DP ReAmp product was cloned into *E. coli* using a TOPO TA Cloning kit (Invitrogen Life Technologies, Carlsbad, USA), using the directions supplied. Briefly, the purified DP ReAmp product was incubated with TOPO treated plasmid (pCR 2.1-TOPO). The cloning reaction was then used to transform TOP10' *E. coli* cells. The cells were then plated onto X-gal treated 50μg/mL Ampicillin LB agar plates. After overnight incubation at 37°C the transformed bacteria with the DP insert are a white colour, while blue coloured colonies do not contain a copy of the DP (Figure 59, p.210). Several clones were selected for sequence verification, and a colony with the correct sequence was used further.

8.4.1.2.1. MiniPrep DNA extraction from Bacteria

Colonies of bacteria were picked off the selective plates and incubated in 5mL of LB broth (50μg/mL Ampicillin) at 37°C, 255rpm for 8hrs. 1.5mL was spun in a benchtop centrifuge at 13000rpm, for 30s, and the supernatant discarded. 100μL of TELT solution and 1μL of DNase free RNase A added, and the pellet resuspended. The cells were incubated at 37°C for 10mins, 100μL of Phenol:chloroform (1:1) were added, vortexed, and spun in a benchtop centrifuge for 1min at 13000rpm. The top aqueous phase was removed to a new tube and 2 volumes of cold absolute ethanol added, and incubated at –20°C for 30mins. It was then spun for 30mins at 13000rpm in a benchtop centrifuge, the supernatant aspirated, and the pellet dried at 42°C. The pellet was resuspended in 25μL of dH_2O. The DNA could then be used to verify the insert by restriction enzyme digestion, as previous, or used for DNA sequencing.

The cloning of the full DP into TOP10' E. coli was repeated several times because of the difficulty in inserting the full DP into the pCR2.1-TOPO plasmid. Once transfected, bacterial colonies with the full DP insert remained white when grown on X-gal treated selective LB agar plates (Figure 59, p.210).

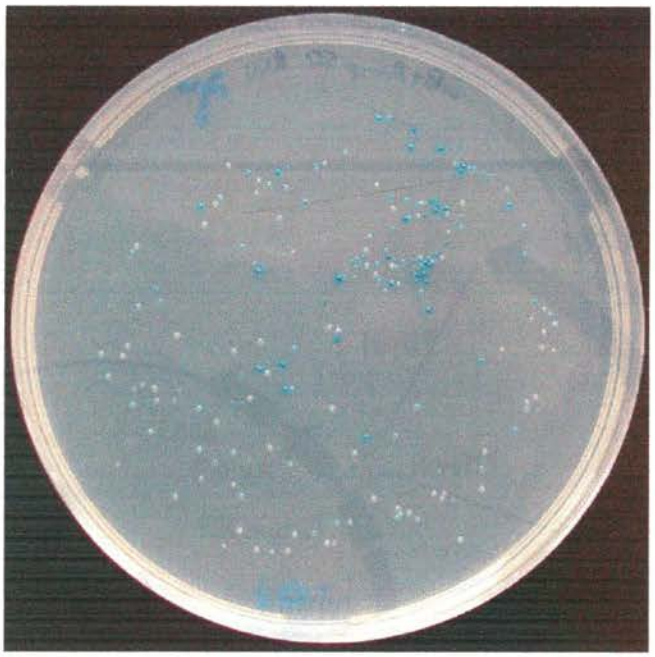


Figure 59. *Photograph of DP transformed colonies.*

21 colonies were sequenced and only two contained the correct 410 bp sequence expected for the full DP. The minor differences in the sequence of the other 19 consisted of one or two incorrect base substitutions, or deletions (Figure 60, p.210). This was expected, as the large 100nt synthesised oligonucleotides are at the limit of what is currently technically possible to synthesise.

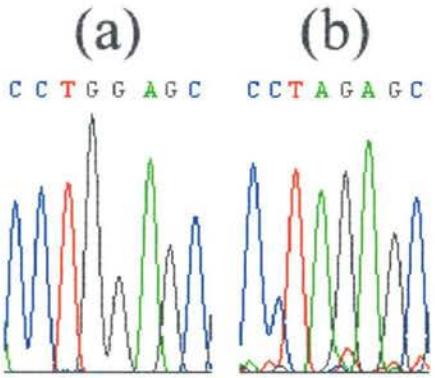


Figure 60. *Example of the differences found in the full DP from a clone.*
(a) Correct sequence (CCT**G**GAGC). (b) Incorrect sequence (CCT**A**AGAGC)

8.4.1.3. Subcloning the DP to produce the DHBV DNA vaccine

To facilitate the processing and presentation of the DP protein, a DNA vaccine vector that contained an ER (Endoplasmic Reticulum) signal was used. The ER signal was expressed before the DP protein and was derived from Adenovirus, however also includes a few extra amino acids at the carboxyl end to act as a spacer before the DHBV epitopes. The DNA vaccine plasmid chosen was pDVERA2 (Figure 61, p.211), (a kind gift of Dr Scott

Thomson), which is a modified ampicillin resistant DNA vaccine plasmid of pDNAVACC (Thomson *et al.*, 1998b).

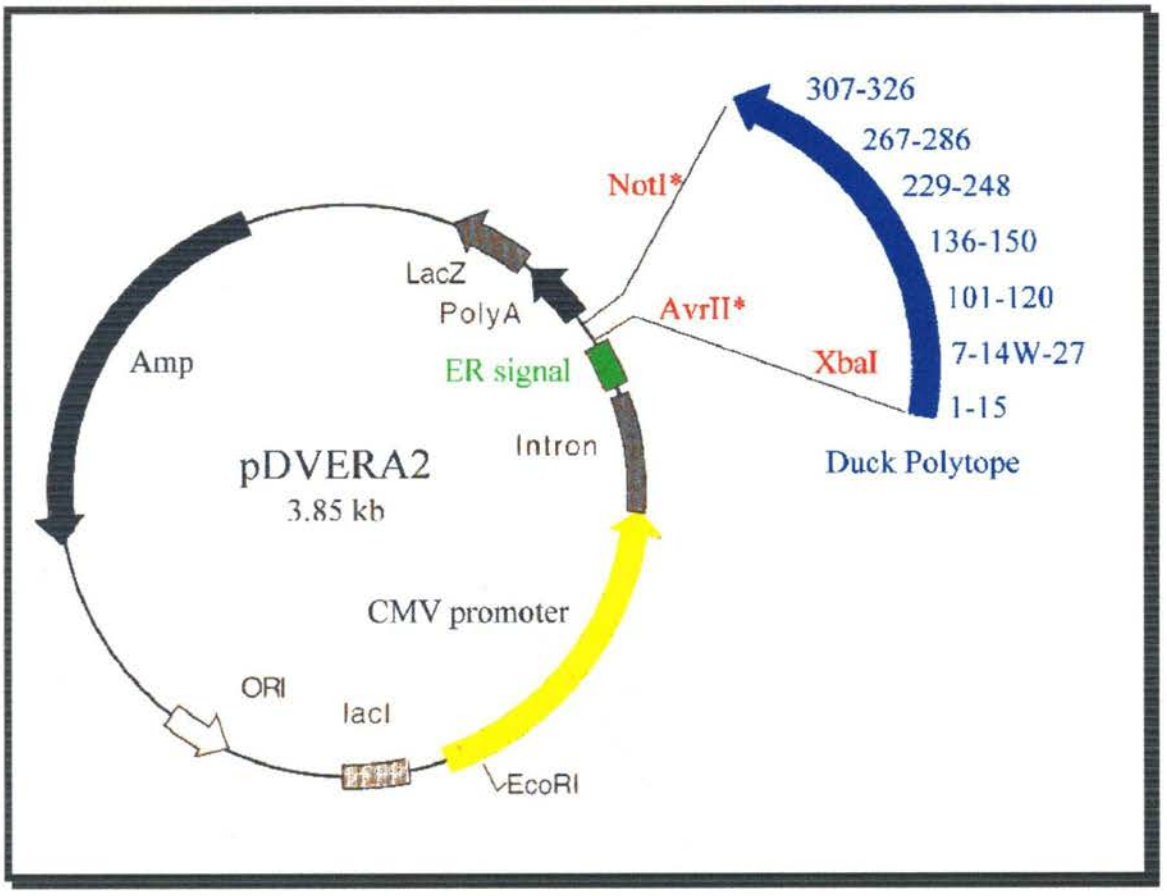


Figure 61. *Plasmid map of pDVERA2 DNA vaccine.*
Blue: DuckPolytope. **Red**: Restriction enzyme sites (* indicates unique site)

The cloned DP extracted DNA was double digested with 1U of each of two restriction enzymes XbaI, and NotI, in 1µL of 10x buffer (XbaI), and 8µL of purified DP at 4°C for 20hrs. The reaction was PEG purified as previous (2.2.2.5, p.71).

The pDVERA plasmid was obtained from Dr. Scott Thomson (kind gift), on a glassfibre mat. The DNA spot was cut out and resuspended by placing it into 10µL of TE (0.1mM EDTA, 10mM Tris, pH 8.0) at RT for 2hrs. It was double digested with 1U of each of two restriction enzymes AvrII, and NotI, in 1µL of 10x buffer (AvrII), and 8µL of resuspended pDVERA plasmid at 4°C for 20hrs. The reaction was PEG purified as previous (2.2.2.5, p.71).

The cut DP was inserted into the cut pDVERA plasmid to form the DHBV DNA vaccine. The fragments of DNA were joined by incubating 4µL of each purified cut fragment with 200U of T4 DNA ligase (New England Biolabs, www.neb.com), and 1µL of 10x buffer at 16°C for 20hrs.

2µl of the cloning reaction was added into a vial ($2x10^6$ cells) of chemically competent *E. coli* (F- *mcr*A .(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*Z.M15 .*lac*X74 *rec*A1 *deo*R *ara*D139 .(*ara-leu*)7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G) (Invitrogen Life Technologies Carlsbad, USA), mixed gently, and incubated on ice for 5 to 30 minutes. The cells were heat-shocked for 30 seconds at 42°C without shaking. The cells were then immediately transferred to ice, and 250µl of room temperature SOC medium added (2% Tryptone, 0.5% Yeast Extract, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$, 20 mM glucose). The cells were shaken at 37°C, 200rpm for 1 hr. 10-50µl was spread on a prewarmed selective LB plate (50µg/mL Ampicillin) and incubated overnight at 37°C.

Several colonies were selected for sequencing as before. A bacterial colony with the correct sequence was then selected and a large amount of plasmid (now DHBV DNA Vaccine) was recovered by MaxiPrep (Qiagen, Melbourne, Australia).

After the full DP was inserted into pDVERA2 to produce the DHBV DNA vaccine 10 clones were sequenced of which 8 were found to have the correct sequence. One clone was selected and several batches of MaxiPreps were produced. They were individually sequenced, and all found to be correct. The pooled DNA vaccine was sequenced both forwards and in reverse, and again found to be correct.

8.4.1.4. Large scale production of DHBV DNA Vaccine

A Qiagen MaxiPrep kit (Qiagen, Melbourne, Australia) was used for large-scale plasmid purification, as per manufacturers recommendations. Briefly, a starter culture was produced from a colony on LB agar (50µg/mL Ampicillin) inoculated into 5mL of LB broth (50µg/mL Ampicillin), and incubated at 37°C, 255rpm for 5-8hrs. 150µL of the starter culture was added to 100mL LB broth (50µg/mL Ampicillin), and incubated at 37°C, 255rpm for 20hrs. The plasmid was then released from the bacteria by chaotropic salt treatment, and adhered to a DEAE-Sepharose column. The column was washed with buffer. The DNA was released from the DEAE-Sepharose column by a buffer with a higher pH. The DNA was then precipitated with isopropanol, and washed with ethanol. The dried DNA pellet was resuspended with 250µL of TE (1mM EDTA, 10mM Tris, pH 8.0). Several batches were produced and individually sequenced to confirm the correct sequence, before being pooled into a large single batch, which was again sequenced. This single batch was used in all experiments. The concentration of the purified DNA vaccine was then determined by spectroscopy (2.2.4, p.73).

8.4.2. Immunogenicity of the DNA Vaccine *in vivo*

8.4.2.1. Vaccination of naïve ducks (DNAvacc1)

Fourteen ducks were randomly divided into two groups: DNA vaccinated, and Control group (Table 58, p.213). They were prebled and tested for DHBV to ensure negativity on day of hatch. On day 7 the DNA vaccinated group was injected *intramuscularly* in 3 sites with 20μg/duck of DNA vaccine plasmid dissolved in 300μL PBS. On day 14 the DNA vaccinated group was injected *intramuscularly* in 2 sites, and *intradermally* in 1 site with 10μg/duck of DNA vaccine plasmid dissolved in 300μL PBS. On day 21 the DNA vaccinated group was injected *intramuscularly*, and *intradermally* in 1 site each with 10μg/duck of DNA vaccine plasmid dissolved in 300μL PBS. The control group was similarly injected each time with PBS.

Group	Ducks	Number
DNAvacc1	7	B67, B68, G57, G97, G98, W39, and W133
Dv1 Controls	7	G92, G93, G100, W42, W118, W120, and W124

Table 58. *Ducks used to determine the immunogenicity of the DNA vaccine (DNAvacc1).*

On day 28, 10mL of blood was drawn into 10mL Heparin/PBS for determination of the PBMC CMI response to the peptides incorporated into the DNA vaccine. The method used was the same as for PBMC previous (7.3.2.1, p.174).

On day 32, all ducks were challenged with 1mL of DHBV201299 serum pool ($2.5x10^{10}$ vge, approximately $10ID_{50}$) injected *intravenously*. The ducks were bled on days 36, 40, 43, 46, 49, 53, 56, and 60-62. The spleens were harvested between days 60 and 62. The spleen was processed as previous (7.3.2.2, p.175). The CMI response was also determined as previous (7.3.2, p.173). All serum and liver samples were tested by dot blot hybridisation and PCR.

8.4.2.2. Neutralisation Assay

A neutralisation assay was performed to determine if DNAvacc1 ducks that were protected from challenge had produced neutralising antibody in response to DNA vaccination. Two challenge protected ducks (G97, and W133), and an unprotected control duck (W39) were chosen. Pre-vaccination test bleeds were tested in parallel for the two protected ducks, and their pre-challenge serum was tested neat and at a 1/10 dilution.

The neutralisation test was set up as follows: Virus from the DHBV051094 serum pool was diluted with PBS in such a way that 100 ID_{50} for a day old duckling was contained in a volume of 20μL. Equal 20μL volumes of test serum and virus were mixed and allowed to stand at RT for 1 hour. The serum/virus mixture was then made up to 300μL with PBS and

intraperitoneally injected into a duckling, with 3 ducklings per group. The ducks were then bled on days 4, 8, 11, and finally on day 15 for groups 1-6. Liver was obtained at sacrifice on day 15. Ducks in groups 7, 8, 9, and 10 (Table 59, p.214), were not killed at this time but were determined to be DHBV positive, and used for the Therapeutic DNA vaccine experiment (DNAvacc2) (8.4.3, p.214). Liver was obtained from group 7, 8, 9, and 10 ducks at sacrifice on day 70.

A virus titration was performed in parallel with the neutralisation assay, to confirm the approximate dosage. Three ducks were inoculated for each amount of DHBV051094 serum pool virus as per Table 59 (p.214). The day old ducks were inoculated by *ip* injection.

Original Duck	Serum	Virus ID_{50} (vge)	Group	Ducks
G97	Pre-vacc	100 ($1x10^5$)	1	P4, P5, P6
	Pre-chall 1:10		2	G56, G57, G58
	Pre-chall		3	Y70, Y72, Y75
W133	Pre-vacc	100 ($1x10^5$)	4	W37, W38, W39
	Pre-chall 1:10		5	B50, B57, B58
	Pre-chall		6	R57, R58, R59
W39	Pre-vacc	100 ($1x10^5$)	7	G90, G91, G92
	Pre-chall		8	Y89, Y90, Y91
Controls	DHBV051094	100 ($1x10^5$)	9	Y58, Y94, Y95
		10 ($1x10^4$)	10	G93, G94, G95
		1 ($1x10^3$)	11	P74, P75
		0.1 (100)	12	R95, R96
		0.01 (10)	13	B83, B84
		nil (0)	14	W98, W99

Table 59. *Neutralisation Assay.*

Ducks G97 and W133 were found to be protected from challenge, while duck W39 was susceptible. Pre-vacc: Serum taken before DNA vaccination (day 0). Pre-chall: Serum taken before DHBV challenge (day 28). Pre-chall 1:10: A 1 in 10 dilution of the day28 serum. Note: the ID_{50} dose is for day old ducks.

8.4.3. Therapeutic use of the DNA vaccine (DNAvacc2)

The eleven persistently infected ducks from groups 7, 8, 9, and 10 of the neutralisation assay experiment (8.4.2.2, p213) were used to determine the therapeutic efficacy of the DNA vaccine. Two groups were formed: one DNA vaccinated, and a control group (Table 60, p.215). DNA sequence data from selected serum and liver samples were obtained for both the core and surface regions as previously described (4.4.2, p.126).

New group	Group	Ducks	Virus ID_{50} (vge)
DNAvacc2	7	G90, G91, G92	100 ($1x10^5$)
	9	Y58, Y94, Y95	
Dv2 Control	8	Y89, Y90, Y91	
	10	G93, G95	10 ($1x10^4$)

Table 60. *Ducks used in the Therapeutic DNA vaccination experiment (DNAvacc2).*

The ducks were treated as per the neutralisation assay experiment until day 15. Ducks were inoculated with either $10ID_{50}$ ($1x10^4$ vge) or $100ID_{50}$ ($1x10^5$ vge) (Table 60, p.215). The ducks were bled and shown to be DHBV DNA positive, then vaccinated on days 19, 26, and 34. The DNA vaccinated group was injected with 50µg DNA vaccine in 300µL PBS per duck in 2 sites, once *intramuscularly*, and once *intradermally*. The controls were injected with PBS alone. The ducks were then bled on days 41, 49, 55, and 70. Liver samples were also obtained on day 70. Serum and liver samples were tested by PCR and dot blot hybridisation.

8.5. RESULTS

8.5.1. DNA Vaccine Production

After production of the DP, it was cloned into E. coli, and of 21 clones sequenced, two were found to be correct, one of which was selected and used further. After subcloning of the DP into pDVERA2, 10 clones were sequenced, eight of which were found to be correct, one of which was used for large-scale production.

Once a single clone containing the correct sequence DNA vaccine was selected, large-scale production and purification were simple procedures. The pooled DNA vaccine was determined to have a concentration of 1.8mg/mL of plasmid DNA vaccine.

8.5.2. Efficacy of the DNA Vaccine *in vivo* (DNAvacc1)

8.5.2.1. Toxicity of the DNA Vaccine

The DNA vaccine was well tolerated by all ducks, without any obvious side effects.

8.5.2.2. Detection of DHBV DNA

As expected all the unvaccinated ducks became infected by the challenge dose of approximately 10 ID_{50}. In contrast, two of the 7 vaccinated ducks (G97, and W133), were completely protected against this reasonably large dose.

The dot blot hybridisation and PCR results are tabulated in Table 44 (p.178). PCR results are unavailable for days 53 and 56, because the serum from these days was collected into

Heparin/PBS to allow separation of PBMC and serum. Unfortunately, this method was found to inhibit the PCR reaction but it had no effect on the dot blot hybridisation results.

Group	Duck	Day 0	28	36	40	43	46	49	53	56	60-62	L
DNAvacc1	B67	0	0						4	4		
	B68	0	0	0	0	0	0	0	0	0	0	0
	G57	0	0						0	0		
	G97	0	0	0	0	0	0	0	0	0	0	0
	G98	0	0						0	0		
	W39	0	0						0	0		
	W133	0	0	0	0	0	0	0	0	0	0	0
Dv1 Controls	G92	0	0						4	4		
	G93	0	0						0	0		
	G100	0	0						0	0		
	W42	0	0						3	3		
	W118	0	0						0	0	0	
	W120	0	0		0	0	0	0	0	0	0	
	W124	0	0						0	0		

Table 61. *Tabulated dot blot hybridisation and PCR results for the DNAvacc1 experiment.*

Dot blot hybridisation and PCR results for the DNAvacc1 experiment. Dot blot results are the numerical value (0=not detected ($\leq x10^6$vge/mL), 1=$1x10^7$vge/mL, 2=$1x10^8$vge/mL, 3=$1x10^9$vge/mL, 4=$1x10^{10}$vge/mL, 5>$2x10^{10}$vge/mL). Shaded blocks indicate DHBV PCR results: positive (>$2x10^3$ vge/mL), negative (<$2x10^3$ vge/mL), clear = not tested by PCR.

The dot blot hybridisation assay and PCR results from the controls indicates that the inoculated dose, although not able to produce high titre persistent infection, was large enough to infect 32 day old ducks, and remain in the liver until the end of the experimental period (day 60-62). All of the control ducks were found to be viraemic by PCR on day 36, with all but two ducks remaining viraemic until the end of the experiment. Duck W118 was found to clear the virus from the serum and be PCR negative on the final bleed (day 62). Duck W120 was only PCR positive in the serum on day 36, and then negative for the rest of the experimental period. All but one of the control ducks were found to be dot blot hybridisation positive in the liver, and the remaining duck (W120), was PCR positive.

In contrast to the control ducks, which were all PCR positive, viraemia was never detected in 3 vaccinated ducks (B68, G97, and W133). Two of these ducks (G97, and W133) were also DHBV negative in the liver, while the third duck (B68), was positive by PCR only, indicating a very low level infection.

Although this result is not statistically significant, it appears that the DHBV DNA vaccine did provide protection to at least two of the seven ducks vaccinated even though the vaccine had not been primarily designed to achieve a humoral response.

8.5.3. Immunogenicity of DNAvacc1

The character of the specific CMI response to DNAvacc1 was assessed by lymphoblastogenesis assays and the humoral response by neutralisation tests.

8.5.3.1. Pre-challenge PBMC CMI response

The post-vaccination, pre-challenge PBMC CMI responses to epitopes incorporated in the DNA vaccine were very poor as measured by the lymphoblastogenesis assay (7.3.2, p.173). The only detectable CMI response was in control duck (W124), which was found to significantly respond to peptides 101-120, and 307-326 ($p<0.05$). Individual duck results are in Appendix 11.9 (p.A43).

8.5.3.2. Post-challenge CMI response

Mitogen response: PBMC and SMC purified from all the ducks were able to respond to PHA stimulation *in vitro* indicating their viability (Table 47, p.182).

Antigen response: One month, (28-30 days) after challenge, the spleen was used to determine the CMI response to the epitopes in the DNA vaccine in the lymphoblastogenesis assay. The results for each duck have been summarised (Table 47, p.182). The full individual duck results are in Appendix 11.9 (p.A43).

There were no significant differences in the *in vitro* response of purified SMC between the vaccinated and the control group. See Appendix (Table 83, p.A43) for statistical analysis. Peptide 7-14W-27 elicited the best *in vitro* response with all of the control group and three of the 7 vaccinated group responding. None of the ducks responded significantly to peptide 71-90.

DNAvacc1 group

Peptide	B67	B68	G57	G97	G98	W39	W133	Peptide	Resp	nonR
1-15								1-15	1	6
7-14W-27								7-14W-27	3	4
71-90								71-90	0	7
101-120								101-120	0	7
229-248								229-248	0	7
267-286								267-286	1	6
307-326								307-326	0	7
SMC PHA								SMC PHA	7	0
SMC LPS								SMC LPS	5	2
PBMC PHA								PBMC PHA	7	0
PBMC LPS								PBMC LPS	6	1

Dv1 Control group

Peptide	G92	G93	G100	W42	W118	W120	W124	Peptide	Resp	nonR
1-15								1-15	3	4
7-14W-27								7-14W-27	7	0
71-90								71-90	0	7
101-120								101-120	1	6
229-248								229-248	2	5
267-286								267-286	3	4
307-326								307-326	1	6
SMC PHA								SMC PHA	7	0
SMC LPS								SMC LPS	4	3
PBMC PHA								PBMC PHA	7	0
PBMC LPS								PBMC LPS	7	0

Table 62. *Summary of post-challenge CMI response to specific immunodominant epitopes in the DNAvacc1 experiment ducks (significant P/N).*

Resp: Number of ducks that responded (significant P/N). NonR: Non-responders.

The response to the peptides appears to be more related to the DHBV status of the duck rather than whether they were vaccinated or not. Vaccinated ducks G97 and W133, which had been protected from infection by the vaccination showed little response to the peptides. Similarly, SMC purified from vaccinated duck B67 and control duck W42 with high level of viraemia, responded poorly to *in vitro* peptide stimulation. Better responses appeared to be related to reduction in DHBV DNA levels. Three (W118, B68 and W120) of the four ducks that demonstrated vigorous polyclonal blastogenesis *in vitro*, had cleared DHBV from the serum but not the liver. The remaining duck with a vigorous blastogenesis response (G93) had low-level viraemia, and may have cleared the infection if the experiment had been carried out for a longer period.

The relationship of the CMI response to DHBV infection is summarised (Table 63, p.219).

Group	Duck	Infection	Peptide 1-15	7-14W-27	71-90	101-120	229-248	267-286	307-326
DNAvacc1	G97	not infected							
	W133								
	B68	**seronegative**							
	G57	persistent infection							
	G98								
	B67								
	W39								
Dv1 Controls	W120	**seronegative**							
	W118								
	G93	persistent infection							
	G100								
	W124								
	G92								
	W42								

Table 63. *Relationship of the CMI response to DHBV infection for the DNAvacc1 experiment.*

DHBV infection is defined as DHBV DNA in serum and liver at euthanasia. Not infected: serum and liver negative throughout. Seronegative: serum negative, liver positive. Persistent infection: serum and liver positive.

8.5.4. Neutralisation Assay

The DNA results of the neutralisation assay have been summarised (Table 64, p.220). They indicate that DNAvacc1 duck G97 was protected from challenge using the DHBV DNA vaccine by means of neutralising antibodies. The neat post-challenge serum of duck G97 was able to neutralise 100 ID_{50} of DHBV, with none of the 3 ducks injected becoming positive, either by dot blot hybridisation or PCR. The 1:10 dilution and the pre-vaccination serum were not neutralising.

The serum from DNAvacc1 duck W133 was unable to prevent DHBV infection, even with neat serum. This would indicate either, that very low levels of antibody are biologically effective in achieving clearance, or that protection from infection was mediated by different mechanisms.

The serum from DNAvacc1 duck W39 was unable to prevent DHBV infection, as expected.

It is evident that the dose of DHBV for this test was well calculated as the two ducks injected with 10 ID_{50} (ducks G93, and G95) (duck G94 died on day 4) both had viraemic infections. One (duck P75) of the two ducks (P74, and P75) injected with 1 ID_{50} had a viraemic infection; the other was not only dot blot hybridisation, but also PCR negative. Both of the ducks (R95, and R96) that were injected with 0.1 ID_{50} were dot blot hybridisation and PCR negative.

Ducks G94 (control 10 ID_{50}) and W99 (control nil) died on day 4. No cause could be determined but most likely due to some genetic abnormality.

Original Duck	Serum	Dose ID_{50} (vge)	group	duck	0	4	8	11	15	L
G97 Protected	pre-vacc	100 ($1x10^5$)	1	P4	0	0	0	0	0	0
				P5	0					
				P6	0					
	pre-chall 1:10	100 ($1x10^5$)	2	G56	0	0	0			
				G57	0					
				G58	0	0	0	0	0	0
	pre-chall	100 ($1x10^5$)	3	Y70	0	0	0	0	0	0
				Y72	0	0	0	0	0	0
				Y75	0	0	0	0	0	0
W133 Protected	pre-vacc	100 ($1x10^5$)	4	W37	0	0	0			
				W38	0	0				
				W39	0	0				
	pre-chall 1:10	100 ($1x10^5$)	5	B50	0					
				B57	0	0	0	0	0	0
				B58	0					
	pre-chall	100 ($1x10^5$)	6	R57	0	0				
				R58	0	0				
				R59	0					
W39 Infected	pre-vacc	100 ($1x10^5$)	7	G90	0					
				G91	0	0				
				G92	0					
	pre-chall	100 ($1x10^5$)	8	Y89	0					
				Y90	0	0				
				Y91	0					
Controls	DHBV051094	100 ($1x10^5$)	9	Y58	0					
				Y94	0	0				
				Y95	0	0	0	0		
		10 ($1x10^4$)	10	G93	0	0	0			
				G94	0					
				G95	0	0				
		1 ($1x10^3$)	11	P74	0	0	0	0	0	0
				P75	0	0				
		0.1 (100)	12	R95	0	0	0	0	0	0
				R96	0	0	0	0	0	0
		0.01 (10)	13	B83	0	0	0	0	0	0
				B84	0	0	0	0	0	0
		Nil (0)	14	W98	0	0	0	0	0	0
				W99	0					

Table 64. *Summary of dot blot hybridisation and PCR results for the Neutralising Antibody experiment.*

Dot blot hybridisation and PCR results for the Neutralising Antibody experiment. Dot blot results are the numerical value (0=not detected ($\leq x10^6$vge/mL), 1=$1x10^7$vge/mL, 2=$1x10^8$vge/mL, 3=$1x10^9$vge/mL, 4=$1x10^{10}$vge/mL, 5>$2x10^{10}$vge/mL). Shaded blocks indicate DHBV PCR results: positive (>$2x10^3$ vge/mL), negative (<$2x10^3$ vge/mL). Black blocks indicate that no sample was available. The liver results for groups 7, 8, 9, and 10 are for day70 (these ducks were used for the DNAvacc2 experiment). Ducks G94, and W99 died on day 4.

8.5.5. Therapeutic efficacy of the DNA vaccine (DNAvacc2)

All of the ducks in the DNAvacc2 experiment were viraemic by dot blot hybridisation previous to DNA vaccination or PBS injection. The controls and the DNA vaccinated groups had a similar average level of viraemia prior to treatment at $4.2x10^8$ and $2.3x10^8$ vge/mL respectively. None of the ducks in the DNAvacc2 experiment were able to completely remove DHBV DNA from the serum and all were dot blot hybridisation positive in the liver at the end of the experimental period.

The maximum viraemia in the DNAvacc2 ducks was either before or during treatment. Three of the six vaccinated ducks (G92, Y58, and Y95) were dot blot hybridisation negative in the serum by day 70, compared with one (G93) of the five control ducks. The average viraemia of the DNA vaccinated ducks decreased by almost a $\log_{10}$ (to ~20% of the pre-treatment level), while the controls increased by a $\log_{10}$ (to ~1000% of the pre-treatment level).

One of the Dv2 control ducks had a biphasic pattern of infection, although it was not dot blot hybridisation negative, it did have very low level viraemia between days 19 and 26.

DNA sequence data for both the preC and Surface forward regions were obtained from the liver of all ducks, as well as from serum of DNAvacc2 ducks G92 (days 11, 19, and 55), Y58 (day 55), Y95 (days 19, and 26), and Dv2 control duck G93 (days 19, and 26). All sequence data obtained from the DNAvacc2 experiment were found to be wild type.

Group	Duck	Day 0	4	8	11	15	*1 19	*2 26	*3 34	41	49	55	70	L
DNA vaccinated2	G90	0												
	G91	0	0											
	G92	0												
	Y58	0												
	Y94	0	0											
	Y95	0	0	0	0									
Control	Y89	0												
	Y90	0	0											
	Y91	0												
	G93	0	0	0										
	G95	0	0											

Table 65. *Tabulated dot blot hybridisation and PCR results for the DNAvacc2 experiment.*

Dot blot hybridisation and PCR results for DNAvacc2 experiment. Dot blot results are the numerical value (0=not detected ($\leq x10^6$vge/mL), 1=1x10^{7}vge/mL, 2=1x10^{8}vge/mL, 3=1x10^{9}vge/mL, 4=1x10^{10}vge/mL, 5>2x10^{10}vge/mL). Shaded blocks indicate DHBV PCR results: positive (>2x10^3 vge/mL), negative (<2x10^3 vge/mL), clear = not tested. Asterisks indicate DNA vaccination injection 1, 2, and 3.

8.6. DISCUSSION

This pilot experiment has a number of technical limitations. In particular the number of ducks used was restricted by the housing facilities available as well as ethical considerations. In retrospect the control injections should have been made with a pDVERA2 plasmid with a nonsense insert, but this could not be achieved in time to take advantage of animal house availability. However, there is no reason to expect that the plasmid itself (without the DHBV epitopes) would have enhanced the immune response to the DHBV epitopes.

An intrinsic problem in using the DHBV model is the increase in natural resistance to DHBV infection as ducklings age. The selection of challenge doses is based on limited data and the very large virus doses needed may in themselves affect the immune response directly by their antigenic mass. The decreased susceptibility of the older ducks (inoculated on day 32) is evident from these results. Even though a much higher dose was used ($2.5x10^{10}$ vge), compared to the young ducks, inoculated on day 1, 4, or 7, with $2.8x10^{3} - 2.8x10^{5}$ vge, none of the older ducks developed the classic highly viraemic persistence which is characteristic of infection in young ducks. Even the administration of the challenge inoculum by the *intravenous* route did not produce a persistent infection with high level viraemia. This reinforces the observations that the older animals tend towards self-limiting acute infection, which is the opposite of the young, where persistence is the norm. This is an intractable limitation of vaccination studies in the DHBV model.

The small animal numbers restricted the ability to sample CMI responses at different time periods after vaccination and challenge. The CMI response is evanescent so this may well have accounted for the negative outcome of the proliferation assays post vaccination. The low CMI responses of the DNAvaccinated2 ducks may be due to the fact that the CMI becomes undetectable soon after resolution of infection in the ducks (Vickery *et al.*, 1999b).

Despite these limitations the experiment yielded several pieces of useful information.

The protective efficacy was unexpected since immunity to DHBV is attributed to anti-surface antibodies. The vaccine was based on surface epitopes but these had been selected for their putative T-cell importance and the overlap with known B-cell epitopes is minor. Moreover, DNA vaccines present peptides mainly via the Th1 pathway and their ability to generate conformational epitopes characteristic of Th2 responses in regarded as poor. However, we were able to demonstrate neutralising antibody in one of the two ducks protected by the vaccine. This ability of DNA vaccination, to protect against hepadnavirus infection, has been shown by others (Triyatni *et al.*, 1998; Du *et al.*, 2003; Zhou *et al.*, 2003). The effectiveness of neutralising protection however, depends on the gene used for DNA

vaccination; a complete preS gene was found to stimulate antibody production similar to a complete S gene construct, but effective protection was significantly lower (Triyatni *et al.*, 1998). Careful consideration must be given to the choice of epitopes expressed by the DNA vaccine.

The average quantity of virus present in serum at day 70 was $2\log_{10}$ lower in the DNAvaccinated group when compared with the unvaccinated controls (ie. the level of viraemia in the DNA vaccinated group was ~1% of that of the control group). This result is not statistically significant due to the low number of ducks used in the experiment. The decision to use only epitopes from the S gene was made on the observation that S-specific responses presaged clearance and supported by the consideration that a CMI response to the surface gene would place extra immune pressure on a single part of the genome already targeted by the humoral response. As such, the virus would have to evade both arms of the immune system in a short region of the genome, and this is more likely to result in a defective mutation.

Much of the CMI research done with hepadnaviruses has focused on the response to the Core gene. However, here too a vigorous immune response does not necessarily lead to elimination of infection. For instance, the CMI response has been mapped for the woodchuck model, in which persistently infected woodchucks were found to be able to respond to several epitopes of the nucleocapsid core protein (Shanmuganathan *et al.*, 1997). So even though there is an experimentally determinable response, it is insufficient to produce clearance of the infection.

The inability of a DNA vaccine to eradicate persistent DHBV infection does not discount that the DNA vaccine may be used therapeutically, as recent research indicates that the use of antivirals in combination with DNA vaccines provide better results (Le Guerhier *et al.*, 2003). The efficacy of the combination of adefovir with DNA-immunisation was compared with the respective monotherapies. DHBV chronically infected Pekin ducks received Adefovir treatment alone or in association with intramuscular immunisation with a plasmid (pCI-preS/S) expressing the DHBV large envelope protein. All of the animals treated with Adefovir demonstrated a marked drop in viraemic titres during administration, but, as appears usual for hepadnavirus drug therapies, was followed by a rebound of viral replication after drug withdrawal. After the third and final DNA boost, the median of viraemia within the duck group receiving the combination therapy tended to be lower compared to that of the other groups. The researchers also suggested that the combination produced an additive effect as a 51% decrease in DHBV DNA was observed in autopsy liver samples from combination therapy group, whereas the monotherapies were found to have

decreased intrahepatic viral DNA by 38 (pCI-preS/S) and 14%, (Adefovir). This effect was found to be sustained for a reasonable length of time as it was observed 12 weeks after the end of therapy (Le Guerhier *et al.*, 2003).

However there is still much to be learned about the administration of combination therapies, which has been demonstrated, by Entecavir and DNA vaccine combination experiments. The drug Entecavir, was orally administered to persistently infected young ducks, from 21 days posthatch for 244 days, which caused a $4\log_{10}$ drop in serum DHBV DNA levels within 80 days, and a slower $2\text{-}3\log_{10}$ drop in serum DHBV surface antigen levels within 120 days. However, the addition of DNA vaccination did not result in any significant effect on viral markers (Foster *et al.*, 2003).

The use of drugs to effectively lower the viral load in combination with other therapies appears to be the next step for most of the therapeutic approaches to clearing hepadnavirus infections, and is not limited to DNA vaccines. Conventional protein vaccines have been found to be partially effective in increasing both the CMI and humoral response of persistently infected woodchucks, in combination with drug therapy (Menne *et al.*, 2002).

However, the concept that virus down regulation and clearance is exclusively achieved by specific anti-viral CMI may be an oversimplification and both specific humoral and non-specific T-cell activity may be required to act in tandem with virus-activated T-cells. Reagents for examining the duck CMI are very limited, so we decided to investigate the relative roles of the two arms of the immune response by studying the effect of bursectomy and thymectomy on the ability of ducks to control hepadnavirus infection.

9. MANIPULATION OF IMMUNE MECHANISMS

9.1. INTRODUCTION

Modulation of the immune system has been achieved by many different methods such as irradiation to remove all of the cells of the immune system (Mumcuoglu *et al.*, 1987; Bocher *et al.*, 1999), selective breeding and transgenic manipulation to produce specialised species (Chisari *et al.*, 1985), use of monoclonal antibodies that target destruction of specific cells (Naessens *et al.*, 1998), and surgical removal of important immune organs (Sugimura and Hashimoto, 1980; Sreter *et al.*, 1996). The immune system of avian species is basically the same as that of mammals but does have some distinct anatomical and developmental features, which permit a surgical approach to experimental modulation of the immune response.

In birds, maturation of B-cells occurs in the Bursa of Fabricius, which is located perianally where it is readily accessible as a compact encapsulated organ. At hatching the secondary lymphoid organs are already populated (albeit minimally), with functional B-cells (Hashimoto and Sugimura, 1976b), however, they are not yet fully matured. In consequence viral infections acquired soon after hatching tend to become persistent. Removal of the Bursa on the day of hatching should retard maturation of the humoral response, and this has been amply demonstrated in the extensive studies on the chick, which originally defined the T- and B-cell lymphocytes and their function (Warner and Szenberg, 1962; Cooper *et al.*, 1965; Magor *et al.*, 1998).

As in mammals, avian T-cell lineages are derived from the thymus. In ducks the thymus is multi-lobed and located in close proximity to the trachea. Removal of the thymus to deplete the mature T-cell population thus presents a technical challenge.

At the time of experimentation there were no immunological markers available to identify duck T- and B-cells, except in flow cytometry where duck T-cells can be identified with anti-human CD3 antibody (Bertram *et al.*, 1996). Despite this limitation the course of

DHBV infection in ducklings after bursectomy and thymectomy can shed light on the relative significance of humoral and cellular immunity in achieving clearance.

If specific CMI to S epitopes is the key response leading to DHBV clearance, impairment of the lymphoblastic response to the immunologically important peptides identified in immunologically intact animals should correlate with an inability to clear DHBV.

9.2. AIMS

(1) To determine the effect of neonatal bursectomy or the thymectomy on the outcome of DHBV infection in 4 week old ducklings.

(2) To correlate the ability of individual 4 week old ducklings to clear DHBV with the CMI response to peptides of the Surface protein and their bursectomised or thymectomised status.

9.3. MATERIALS AND METHODS

These experiments were done in conjunction with Jim Pouliopoulos, as part of his Masters degree program, and so the day-to-day animal duties, harvesting of cells, and the lymphoblastogenesis assay work was shared.

9.3.1. Surgical Protocol

All anaesthesia and surgery was kindly performed by Dr. Anand Deva, Dr. Robert Dixon, and Dr. Karen Vickery.

Ducks were premedicated with 1mg/kg of ketamine hydrochloride, intramuscularly. They were induced and maintained on inhalational anaesthetic (Isofluorane). The immediate surgical area was plucked and the surrounding area shaved and the skin surgically prepared with 70% ethanol, and povidone-iodine (Faulding Pharmaceuticals Salisbury, South Australia). All surgical instruments were autoclaved before use (holding time 121°C, 15 lbs, 20mins).

Postoperatively all ducklings were given 2mL Hartmans' solution intraperitoneally. Postoperative analgesia was provided by wound infiltration with 0.25% bupivacaine with adrenalin (2.5µg/mL). An aerosol dressing spray (Opsite, Smith and Nephew Hull, UK) was applied to wounds and ducklings were left to recover in a heated room. Postoperative antibiotic prophylaxis was provided by Tetracycline in the drinking water for 7 days.

The surgical methods outlined in the Handbook of Experimental Immunology (Weir, 1978) were followed.

9.3.2. Control ducks

The twenty-four naïve negative control ducks were the same ducks as described in detail in Chapter 7 (7.3.1.1, p.170). These ducks were never in contact with DHBV (Table 66, p.228).

There were twenty-five positive control ducks (Table 66, p.228). Twelve ducks (G531, G58, G631, G72, G89, P72W48, P631, V2R, W105, W106, W107, and W111) were the same ducks as described in detail in Chapter 7 (7.3.1.3, p.170). Another thirteen ducks (G86, G511, G991, P17, P54, P57, P531, W34, W43, W48, W103, W139, and W451) were similarly treated. Ducks were infected with DHBV at 4 weeks of age and euthanased 43 days later. Ducks G86, G511, G991, P54, P57, P531, W34, W43, W48, W103, and W451, were inoculated with $2.0x10^9$ vge (ID_{50} equivalent) of DHBV from serum pool DHBV200499, while ducks G58, G531, P17, P631, P72W48, V2R, W105, W106, W107, W111, and W139, were inoculated with the same amount of DHBV from serum pool DHBV200197. The two different serum pools contained the same concentration of DHBV DNA ($2.0x10^{10}$ vge/mL), and were found to have the same experimental properties.

Ducks G72, G89, and G631, were inoculated with ten times the standard dose ($2.0x10^{10}$ vge from serum pool DHBV200197, approximately $10ID_{50}$). These ducks were used for histological and cell count data, as well as the lymphoblastic antigen response (Chapter 7, p.170), as they were found to be similar to the normal positive controls. However, these ducks could not be used for the outcome results, because they have received a larger dose of DHBV and dose is an important factor in the outcome of the infection.

Type	Batch	Ducks	Number
Bursectomised	Bursect	10	W101, W109, W131, W132, W140, and W104, W110, W121, W130, and W145
Thymectomised	Thymect1	4	W122, W125, W126, and W147
	Thymect2	9	W151, W152, W153, W156, W157, W160, W167, W168, and W170
Positive Control		25	G86, G511, G991, P54, P57, P531, W34, W43, W48, W103, and W451 (DHBV200499) G58, G531, P17, P631, P72W48, V2R, W105, W106, W107, W111, and W139 (DHBV200197)
			G72, G89, and G631 (high dose group)
Negative Control		24	1A, 1B, 1C, 1D, 1E, 1F, 1G, 1H, 1I, 1J, 1K, 1L, 2A, 2B, 2C, 2D, 2E, 2F, 2G, 2H, 2I, P24P53, V2T, and V2U

Table 66. *Ducks used in the Bursectomy and Thymectomy experiments.*
Ducks G72, G89, and G631, were given 10x the standard inoculum; as such they could not be used for the outcome of infection results, leaving 22 ducks in the positive control group.

9.3.3. Bursectomised ducks

Ducks (W101, W104, W109, W110, W121, W130, W131, W132, W140, and W145) (Table 66, p.228) were bursectomised in ventral recumbency, on day of hatch. A horizontal 5 to 7mm long incision was made just ventral the tail where the lower edge of the last vertebra could be felt. The bursa was grasped with dissecting forceps and gently freed from its attachments to the upper surface of the cloaca, with care not to the damage blood vessels or the overlying ureters and genital tubes. The bursa was excised as close as possible to its cloacal attachment. The incision was closed with 3 simple interrupted stitches using monofilament 4/0 suture.

All bursectomised ducks were challenged on day 28 with $2.0x10^9$ vge of DHBV: ducks W101, W109, W131, W132, and W140, were inoculated with serum pool DHBV200197, while ducks W104, W110, W121, W130, and W145, were inoculated with serum pool DHBV200499. Both serum pools contained the same concentration of DHBV ($2.0x10^{10}$ vge/mL) (Methods and Materials, 2.2.7, p.74). This dose was interpolated to be equivalent to $1ID_{50}$, as based on previous data for 26 day old ducks (Vickery and Cossart, 1996). Serum samples were obtained pre challenge and twice weekly (every 3-4 days) post challenge. The spleen was harvested and a liver sample obtained at euthanasia on day 70.

9.3.4. Thymectomised ducks

The one day old ducks were thymectomised in two batches (Table 66, p.228), due to surgical time constraints.

For thymectomy, the day old ducklings were placed ventral side down and a pillow of gauze was placed under the neck so that the cervical spine was horizontal to the table. A dorsal midline incision was made from the scapular region to the base of the skull. A skin flap was made on one side of the neck. Each thymic lobe was separated from the surrounding fascia and removed. The last lobe was visualised and removed by gently pulling the jugular vein from the thoracic cavity. The lobes on the other side of the neck were removed in a similar fashion. The wound was closed using monofilament (4/0) continuous subcuticular suture. In a few ducks there was difficulty in removing the last thymic lobe.

The ducks were inoculated on day 29 or 30, with 100µL of DHBV200197 ($2.0x10^9$ vge, an ID_{50} equivalent), bled twice weekly (every 3-4 days) until day 69 or 70, when the spleen was harvested, and blood and liver samples obtained at euthanasia (day 43).

9.3.5. Assays performed on Samples

All serum and digested liver samples were serially diluted and tested for DHBV DNA by dot blot hybridisation to determine level of viraemia. If negative by dot blot hybridisation, serum was tested by PCR (if sufficient serum remained).

Ducks were weighed to assess whether the surgery adversely affected their growth.

The CMI response to peptides and mitogens was determined by the lymphoblastogenesis assay as previously described (7.3.2, p.173).

The whole spleen minus a small section for histopathological analysis was purified. An estimate of the total splenic lymphocytes was obtained by counting SMC following purification for cell culture.

9.3.5.1. Histopathology

Liver, spleen, as well as residual thymic and surrounding fascial tissue were obtained for histopathology at euthanasia. The tissues were processed as described (2.2.10, p.77).

Histopathology was also performed on duck groups from Chapter 7: Negative control group, Protein vaccinated group, and the Positive control group.

The grading of liver, thymus, and spleen samples by code was generously performed by Dr. Ted Wills from Anatomical Pathology (Central Area Health Services). Inflammation of the liver was graded in accordance to that previously described (Marion *et al.*, 1984) (Table 67, p.230).

Inflammation	Description
Normal	No inflammatory cells or occasional foci of inflammatory cells in portal tracts or parenchyma.
Slight	Occasionally observed in normal uninfected ducks.
Mild	Conspicuous accumulation of inflammatory cells in portal tracts with or without scattered focal necrosis, increase in bile ductules and increase in sinusoid cells.
Moderate	Inflammation as above, but including inflammatory cell extension into the parenchyma along septae with or without piecemeal necrosis.
Severe	Accompanied by regenerative nodules, extensive septae formation, or areas of collapse.

Table 67. *Description of histological inflammation grading.*

Interpretation was fairly strict: one or two portal tracts with a few inflammatory cells in them may be within normal limits, but was graded as slight. Statistical comparisons using the

Fisher's exact test were performed utilising the combined normal and slight changes as normal, and mild to severe changes as an indication of inflammation.

Splenic architecture was graded as having normal or reduced follicles.

9.3.5.2. Cell counts

A Whole Blood Cell count (WBC) was performed to determine the total leukocytes in the blood. Natt and Herrick's method was used to enumerate total leukocytes (2.2.9.2, p.76). Leukocytes and lymphocytes stain darkly while erythrocytes and thrombocytes are lightly stained.

The SMCs and PBMCs were counted by Trypan blue exclusion (2.2.9.1, p.76). These counts were primarily used to determine the cell concentration for plating, but were also analysed.

9.3.5.3. Statistical analysis

The lymphoblastogenesis assay was analysed, and interpreted, in the same manner as described previously (7.3.3, p.177). Briefly, Fisher's exact test was used to compare the number of responders for each group; a responder being statistically higher (Student's t-test, 2 tailed, 2 sample) than the control wells by greater than 1000cpm. A p value of <0.05 was considered significant.

For histopathology and cell counts, a Student's t-test, (or Mann-Whitney Rank Sum test, when normality failed), was used comparing the mean of the individual counts; p value of <0.05 was considered significant.

9.4. RESULTS

9.4.1. Surgical procedures

Almost all ducks that underwent the surgical procedures of either bursectomy, or thymectomy, survived and were observed to be healthy and exhibited normal behaviour. One bursectomised duck died while being operated on, while ten survived to provide experimental data.

9.4.2. Duck Body Weight

Bursectomised and thymectomised ducks grew at the same rates as controls, indicating that the surgery did not adversely affect their growth.

9.4.3. Outcome of Infection

9.4.3.1. Negative control ducks

None of the twenty-four naïve negative control ducks were found to be DHBV DNA positive by dot blot hybridisation or PCR for any of the samples tested. A more detailed description was given in Chapter 7 (7.4.1.1, p.177).

9.4.3.2. Positive control ducks

At sacrifice, ten of the twenty-two positive control ducks (G86, G511, G531, G991, P17, P54, P57, P72W48, W103, and W106), were liver negative, while the other twelve ducks (G58, P531, P631, V2R, W34, W43, W48, W105, W107, W111, W139, and W451), were found to be liver positive for DHBV DNA (Table 68, p.232).

Of the ten DHBV DNA liver negative ducks, six (ducks G991, P17, P54, P72W48, W103, and W106) were found to be viraemic on at least one occasion by PCR, indicating that the ducks were at least transiently infected. The other four liver negative ducks remained uninfected throughout. All of the twelve DHBV DNA liver positive ducks were viraemic on one or more occasions. The dot blot hybridisation and PCR results for the positive control group are tabulated (Table 68, p.232).

Ducks	Days post inoculation													
Liver negative	0	4	7	11	14	19	21	24	27	29	34	37	43	L
G86	0	0	0	0	0	0	0			0			0	0
G511	0	0	0	0	0	0	0			0			0	0
G531	0	0	0	0	0	0	0			0			0	0
G991	0	0		0	0	0	0			0			0	0
P17	0	0			0	0	0			0			0	0
P54	0	0	0	0	0	0	0			0			0	0
P57	0	0	0	0	0	0	0			0			0	0
W103		0		0	0	0	0	0	0	0	0	0	0	0
W106		0			0	0	0	0	0	0	0	0	0	0
P72W48	0	0			0	0	0	0	0	0	0	0		0
Liver positive	0	4	7	11	14	19	21	24	27	29	34	37	43	L
G58	0	0	0		0	0	0			0			0	5
P531	0	0	0		2	0	2			3			0	5
P631	0	0	0	0	0	0	0			0			2	4
W34	0	0	0		0	3	1			2			4	5
W43	0	0			0	2	2			2			2	5
W48	0	0	0		0	0	1			3			0	4
W139	0	0		0	0	0	0			0			0	
W451	0	0	5		5	5	2			3			5	5
V2R	0					0								
W105		0			0	0	0	0	0	0	0	0	0	
W107		0			0	0	0	0	0	0	0	0	0	
W111		0			0	0	0	0	1	2	1	1	2	

Table 68. *Tabulated dot blot hybridisation and PCR results for the Positive control ducks.*

Liver negative ducks are in the top table, while liver positive ducks are in the bottom table. Dot blot results are the numerical value (0=not detected ($\leq x10^6$vge/mL), 1=$1x10^7$vge/mL, 2=$1x10^8$vge/mL, 3=$1x10^9$vge/mL, 4=$1x10^{10}$vge/mL, 5>$2x10^{10}$vge/mL, +=positive>$1x10^7$vge/mL). Shaded blocks indicate DHBV PCR results: positive (>$2x10^3$ vge/mL), negative (<$2x10^3$ vge/mL), clear = not tested. L=Liver.

9.4.3.3. Bursectomised ducks

All of the bursectomised ducks (10/10) were DHBV positive in the liver at euthanasia. All bursectomised ducks were positive for DHBV DNA in the serum on multiple occasions, 9 were quantifiable by dot blot hybridisation; viraemia in one duck (W110) was only detectable by PCR. Four of the ducks (W104, W110, W132, and W145) cleared viraemia prior to euthanasia. Most of the bursectomised ducks (8/10) had viraemia levels that reached 10^7 vge/mL. The Bursectomy duck results are summarised (Table 44, p.178).

Duck	Days Post Inoculation								
	0	5	8	12	16	25	30	37	L
W101	0	0	1	4	5	1	1	1	5
W104	0	0	2	0	1	1	0	0	5
W109	0	0		5	2	1	1	1	5
W110	0			0	0	0	0	0	
W121	0	0	5	1	1	2	2	2	5
W130	0	0	5	0	2	4	4	4	5
W131	0	0	0	4	1	1	1	1	5
W132	0	0	0	1	1	0	1	0	5
W140	0	0	1	1	1	2	1	2	5
W145	0	0	1	3	1	0	1	0	5

Table 69. *Tabulated dot blot hybridisation and PCR results for the Bursectomy experiment.*

Dot blot results are the numerical value (0=not detected ($\leq 1x10^6$vge/mL), 1=$1x10^7$vge/mL, 2=$1x10^8$vge/mL, 3=$1x10^9$vge/mL, 4=$1x10^{10}$vge/mL, 5>$2x10^{10}$vge/mL). Shaded blocks indicate DHBV PCR results: positive (>$2x10^3$ vge/mL), negative (<$2x10^3$ vge/mL), clear = not tested. L=Liver.

9.4.3.4. Thymectomised ducks

Only three of the thirteen thymectomised ducks (W126, W152, and W160) were DHBV positive in the liver at euthanasia. Only one of the three liver positive thymectomised ducks (W126) was viraemic throughout the experimental period while the other two ducks were never viraemic. This duck had an initially high peak, followed by a trough and a second peak approximately one $\log_{10}$ lower than the original. The amount of circulating virus then fell and was maintained at less than $1x10^7$ vge/mL until the termination of the experiment. Five of the ducks (W122, W147, W153, W157, and W170) that were DHBV negative in the liver at euthanasia were transiently viraemic by PCR 4 to 11 days post inoculation. The Thymectomy results are summarised (Table 70, p.234).

	Days Post Inoculation												
Duck	**0**	**4**	**7**	**11**	**14**	**17**	**20**	**27**	**30**	**34**	**37**	**40**	**L**
W122	0	0	0	0	0	0	0	0	0	0	0	0	0
W125	0	0	0	0	0	0	0	0	0	0	0	0	0
W126	0	0	0	5	5	1	2	2	3	3	1	1	5
W147	0	0	0	0	0	0	0	0	0	0	0	0	0
Duck	**0**	**5**	**7**	**11**	**14**	**18**	**22**	**26**	**29**	**34**	**36**	**40**	**L**
W151	0	0	0	0	0	0	0	0	0	0	0	0	0
W152	0	0	0	0	0	0	0	0	0	0	0	0	5
W153	0	0	0	0	0	0	0	0	0	0	0	0	0
W156	0	0	0	0	0	0	0	0	0	0	0	0	0
W157	0	0	0	0	0	0	0	0	0	0	0	0	0
W160	0	0	0	0	0	0	0	0	0	0	0	0	5
W167	0	0	0	0	0	0	0	0	0	0	0	0	0
W168	0	0	0	0	0	0	0	0	0	0	0	0	0
W170	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 70. *Tabulated dot blot hybridisation and PCR results for the Thymectomy experiment.*

Dot blot results are the numerical value (0=not detected ($\leq x10^6$vge/mL), 1=$1x10^7$vge/mL, 2=$1x10^8$vge/mL, 3=$1x10^9$vge/mL, 4=$1x10^{10}$vge/mL, 5>$2x10^{10}$vge/mL). Shaded blocks indicate DHBV PCR results: positive (>$2x10^3$ vge/mL), negative (<$2x10^3$ vge/mL), clear = not tested. L=Liver.

9.4.3.5. Analysis of the Outcome of Infection

No significant difference in the infectivity of serum pools DHBV200197 and DHBV200499 was found either within the positive control group (p=0.434), or the bursectomy group (p=1.000).

All ten of the bursectomised ducks (10/10) failed to clear DHBV infection from the liver, while 66% (12/18) of positive controls that were ever found to be viraemic, remained infected; although this was not quite significant (p=0.062). When the outcome of the infection in the bursectomised duck groups was compared with the positive groups given the same DHBV serum pool, there was no statistical difference; DHBV200197 (7/11) (p=0.245), DHBV200499 (5/11) (p=0.093). However, by combining the results of both serum pools, the bursectomised group (10/10) was significantly more likely to remain infected when compared with the inoculated control group (12/22) (p=0.013).

Only 23% of thymectomised ducks (3/13), remained infected, which was significantly better than the positive controls that were ever found to be viraemic (12/18) (p=0.029); but when

compared with positive control ducks administered with the same serum (7/11), was not significant (p=0.095), neither was it significant when compared to the total positive control ducks (12/22) (p=0.089).

The bursectomised ducks were significantly more like to remain infected that the thymectomised ducks (p<0.001).

9.4.4. Kinetics of Infection

9.4.4.1. Bursectomised ducks

Nine of the ten bursectomised ducks had serum titres of DHBV quantified by dot blot hybridisation. All of the bursectomised ducks had high levels of DHBV DNA in the liver at euthanasia. When the course of infection is more closely analysed by graphing the dot blot hybridisation results (Figure 62, p.236), the bursectomised ducks exhibited four patterns of viraemia: (a) Single large peak followed by persistent viraemia of approximately $1x10^6$ vge/mL, (b) Biphasic, (c) low level highly variable viraemia, and (d) low level, only PCR positive viraemia.

(a) Four ducks (W101, W109, W121, and W131), developed a single high peak of viraemia with titres above $1x10^{10}$ vge/ml; the level of DHBV DNA then gradually fell to approximately $1x10^7$ vge/mL.
(b) Duck W130 exhibited a biphasic response, similar to that originally described in Chapter 3.
(c) Low level highly variable viraemia was demonstrated by ducks W104, W132, W140, and W145. These ducks exhibited fluctuating viraemia with peaks of up to $1x10^7$ - $1x10^8$.
(d) The only detectable viraemia for duck W110 was by PCR, indicating a low level infection, however the liver contained high levels of DHBV DNA.

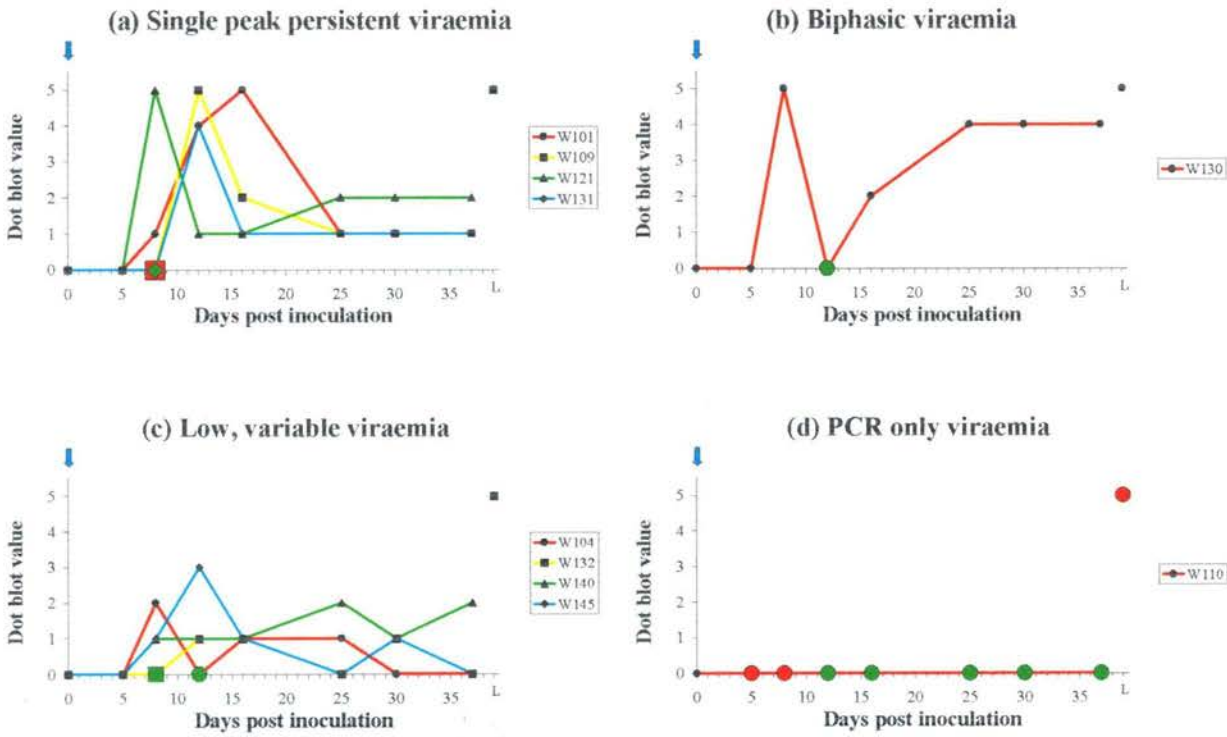


Figure 62. *Graphic results for Bursectomy experiment ducks.*

Ducks with similar patterns of viraemia (as described in 9.4.4.1, p.235) are grouped. All ducks were liver positive. Dot blot results are the plotted numerical value (0=not detected ($\leq x10^6$vge/mL), 1=$1x10^7$vge/mL, 2=$1x10^8$vge/mL, 3=$1x10^9$vge/mL, 4=$1x10^{10}$vge/mL, 5>$2x10^{10}$vge/mL). The blue arrow indicates when the ducks were inoculated. PreS-S PCR results are indicated by large data points: green = PCR negative, red = PCR positive, small black = not tested.

9.4.4.2. Thymectomised ducks

Of the six viraemic thymectomised ducks, DHBV DNA could be quantified in only duck W126 (Figure 63, p.236). This duck had an initial high peak of $1.2x10^8$ vge/mL, followed by a trough and a second peak approximately one $\log_{10}$ lower than the first peak. The amount of circulating virus then fell and was maintained at approximately 10^6 vge/mL until the termination of the experiment.

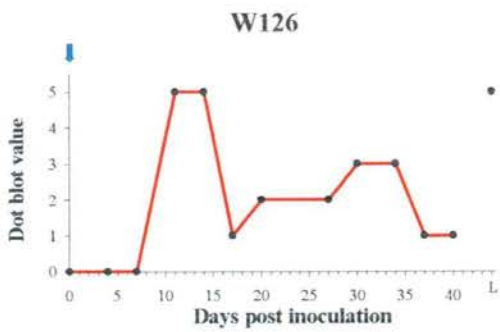


Figure 63. *Graphic results for Thymectomy duck (W126).*

Dot blot results are the plotted numerical value (0=not detected ($\leq x10^6$vge/mL), 1=$1x10^7$vge/mL, 2=$1x10^8$vge/mL, 3=$1x10^9$vge/mL, 4=$1x10^{10}$vge/mL, 5>$2x10^{10}$vge/mL). The blue arrow indicates when the duck was inoculated.

9.4.4.3. Positive control ducks

As expected for a relatively low inoculation dose of approximately $1ID_{50}$ about half (55%) were DHBV DNA positive in the liver at euthanasia. Another characteristic of the low dose was relatively low viraemia, however several of the infection patterns seen in Chapter 3, are evident. Ducks G72, and W451, both developed a biphasic pattern, while duck P531 developed a fluctuating viraemia.

9.4.5. Histology

Results for individual ducks can be found in the Appendix (Table 85, p.A129).

9.4.5.1. Liver histology

The results of histopathological examination of the liver have been summarised (Table 71, p.237).

Group	Total	Normal No. (%)	Inflamed No. (%)
Negative control	23	22 (96%)	1 (4%)
Positive control	17	12 (70%)	5 (30%)
Bursectomised	9	4 (44%)	5 (56%)
Thymectomised	13	11 (85%)	2 (15%)

Table 71. *Summary of histopathological results for the Liver.*
All ducks considered to have liver inflammation were also DHBV DNA positive in the liver (except for negative control duck). Inflammation was considered mild or above, as described in Table 66 (p.228).

In comparison to normal non-challenged ducks (negative control group), only the bursectomised ($p=0.003$) group showed evidence of increased inflammatory changes due to the infiltration of portal tracts by lymphocytes. However the infected control (positive control group) ($p=0.067$) demonstrated a possible trend.

A greater proportion of bursectomised ducks had inflammatory infiltrates within the liver than positive control ducks, however no significant difference can be demonstrated between these groups ($p=0.234$). The bursectomised ducks did not statistically have increased inflammation compared to the thymectomised ducks, but a trend was evident ($p=0.074$).

DHBV infection was significantly associated ($p<0.001$) with liver disease. Except for the negative control duck, all ducks that had inflammation of the liver were also DHBV DNA positive in the liver. Of the ducks considered to have normal liver histology, half of the positive control group (6/12), all of the bursectomised group (4/4), and only one of the thymectomised group (1/11), were DHBV DNA positive in the liver.

9.4.5.2. Spleen histology

The results of histopathological examination of the spleen have been summarised (Table 72, p.238).

Group	Total	Normal No. (%)	Reduced No. (%)
Negative control	18	15 (84%)	3 (16%)
Positive control	17	9 (53%)	8 (47%)
Bursectomised	9	4 (44%)	5 (56%)
Thymectomised	12	8 (67%)	4 (33%)

Table 72. *Summary of histopathological results for spleen follicles.*

The splenic architecture in the bursectomised ducks showed a reduction in follicles in comparison to negative control ducks, but was not significant (p=0.072). The frequency of positive control ducks with splenic alterations was also elevated, but not statistically significant from the negative controls (p=0.075).

Of the 8 positive control ducks with reduced follicles, 3 were liver positive (only one of these three also had liver inflammation). The one negative control duck with mild liver inflammation also had reduced splenic follicles. All five of the bursectomised ducks with reduced follicles were liver positive, and two of these also had liver inflammation. None of the thymectomised ducks with reduced follicles were liver positive, or had liver inflammation.

9.4.5.3. Thymus histology

All thymic lobes that were extracted from the day old ducks were similar to that observed in Figure 64 (p.238).

(a) (b)

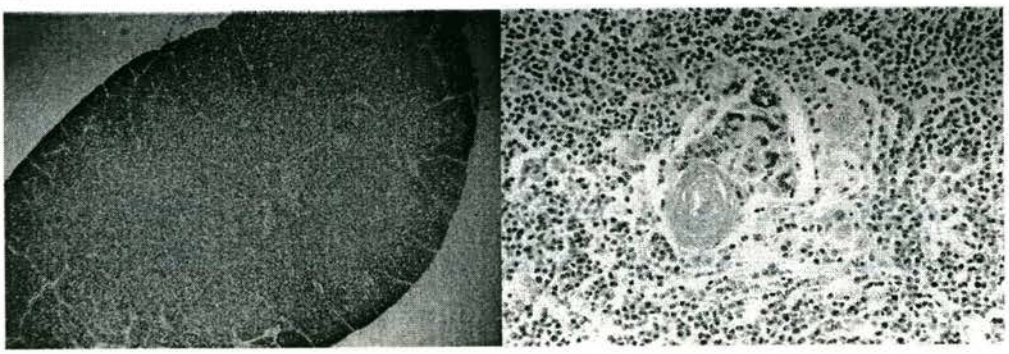


Figure 64. *Histological example of neonatal thymus.*
(a) Low power 100x (b) High power 400x showing Hassal's corpuscles.

There are some notable structures that show structural similarity to the human thymus. The densely stained cortex (peripheral zone) lobes are clearly separated by connective tissue septa and a lighter staining central region, the medulla. Within the medulla, Hassal's corpuscles were prominent features.

Both the thymectomised and positive control groups exhibited thymic involution indicated by replacement of the thymic parenchyma with adipose tissue. All that remains of thymus from adult ducks are small irregular strands of tissue composed of shrunken epithelial cells and lymphocytes. Hassal's corpuscles are not easily discernible. None of the thymectomised ducks was found to contain any thymic tissue at euthanasia, while only 1 of the 13 positive control ducks as found to contain any thymic tissue at euthanasia: there was no statistical difference.

9.4.6. Cell counts

Results for individual ducks can be found in the Appendix (Table 86, p.A130), as can the summarised group mean results. For easy comparison the summarised group mean cell counts has been graphed (Figure 65, p.239).

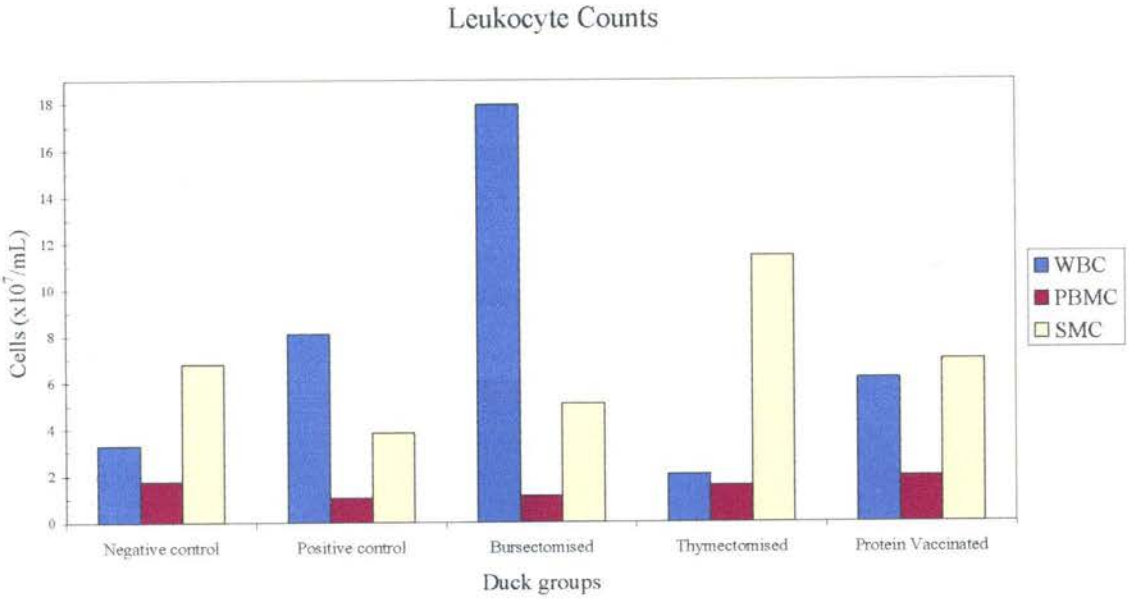


Figure 65. *Summary of the means of the Cell counts.*

Total leukocyte counts (WBC) were significantly elevated in bursectomised ducks when compared with the negative controls ($p<0.001$). The WBC was significantly depressed in thymectomised ducks in comparison to the positive control ($p=0.005$), immune (protein vaccinated ducks) ($p=0.003$), and the negative control groups ($p=0.048$). Although the DHBV positive ducks appeared to have a greater number of leukocytes than DHBV negative

ducks, it was not quite significant (p=0.055). The average WBC for the immune group (protein vaccinated ducks), was between that of the positive and negative controls.

This trend was maintained when the results of the negative, positive, and immune groups are pooled and considered to be a group with a normal immune system, then the bursectomised ducks have elevated WBC counts (p<0.001), and the thymectomised ducks have decreased WBC counts (p=0.001).

There was no significant difference in the PBMC counts between any of the groups, as they were all approximately equivalent.

The thymectomised group had an elevated SMC count in relation to all other groups: negative control (p=0.001), positive control (p<0.001), bursectomised (p<0.001), and immune (p=0.004).

Again, when the results of the negative, positive, and immune groups are pooled and considered to be a group with a normal immune system, then the bursectomised ducks have similar SMC counts (p=0.546), while the thymectomised ducks have an elevated number of splenic cells (p<0.001).

When the composition of the circulating leukocytes is analysed, an interesting picture emerges. Although all groups had roughly the same number of circulating mononuclear cells (PBMC), the WBC counts varied enormously. Thus the percentage of PBMC in the total blood leukocyte population is different (Figure 66, p.240). In ducks, the non-PBMC cells in the blood circulation are considered to be mostly heterophils.

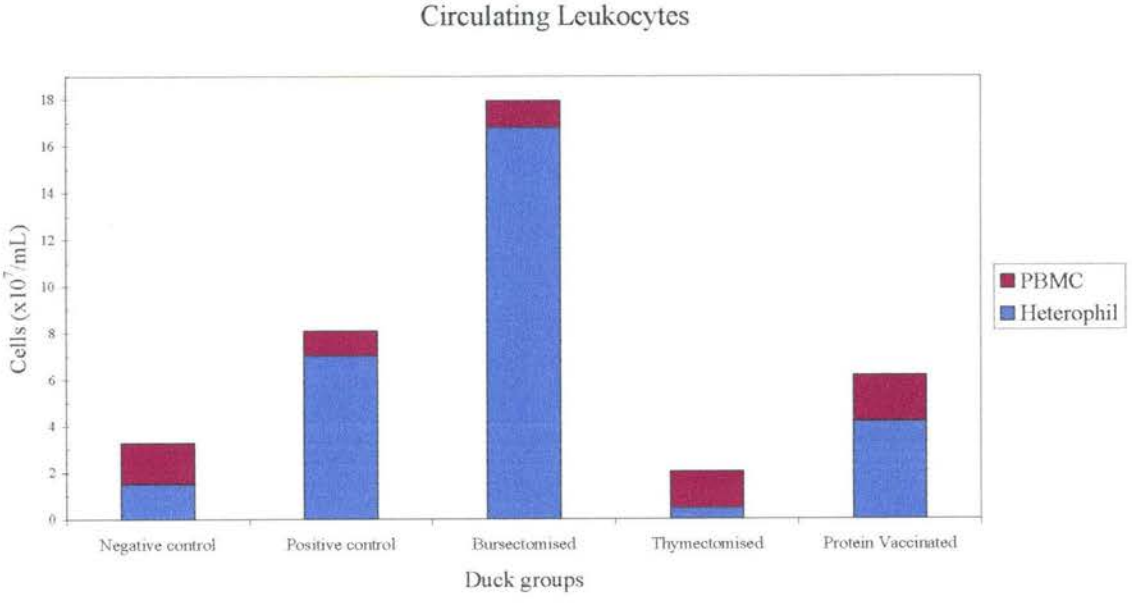


Figure 66. *PBMCs as a subpopulation of the total blood leukocytes.*

It is clearly demonstrated in Figure 66 (p.240), that approximately half of the total leukocytes in the blood of the negative controls are PBMCs, while in the positive controls they are only about 15% of the total population. For the bursectomised group the PBMCs are only approximately 5% of the total population, while in the thymectomised group they comprise approximately 75% of the cells. It is interesting to note that the counts for the immune ducks (protein vaccinated) are in between that of the negative and positive control groups.

9.4.7. CMI Response results

9.4.7.1. Bursectomised Ducks

The results from the Bursectomised ducks, for the significant P/N analysis have been summarised, (Table 73, p.242). The full results for each duck are in the Appendix (11.9, p.A43).

The bursectomised response to mitogens cannot be fully appreciated as data were only available for three ducks (W109, W121, and W130).

Due to a problem with obtaining enough of peptides 1-14, 7-14W-27, 7-14R-27, 22-41, 229-248, and 307-326, there is no CMI response data for these peptides. CMI response data were only available for seven ducks (W101, W109, W121, W130, W131, W132, and W145). There was no clear pattern of response to any of the peptides tested, however one point to keep in mind, is that none of the bursectomised ducks responded to peptide 71-90 (one of the immunologically important peptides incorporated into the DNA vaccine). Only one duck responded well to peptide stimulation (W132). In this duck, *in vitro* testing corresponded with dot blot hybridisation seroconversion from positive to negative.

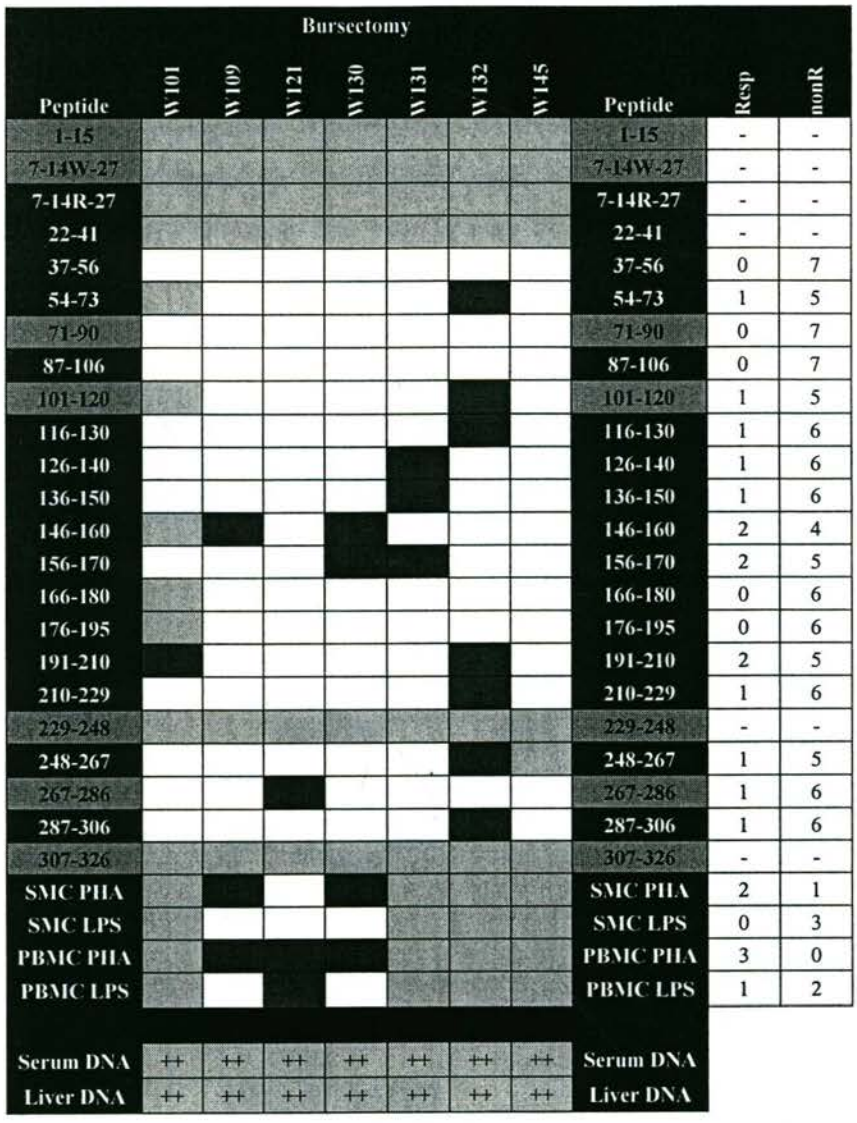


Table 73. *Summary of CMI response of Bursectomy ducks to Surface ORF peptides (significant P/N).*

Resp: Number of ducks that responded (significant P/N) ( ). NonR: Non-responders (blank box). Empty shaded box ( ): not tested. DHBV DNA summary: Dot blot hybridisation positive (++), negative (-). The peptides selected for the DHBV DNA vaccine (Chapter 7, p.170), are in black text with light blue background.

9.4.7.2. Thymectomised Ducks

The results from the Thymectomised ducks, for the significant P/N analysis have been summarised, (Table 74, p.243). The full results for each duck are in the Appendix (11.9, p.A43).

The response to the mitogen PHA, was good and compares well with the other CMI response experiments. The LPS response was poor, but comparable to that of the positive controls.

The one thymectomy duck with quantifiable viraemia (W126), did not respond to a single peptide, but the SMC were viable and able to respond to PHA. This correlates with the positive controls which did not respond well to the peptides either.

More than half (7/13), of the thymectomised ducks responded to peptide 210-229; considered non-specific as several of the other CMI groups also responded to this peptide (negative, and protein vaccinated). This epitope was found to have sequence similarity to a streptococcal species (Chapter 6), which could result in cross reactivity with the DHBV peptide. The immune response to this peptide may be humoral, with the production of antibodies, and it is possible that the thymectomised ducks (with higher proportion of B-cells), are able to respond to a B-cell epitope in the lymphoblastogenesis assay.

	Thymectomy															
Peptide	W122	W125	W126	W147	W151	W152	W153	W156	W157	W160	W167	W168	W170	Peptide	Resp	nonR
1-15		■										■		1-15	2	11
7-14W-27		■				■	■					■		7-14W-27	4	9
7-14R-27	■											■		7-14R-27	2	11
22-41	■					■						■		22-41	3	10
37-56							■							37-56	1	12
54-73														54-73	0	13
71-90						■	■			■		■		71-90	4	9
87-106														87-106	0	13
101-120														101-120	0	13
116-130					■			■						116-130	2	11
126-140							■							126-140	1	12
136-150		■			■							■		136-150	3	10
146-160														146-160	0	13
156-170														156-170	0	13
166-180		■											■	166-180	2	11
176-195														176-195	0	13
191-210						■						■		191-210	2	11
210-229		■			■	■	■	■				■	■	210-229	7	6
229-248														229-248	0	13
248-267		■							■			■	■	248-267	4	9
267-286														267-286	0	13
287-306														287-306	0	13
307-326														307-326	0	13
SMC PHA		■	■	■	■	■	■	■	■	■	■	■	■	SMC PHA	12	1
SMC LPS		■		■								■	■	SMC LPS	4	9
PBMC PHA	■	■		■	■	■	■	■		■	■	■	■	PBMC PHA	11	2
PBMC LPS		■		■							■			PBMC LPS	3	10
Serum DNA	+	-	++	+	-	-	+	-	+	-	-	-	+	Serum DNA		
Liver DNA	-	-	++	-	-	++	-	-	-	++	-	-	-	Liver DNA		

Table 74. *Summary of CMI response of Thymectomised ducks to Surface ORF peptides (significant P/N).*

Resp: Number of ducks that responded (significant P/N) (■). NonR: Non-responders (blank box). DHBV DNA summary: Dot blot hybridisation positive (++), PCR positive only (+), negative (-). The peptides selected for the DHBV DNA vaccine (Chapter 7, p.170), are in black text with light blue background.

None of the thymectomised ducks responded to the antigenically important peptides 101-120, 229-248, 267-286, 307-326. This lack of response is expected, as these should be T-cell epitopes, and the thymectomised ducks have a reduced ability to respond to such

epitopes. Interestingly, several ducks (4/13), responded to peptide 71-90, which could be a B-cell epitope (as none of the bursectomised ducks responded to this peptide).

The results from both the Bursectomy and Thymectomy experiments were analysed and compared to each other (Table 75, p.244), and compared to other CMI response experiments (Table 76, p.245, and Table 77, p.246).

	Thymectomy group		Bursectomy group			Fisher Exact	
Peptide	Resp	nonR	Resp	nonR	Peptide	P	< 0.05
1-15	2	11	-	-	1-15	-	
7-14W-27	4	9	-	-	7-14W-27	-	
7-14R-27	2	11	-	-	7-14R-27	-	
22-41	3	10	-	-	22-41	-	
37-56	1	12	0	7	37-56	1.000	
54-73	0	13	1	5	54-73	0.316	
71-90	4	9	0	7	71-90	0.249	
87-106	0	13	0	7	87-106	ns	
101-120	0	13	1	5	101-120	0.316	
116-130	2	11	1	6	116-130	1.000	
126-140	1	12	1	6	126-140	1.000	
136-150	3	10	1	6	136-150	1.000	
146-160	0	13	2	4	146-160	0.088	
156-170	0	13	2	5	156-170	0.111	
166-180	2	11	0	6	166-180	0.544	
176-195	0	13	0	6	176-195	ns	
191-210	2	11	2	5	191-210	0.587	
210-229	7	6	1	6	210-229	0.158	
229-248	0	13	-	-	229-248	-	
248-267	4	9	1	5	248-267	1.000	
267-286	0	13	1	6	267-286	0.350	
287-306	0	13	1	6	287-306	0.350	
307-326	0	13	-	-	307-326	-	
SMC PHA	12	1	2	1	SMC PHA	0.350	
SMC LPS	4	9	0	3	SMC LPS	0.529	
PBMC PHA	11	2	3	0	PBMC PHA	1.000	
PBMC LPS	3	10	1	2	PBMC LPS	1.00	

Table 75. *Summary of the statistical analysis of the Bursectomy and Thymectomy groups (significant P/N).*

The red shade indicates a possible trend (P<0.10). ns: non significant. The peptides selected for the DHBV DNA vaccine (Chapter 7, p.170), are in black text with light blue background.

Peptide	Bursectomy Resp	Bursectomy nonR	Negative Resp	Negative nonR	Fisher Exact P	Fisher Exact < 0.05	Positive Resp	Positive nonR	Fisher Exact P	Fisher Exact < 0.05	Protein Vacc Resp	Protein Vacc nonR	Fisher Exact P	Fisher Exact < 0.05	Peptide
1-15	-	-	1	23	-		1	5	-		4	11	-		1-15
7-14W-27	-	-	5	19	-		1	5	-		10	5	-		7-14W-27
7-14R-27	-	-	4	20	-		1	5	-		11	4	-		7-14R-27
22-41	-	-	4	20	-		1	5	-		6	9	-		22-41
37-56	0	7	1	23	1.000		0	12	ns		4	11	0.263		37-56
54-73	1	5	2	22	0.501		0	12	0.333		5	10	0.623		54-73
71-90	0	7	0	24	ns		0	12	ns		3	12	0.523		71-90
87-106	0	7	4	20	0.550		1	11	1.000		3	12	0.523		87-106
101-120	1	5	1	23	0.366		0	12	0.333		6	9	0.613		101-120
116-130	1	6	2	22	0.550		0	12	0.368		3	12	1.000		116-130
126-140	1	6	3	21	1.000		0	12	0.368		3	12	1.000		126-140
136-150	1	6	1	23	0.406		1	11	1.000		5	10	0.616		136-150
146-160	2	4	4	20	0.571		0	12	0.098		1	14	0.184		146-160
156-170	2	5	4	20	0.596		0	12	0.123		3	12	0.637		156-170
166-180	0	6	3	21	1.000		0	12	ns		2	13	1.000		166-180
176-195	0	6	3	21	1.000		0	12	ns		4	11	0.281		176-195
191-210	2	5	0	24	0.045	*	0	12	0.123		2	13	0.565		191-210
210-229	1	6	9	15	0.379		2	10	1.000		9	6	0.074	*	210-229
229-248	-	-	7	17	-		0	6	-		9	6	-		229-248
248-267	1	5	2	22	0.501		1	10	1.000		4	11	1.000		248-267
267-286	1	6	3	21	1.000		0	12	0.368		7	8	0.193		267-286
287-306	1	6	2	22	0.550		0	12	0.368		4	11	0.637		287-306
307-326	-	-	4	20	-		0	6	-		7	8	-		307-326
SMC PHA	2	1	24	0	0.111		7	5	1.000		15	0	0.176		SMC PHA
SMC LPS	0	3	18	6	0.029	*	2	10	1.000		11	4	0.043	*	SMC LPS
PBMC PHA	3	0	18	0	1.000		7	1	1.000		6	0	1.000		PBMC PHA
PBMC LPS	1	2	4	14	1.000		2	6	1.000		0	6	0.333		PBMC LPS

Table 76. *Summary of the statistical analysis of the Bursectomy and other CMI response groups (significant P/N).*

The asterisk indicates a significant difference (P<0.05) while the red shade indicates a possible trend (P<0.10). ns: non significant. The peptides selected for the DHBV DNA vaccine (Chapter 7, p.170), are in black text with light blue background.

Peptide	Thymectomy Resp	Thymectomy nonR	Negative Resp	Negative nonR	Fisher Exact P	Fisher Exact < 0.05	Positive Resp	Positive nonR	Fisher Exact P	Fisher Exact < 0.05	Protein Vacc Resp	Protein Vacc nonR	Fisher Exact P	Fisher Exact < 0.05	Peptide
1-15	2	11	1	23	0.278		1	5	1.000		4	11	0.655		1-15
7-14W-27	4	9	5	19	0.691		1	5	1.000		10	5	0.128		7-14W-27
7-14R-27	2	11	4	20	1.000		1	5	1.000		11	4	0.003	*	7-14R-27
22-41	3	10	4	20	0.678		1	5	1.000		6	9	0.435		22-41
37-56	1	12	1	23	1.000		0	12	1.000		4	11	0.333		37-56
54-73	0	13	2	22	1.000		0	12	ns		5	10	0.044	*	54-73
71-90	4	9	0	24	0.011	*	0	12	0.096	*	3	12	0.670		71-90
87-106	0	13	4	20	0.276		1	11	0.480		3	12	0.226		87-106
101-120	0	13	1	23	1.000		0	12	ns		6	9	0.018	*	101-120
116-130	2	11	2	22	0.602		0	12	0.480		3	12	1.000		116-130
126-140	1	12	3	21	1.000		0	12	1.000		3	12	0.600		126-140
136-150	3	10	1	23	0.115		1	11	0.593		5	10	0.686		136-150
146-160	0	13	4	20	0.276		0	12	ns		1	14	1.000		146-160
156-170	0	13	4	20	0.276		0	12	ns		3	12	0.226		156-170
166-180	2	11	3	21	1.000		0	12	0.480		2	13	1.000		166-180
176-195	0	13	3	21	0.538		0	12	ns		4	11	0.102		176-195
191-210	2	11	0	24	0.117		0	12	0.480		2	13	1.000		191-210
210-229	7	6	9	15	0.489		2	10	0.097	*	9	6	1.000		210-229
229-248	0	13	7	17	0.038	*	0	6	ns		9	6	0.001	*	229-248
248-267	4	9	2	22	0.157		1	10	0.327		4	11	1.000		248-267
267-286	0	13	3	21	0.538		0	12	ns		7	8	0.007	*	267-286
287-306	0	13	2	22	0.532		0	12	ns		4	11	0.102		287-306
307-326	0	13	4	20	0.276		0	6	ns		7	8	0.007	*	307-326
SMC PHA	12	1	24	0	0.351		7	5	0.073	*	15	0	0.464		SMC PHA
SMC LPS	4	9	18	6	0.015	*	2	10	0.645		11	4	0.056	*	SMC LPS
PBMC PHA	11	2	18	0	0.168		7	1	1.000		6	0	0.544		PBMC PHA
PBMC LPS	3	10	4	14	1.000		2	6	1.000		0	6	0.517		PBMC LPS

Table 77. *Summary of the statistical analysis of Thymectomy and other CMI response groups (significant P/N).*

The asterisk indicates a significant difference (P<0.05) while the red shade indicates a possible trend (P<0.10). ns: non significant. The peptides selected for the DHBV DNA vaccine (Chapter 7, p.170), are in black text with light blue background.

9.5. DISCUSSION

We hypothesised that co-ordination of the cellular and humoral arms of the immune system are required for hepatitis B virus clearance. The lack of coordination of the two arms, or abrogation of either arm, should result in persistence of HBV and the development of chronic infection.

In this study we investigated the effect of the abrogation of either the humoral arm (by bursectomy) or the cellular arm (by thymectomy) of the immune system. In studies of mouse immunology, T-cell deficiency is achieved by combining thymectomy, subjecting the animal to irradiation, and re-constituting the B-cell population by allograft from the same mouse strain to re-establish B-cell competence. This method confers total ablation of intra or extra-thymic T-cells, however, it was impractical for use in these experiments due to the unknown degree of genetic variability in our outbred animal population, and limited knowledge of cell markers for the *in vitro* expansion and selection of lymphocyte subsets. Consequently, the effect of residual thymic function has not been entirely excluded. Development of the thymus in birds begins at day 5 of incubation as an outgrowth of the pharyngeal pouches. Precursor cells originating from blood-borne lymphoblasts within the yolk sac, enter the thymus from 7 days of incubation (Jotereau *et al.*, 1980), and differentiate into T-lymphocytes within the special microenvironment of the thymus. The T-lymphocytes that are incapable of recognising self-antigen undergo extensive proliferation within the thymus independently of antigenic stimulation. Successive waves of thymocyte precursors enter the thymus and undergo both positive and negative clonal selection, and subsequently populate the lymphoid organs.

Within the developing chick, *in situ* expansion of cortical TcR1 cells is minimal. These cells however, rapidly disperse throughout the body, and are found in the spleen by embryonic day 15, and intestine and bursa a day later. TcR1 cells comprise approximately 20-50% of circulating T-cells in adult chickens, and are located in the red pulp of the spleen; two thirds of the cells express CD8 (Cooper *et al.*, 1991). TcR1 cells do respond to PHA, but not as well as other T-cells, they can be cytotoxic, and may include a subset of suppressor cells (Quere, 1992). Development of TcR2, and TcR3 T-cells, is moderately compromised by thymectomy, however, TcR1 cells are severely compromised, suggesting a continual thymic seeding of the peripheral TcR1 population (Chen *et al.*, 1989).

In ducks, bursectomy can be successfully performed surgically, whereas in the mouse it is achieved by γ-irradiation, or antibodies to B-cells. Surgical removal *in ovo* has been shown to severely limit B-cells from the chicken (Huang and Dreyer, 1978). Bursectomy at embryonic day 18, leads to complete elimination of B-cells, while our bursectomy was

performed at day of hatch (embryonic day 21), which should significantly reduce the number of B-cells.

The positive control ducks were given a dose of DHBV that would result in approximately half of the control ducks becoming chronically infected as characterised by DHBV infection of the liver at euthanasia. The outcome of the dose was very close to that expected, with 12/22 ducks liver positive.

As expected, the abrogation of the humoral arm of immunity led to persistence of infection in all ten ducks. Thymectomy had a marginal but non-significant ($p = 0.089$) effect on the prevention of persistent infection with 10/13 thymectomised ducks liver negative, compared with 10/22 control ducks clearing DHBV infection.

The bursa in the duck is a long cylindrical organ attached to the dorsum of the cloaca by a thin stalk and there is little difficulty in ensuring its complete removal; up to 98% of ducks have no residual bursal material following neonatal bursectomy (Hasek *et al.*, 1972). The thymus is however more difficult to completely remove as like the chicken, it is a lobulated organ lying along the jugular vein, and both the number of lobes and their location can vary from duck to duck, which increases the chance that some thymic material may remain post thymectomy. It has been found that about 5% of neonatally thymectomised chickens had detectable thymic tissue at autopsy (Cooper *et al.*, 1966b). We found little evidence of residual thymic material in our thymectomised ducks, although there was clear evidence of major thymic involution in the adult positive control ducks. Residual thymic material has been reportedly found in all thymectomised chickens (Warner and Szenberg, 1962). Despite this, these chickens still failed to reject implanted homografts in the normal fashion. Even without the complete removal of all thymic material, all thymectomised ducks would have suffered a relative loss of T-cells compared to the normal controls or the bursectomised group. Since the thymectomised duck lymphocytes were able to responded sufficiently to PHA, it is possible that some of the thymectomised ducks had sufficient thymic material to produce effective T-cells, that a lymphocyte population which had already migrated through the thymus prior to hatch was able to produce the response, or that duck PHA sensitive lymphocytes can originate from extrathymic sources such as the liver and spleen (although such cells may not be sufficiently matured). It has been shown that stimulated cultures from normal ducks were supported by macrophage adhesion whereas cultures deficient of macrophages were less capable of proliferating; avian macrophages also respond to PHA (Higgins and Teoh, 1988). Although PHA is a polyclonal antigen which is capable of stimulating and re-stimulating multiple T-cells, it was suggested that survival was dependent on cell to cell contact (Higgins and Teoh, 1988), and induction of lymphokine release

including IL-2 resulting in transformation and prolonged survival (Vickery and Cossart, 1996).

Neonatal thymectomy has previously been reported to cause depression of the total leukocyte count in ducks (Sugimura *et al.*, 1975). This was evident in our results from a significant decrease in the total leukocyte count in the thymectomised ducks when compared with immunologically normal positive control ducks ($p<0.005$) and the negative control ducks ($p=0.048$), indicating a reasonably successful removal of the thymus. In comparison, the total leukocyte count was elevated in bursectomised ducks ($p<0.001$) possibly indicating a status of ongoing infection.

In an endeavour to determine whether the change in total leukocyte count was due to a decrease in circulating lymphocytes, the PBMC counts following cell culture purification were used. There are several sources of error for these counts, such as occasionally 10mL of blood could not be obtained, and cells are lost during the purification procedure, but overall these errors should have been equal for all groups, making the data usable. Overall, the circulating lymphocyte number was unaffected by bursectomy, or thymectomy, when compared to controls. This was similar to experiments in the chicken where depletion in T-cells caused a compensatory increase in B-cells, and *visa versa* (Wick *et al.*, 1975). So, although thymectomised chickens had decreased T-cells, and bursectomised chickens had decreased B-cells, the overall number of circulating lymphocytes remained the same. Due to a technical difficulty, blood smears for counting the blood cell percentages were lost, preventing a detailed comparison of T-cell numbers.

A correlation between the patterns of acute infection and outcome was established. Ducks that had low level viraemia, were more likely to clear the virus from the serum and/or liver, than ducks with high, or prolonged viraemia. The biphasic pattern was again seen and was associated with a failure to clear the infection from the liver. Although viraemia was more pronounced in the bursectomised group than the positive controls, the peak level of viraemia was comparable. Ongoing infection was characterised by a higher incidence of inflammatory responses within the liver: all ducks with liver inflammation were DHBV DNA liver positive (except for the single negative control duck). Little evidence of inflammation was seen in ducks that cleared the infection, which suggests clearance by curing rather than cell death, and inflammation caused by cellular (Th1 or macrophage) rather than antibody induced mechanisms.

The hypothesis currently proposed by Chisari, is that control of hepadnaviral replication, and clearance of infection occurs before liver damage and is mediated by soluble factors such as

IFN, or TNF. The experimental evidence for this theory is based on transgenic mouse studies, and a limited number of chimpanzee studies (Guidotti *et al.*, 1994; Chisari and Ferrari, 1995; Guidotti *et al.*, 1999; Thimme *et al.*, 2003; Wieland *et al.*, 2003). Although no severe liver damage was seen in our ducks, limited inflammation was associated with the thymectomised, and positive control ducks that failed to clear the infection, suggesting that the cells are cured, not destroyed. DHBV infection was significantly associated ($p<0.001$) with hepatitis, as has previously been shown (Vickery *et al.*, 1989).

High viral titres early in the infection phase, particularly within the first two weeks were found to be an early marker of chronic infection, while viraemia was self-limiting by no later than 3 weeks following inoculation in control ducks in which DHBV was cleared from the liver. B-cell production of sAg neutralising antibody is known to correlate with a reduction of viraemia late in the time course of acute infection. However, the production of this virus specific antibody, which is critical for complexing and clearing viral particles and preventing reinfection of susceptible cells, is a T-cell dependent process. Although neonatally thymectomised chickens are incapable of rejecting homografts, they are able to mount a non-specific antibody response (Warner and Szenberg, 1962; White and Timbury, 1973), but the level of specific viral antibodies is decreased (White and Timbury, 1973).

No statistical difference in response to mitogens was observed between PBMC and SMC cells except from the thymectomised ducks, (excluding the bursectomised group, which only consisted of three ducks). A quantitative increase in response to PHA was observed with ducks that have cleared DHBV from serum in comparison to ducks with infected livers. PBMCs of human chronic carriers have been shown to become insensitive to PHA (Scudeletti *et al.*, 1986; Nouri-Aria *et al.*, 1988), while others have demonstrated that lymphocyte transformation by PHA was normal in patients with Hepatitis B, chronic active hepatitis, asymptomatic carriers, and patients with chronic persistent hepatitis (Wicks *et al.*, 1975). CMI suppression, implicating defective T-cells, or accessory inhibitory cells or pathways, may be associated with ducks exhibiting evidence of prolonged liver infection.

Persistent infection is normally associated with low level immune response, however in the immune modulated ducks, although the bursectomised ducks were viraemic and liver positive, the number of lymphoblastic responses was not less than the thymectomised ducks, most of which had cleared the infection. Unexpectedly, the bursectomised ducks even showed a trend towards responding to peptide 146-160, however the relatively small numbers involved do not make any conclusions possible. Whether the low response from the thymectomised ducks was due to the rapid decrease in cellular response over time, or

indicative of other clearance mechanisms is unknown. Further studies involved in measuring the immune response of thymectomised ducks sooner after challenge are required.

The bursectomised ducks were only able to produce a significantly different response to one peptide (peptide 191-210, when compared to the negative control group) (Table 76, p.245). The small number of ducks in the bursectomised group decreases the significance of any difference.

Analysis of the lymphoblastic response of the thymectomised ducks with that of the other groups produces some interesting differences (Table 77, p.246). The thymectomised ducks responded significantly better to peptides 71-90, and 229-248, than the negative controls. The protein vaccinated ducks responded similarly to peptide 229-248, when compared with the negative controls, but they did not significantly respond to peptide 71-90, when analysed by the sig P/N method. However, both of these peptides were incorporated into the DNA vaccine (8.3, p.202). Further comparison of the thymectomised ducks with the protein vaccinated ducks, indicates that the thymectomised ducks did not respond as well to peptides 7-14WR-27, 54-73, and 101-120, as the protein vaccinated compared to negative controls. Even though the outcome of the protein vaccinated and the thymectomised ducks was similar, their lymphoblastic response to various epitopes on the DHBsAg was significantly different. These studies are unable to determine what the difference in the response is due to, but it may be that the removal of the majority of TcR1 T-cells (by thymectomy), may have led to the removal of suppressor cells (the majority of which are TcR1 cells), which allowed a more effective immune response to be generated.

The lack of response by the thymectomised ducks to peptides 101-120, 229-248, 267-286, and 307-326, is a good indication that these epitopes are T-cell epitopes, or at least T-cell dependent. The lack of response by the bursectomised ducks to peptide 71-90, is not significant as the group as a whole did not respond well to any peptides, but as the thymectomised ducks responded quite well (4/13), it is possible that this peptide contains a B-cell epitope, and that the lymphoblastogenesis assay was able to detect B-cell proliferation, rather than just for T-cells. Peptide 71-90 is in the preS region that contains may other B-cell epitopes, and it may have been detected in the thymectomised ducks because of an increased B-cell response.

The down regulation of costimulatory molecules expressed on APC may indicate T-cell suppression, which may be associated with the role of activated T suppressor cells; found to have a specific phenotype in the murine model (Sakaguchi *et al.*, 1996). These suppressor cells have an IL-2 receptor alpha-chains (Roitt and Delves, 2001), this phenotype of T-cell

inhibits the up-regulation and production of IL-2, thus suppressing the proliferation of responding CD4+ and CD8+ T-cells and ultimately, effecting production of TNF-α and IFN-γ which mediate the mutual antagonism of Th1 and Th2 subsets. The mechanism of suppression is considered to be cell-contact dependent (Dieckmann *et al.*, 2002), and also impairs co-stimulatory pathways for activated B-cells. The co-stimulation of activated B-cells by T helper cells (Th2) may be thus blocked and could explain the absence of anti-HBs in chronically infected patients.

In conclusion the loss of the humoral immune system by bursectomy leading to persistent infection with higher levels of virus replication suggests that the CMI response alone is insufficient to clear hepadnavirus infection. However, thymectomy at hatch had little effect on the outcome of infection. This unexpected result may indicate that sufficient thymic material remained, the T-cell effectors of clearance have already passed through the thymus prior to hatch, the innate immune responses are increased in thymectomised animals, or as has been shown in the chicken thymectomy results in augmentation of humoral immunity.

10. General Discussion

These studies were initiated to gain insight into the interaction between the surface protein of DHBV and the immune system. It was hoped that this would lead to a new understanding of the mechanism of virus clearance and possibly even to the design of a therapeutic vaccine which might be effective in established carriers.

A temporal association between the appearance of DHBV surface antigen specific lymphoblastic proliferation and clearance had already been observed using native S protein as the test antigen (Vickery *et al.*, 1997; Vickery *et al.*, 1999a; Vickery *et al.*, 1999b). These findings were extended in an experimental system where inoculation of ducks at a defined age with a specific virus dose would reliably produce virus clearance in some members of the cohort and persistence in others. During standardisation of this model system a novel biphasic pattern of infection was observed in a proportion of inoculated ducks. The rapid fluctuation, both up and down, in the level of viraemia in the absence of massive liver damage implied a dynamic interaction between the immune system and virus replication. The literature provided some support for this hypothesis, particularly studies of hepatitis B transgenic mice where very rapid suppression of viral synthesis was achieved by administration of interferon (Guidotti *et al.*, 1996b; Guidotti *et al.*, 2002). In the duck the detailed histological studies by Jilbert and co-workers, showed dramatic reduction of DHBV antigens and DNA in the liver without massive lymphocyte infiltration, or cell death (Jilbert *et al.*, 1992). They therefore attributed this down regulation to cytokine activity rather than cell mediated cytotoxicity.

To investigate the mechanism of this regulation and how it might lead to viral clearance it was decided to compare the sequence of viruses circulating at different phases of infection. It was hypothesised that immune pressure might select virus variants of either enhanced or diminished replicative efficiency. A particular mutation (T=>A double substitution at nt 731 and 732) was found in two different ducks both of which had self-limited infection. No other nucleotide substitutions were observed in any of the 38 other ducks. This mutant could not be passaged directly from the serum of these ducks, nor could it be transmitted by inoculation of a full length clone. Taken together this implies that immune selection of a defective variant may be one mechanism of hepadnavirus clearance.

The location of this mutation at the extreme 5' end of the pre-S gene outside the normal coding sequence suggests that it may have a regulatory role on virus replication, and it would be expected to interact with IFN, the putative effector cytokine. Little is currently understood about duck cytokines or their response elements, though gradual progress is being made in cloning and sequencing duck immunoglobulin and cytokine genes (Ziegler and Joklik, 1981a; Higgins *et al.*, 1993; Higgins and Warr, 1993; Schultz *et al.*, 1995; Schultz and Chisari, 1999; Huang *et al.*, 2001). The cDNA of Duck IFN-gamma contains a 495 bp ORF that encodes a putative 164 aa protein that shares 67% identity with chicken IFN-gamma, but only 30-35% identity with mammalian IFN-gamma (Huang *et al.*, 2001). This low sequence homology between duck cytokines and chicken or mammalian cytokines has been experimentally paralleled in showing that chicken or mammalian cytokines have low cross-reactivity with the duck system (Higgins *et al.*, 1993; Huang *et al.*, 2001). Commercially available cytokines are therefore not particularly useful in the investigation of DHBV and until duck IFN can be obtained by gene expression the non-specific immune response, which is highly significant in hepadnavirus clearance, cannot be investigated further.

The mutation of interest was not present in all of the ducks with virus clearance, so the peptides important in the specific sAg CMI response associated with clearance was defined using the lymphoblastogenesis assay. This approach was dictated by the lack of reagents for ELISPOT or identification of T cell lineages in the duck. The Surface protein sequence of DHBV was initially subjected to computational analysis based on hydrophobicity, surface probability, and antigenicity, to attempt to select immunogenic peptides. This showed that there were several hydrophobic regions towards the end of the S region which are considered to be the transmembrane domains, while the preS region was predominantly hydrophilic, in keeping with the current consensus that it is the region responsible for receptor binding.

A battery of twenty-three overlapping peptides was synthesised, including the native and mutant variant sequence for peptide 7-21 (7-14W-27 and 7-14R-21, respectively). When these peptides were tested using peripheral blood mononuclear cells and splenic mononuclear cells from naïve, infected and immunised ducks stimulatory responses were found in individual ducks in all three groups. Database similarity searches of all the peptides revealed that they all had homology with other DHBV strains, while a few were found to have varying degrees of relation to Snow Goose, Crane, Heron, Stork, Human (and other mammalian hepadnaviruses). It was interesting to discover that peptide 176-195 had some similarity with a murine T-cell receptor, while peptide 210-229 was related to a streptococcal protein. The significance of these relationships was not determined, but does open some intriguing possibilities, such as it may be possible that the Surface protein is able to interfere

with the host's immune response. Immunomodulation is known for several viruses and may explain the lack of immune response in persistent infection.

The persistently infected ducks failed to significantly ($p<0.05$) respond to any of the sAg peptides when compared with the negative controls. Two different methods of analysis (>5000cpm and sig P/N, section 7.3.3, p.177) both showed that immune and challenged ducks had a significant ($p<0.05$) response to peptides 7-14W-27, 7-14R-27, 71-90, 101-120, and other peptides that where found to be also important ($p<0.10$) were 1-15, 37-56, 229-248, 267-286, and 307-326. The significant peptides included the peptide spanning the mutant (7-14R-27), described above. After initial interpretation of the results (>5000cpm) peptides 1-15, 7-14W-27, 71-90, 101-120, 229-248, 267-286, and 307-326 were designated "peptides of immunological importance" and it was decided to test this interpretation by incorporating them in a DNA vaccine which was designed to stimulate a specific CMI response. It was noted that one of these peptides (101-120) overlapped known DHBV B cell motifs defined as naturally occurring DHBV antibody epitopes (Chassot *et al.*, 1994).

The DNA vaccine was constructed in the plasmid pDVERA2 (generously provided by Scott Thomson) by a three step process of producing the DuckPoly (containing the coded peptides), cloning of the DP, and subcloning of the DP into the DNA vaccine plasmid. It was tested for T cell immunogenicity in naïve ducks by assaying the response of PBMCs to the seven "immunologically important peptides" in the lymphoblastogenesis assay 7 days after a third injection of vaccine at which time they were challenged with $2.5x10^{10}$ vge of DHBV. They were euthanased and their SMC assayed by lymphoblastogenesis assay a month (28-30 days) later. Persistently infected ducks were vaccinated with a similar schedule and observed for three subsequent weeks before they were killed and lymphoblastogenesis assays performed on the splenic mononuclear cells. The CMI response on all occasions was disappointing, but in retrospect this might have been predicted by the choice and timing of the tests. The use of PBMC means that only low cell numbers are available and there is the probability that stimulated cells will be localised in the liver and hence underrepresented in the circulation. The decision to observe challenge results on the naïve vaccinated ducks and to follow the effect of vaccination on viraemia in the persistently infected groups resulted in a significant time lapse between the last antigenic stimulus and testing. This probably exceeded the limits of detectability of responses using *in vitro* testing, because antigen-specific responses quickly fall to baseline levels (Vickery *et al.*, 1999b).

An unexpected outcome of the DNA vaccination experiment was the generation of protective immunity to challenge. Although noted in the modelling process, the overlap of a

single neutralising B cell epitope (Chassot *et al.*, 1994), within one of the T cell epitopes (peptide 101-120) used in the DNA vaccine, was not considered to be enough to elicit such a strong response. However, neutralising antibody was formally detected in the serum of one of the two protected ducklings but insufficient serum was available to pursue this issue further. A DHBV DNA vaccine has previously been shown to provide protective immunity (Triyatni *et al.*, 1998), and it seems probable that our DNA vaccine was able to stimulate B-cells, as well as the anticipated T-cell response, and that a very effective protective DNA vaccine could be developed by incorporating a better spectrum of B cell epitopes. It could be an advantage to design a polytope with both T and B cell epitopes to induce a co-operative humoral and cellular response.

The effector mechanism responsible for hepadnavirus clearance has long been assigned to a cell mediated immune response, but it has not been clear if the same antigenic specificity is responsible for clearance and hepatocyte damage. In HCV infection, virus-specific CTLs limit viral replication in patients with chronic HCV infection (Freeman *et al.*, 2003). There are good indications that capsid antigens induce hepatitis and cirrhosis in both human hepatitis B and woodchuck HBV (Burrell *et al.*, 1984; Zoulim *et al.*, 1996). The situation regarding clearance is less defined, but there is almost certainly a need for an anti-surface response capable of protecting uninfected hepatocytes whatever the mechanism of down regulation of virus replication. An experiment using antiviral treatment to inhibit virus growth in established DHBV carriers, followed by DNA vaccination could clarify this issue, and within the last year several groups have attempted this with varying degrees of success (Foster *et al.*, 2003; Le Guerhier *et al.*, 2003).

Treatment of HBV in man uses a strategy of antiviral treatment plus administration of interferon over a long period (Bahar *et al.*, 2003; Cooksley *et al.*, 2003; Heathcote, 2003; Yalcin *et al.*, 2003). There is no consensus about the detection of a specific CMI in individuals responding to treatment. The lack of reagents for identification of duck lymphocyte classes has been a great impediment to studies of this type in experimental DHBV infection, but the practicability of modulating the immune response by surgical removal of the bursa or thymus makes it possible to assign effector roles to the different arms of the immune system.

Bursectomised ducklings were unable to clear DHBV, whereas paradoxically, thymectomised and control birds cleared infection at comparable rates. These findings provide substantial support for the hypothesis that production of neutralising antibodies is an essential component of viral clearance. The observed Surface protein specific lymphoblastogenesis response could therefore be significant in the context of B cell

stimulation rather than in effecting clearance of infected cells, or directly down-regulating virus replication.

The technical difficulties of surgical thymectomy in duck hatchlings may have permitted survival of a T cell population (Cooper *et al.*, 1966b), sufficient to achieve clearance by generation of specific T cell responses, but a more probable explanation is the over riding importance of non-specific CMI in down regulating virus replication (Wieland *et al.*, 2003). The T cells involved in innate immune responses escape from the thymus in significant numbers pre-hatch and would thus be available in even rigorously thymectomised ducks. Effective therapeutic vaccines may therefore need to stimulate IFN responses by incorporating appropriate motifs, and viral polytopes encoding B cell rather than T cell peptides alone (Min *et al.*, 2001).

The findings from this investigation raise many new questions, and there are several pathways along which further research could be directed. The current findings have limited statistical significance because of the considerable variation in individual response of ducks in the same experimental group. While larger numbers may well increase the statistical significance, it would also be influenced by the outbred state of the ducks presently available. Currently, there are no commercially available lineages of ducks that can be used for experimental purposes, which means that the individual responses of the currently used outbred ducks vary substantially. The use of better genetically defined ducks would allow fewer to be used in each experiment, and allow more specific research to be undertaken on individual components of the immune system.

There is a growing understanding of the molecular biology of the duck immune system (Jacobs *et al.*, 1997; Magor *et al.*, 1999). Duck interferons have been under investigation for a long time, initially by use of partially purified supernatant (Ziegler and Joklik, 1981b), and more recently by using recombinant proteins produced in *E. coli* (Schultz *et al.*, 1995), which include duck IFN gamma (Schultz and Chisari, 1999).

However, the burgeoning discovery, and characterising of duck lymphokines (Higgins *et al.*, 1993; Huang *et al.*, 2001), opens a new world of possibilities. Many of the techniques that have so far been unavailable are or will soon be open to use in the duck model system. One of the most powerful techniques that would become available with the discovery of these duck proteins will be the ability to produce monoclonal antibodies to them. The production of such MAb would allow for a more detailed breakdown of the composition of the types of PBMCs that are in the liver and circulation during the various time periods of the various infection patterns. It is possible that certain subsets of PBMCs will be associated with

different liver pathology, and such information would allow for better prognosis of the infection in individuals. Knowing the cell types associated with clearance would lead to a better understanding of the mechanisms involved, and may lead to the use of certain cytokines (those secreted by cell types associated with clearance) in more effective treatment.

The expression of the new duck lymphokines in the liver would be of interest. Microchip gene arrays have opened up many new opportunities to observe the regulation of genes and the produced proteins (Schlaak *et al.*, 2002). Utilising such a system would enable us to examine the genes that are up regulated during infection in not only the white blood cells of the duck but also in hepatocytes, which may lead to discovering which genes are affected by the various cytokines, and what role they play in clearing the infection from the cell.

Another interesting aspect that was discovered during the current study was the possible sequence similarity of peptide 176-195 with part of a murine TcR. Other research found that a synthetic hydrophobic peptide (called core peptide) derived from the transmembrane sequence of the TcR alpha chain has been shown to inhibit T-cell mediated inflammation, shown to suggest that peptide inhibition is affected by its structure and charge interactions, and may involve common signalling molecules in T, B and natural killer cells (Huynh *et al.*, 2003). The concept that hepadnaviruses could be immunomodulatory has not been given much consideration, and would have implications for design of newer therapeutic treatment, and may be another factor in determining the outcome of infection.

The discovery of "newer" duck interleukins will open the door for studies of IL-12, which is of great interest in other chronic infections. IL-12 production is reduced in HIV infection, and recombinant human IL-12 (rhIL-12) augments *in-vitro* HIV-specific proliferative responses in PBMC from HIV-seropositive individuals. Later studies also demonstrated that rhIL-12 (recombinant human IL-12) augments *in-vitro* HIV-specific CTL activity (Young *et al.*, 2001). The use of naturally occurring antivirals should produce treatments that are less toxic than the current nucleoside analogues, and hopefully decrease the rate of treatment failure (Okamoto *et al.*, 2003).

The use of DNA vaccines has many advantages, and the current study encourages further work towards a therapeutic vaccine. The preliminary findings from this study are that our unadjuvanted DNA vaccine was able to induce both a CMI and protective antibody response. The 90% reduction in serum DHBV DNA levels a month after cessation of treatment, is comparable with early trials of therapeutic agents (Omata *et al.*, 1986; Sherker *et al.*, 1986; Tsiquaye *et al.*, 1986). One of the greatest challenges to DNA vaccination is delivery. In the

current study, the vaccine was injected with a standard syringe and administered on an *intramuscular* and *intradermal* schedule, similar to the first full viral DNA infections (Will *et al.*, 1982). In hindsight, neutralising antibodies may have been induced by the *id* injection. Production of neutralising antibody has been shown to be enhanced by *id* injection of a HBV protein vaccine, above that of the normal *im* administration (Wilkins and Cossart, 1990), which would indicate that administration *id* generally provides better immunogenicity that *im*. Future experiments should be used to test the two different administration methods to determine which produces a better response. The role of administration in the use of DNA vaccines cannot be underplayed, as much time and money has been invested in different delivery systems, such as the gene gun approach (Williams *et al.*, 1991; Tang *et al.*, 1992).

The DNA vaccine could also be combined with some of the newly discovered duck cytokines. The use of cytokines such as IFN is the new standard for treatment of chronic hepatitis infections, and it inclusion in the DNA vaccine could provide the necessary mechanism for an effective response, although careful selection of the appropriate IFN would have to be investigated as the closely related chicken appears to have several forms of IFN (Sick *et al.*, 1996). The use of the DNA vaccine could also be combined with drug therapy. Drug therapy could be used to lower the level of viraemia, and then the DNA vaccine could be used to augment the immune response.

Although HBV has had an effective vaccine for preventative treatment for many years now, there are still a large number of carriers in the world. Treatment of these carriers may allow for decreased morbidity of individuals, and decreased morbidity of the carrier community as a whole, and would be a worthwhile endeavour for its own sake. But the study of viral interaction with the immune system has produced much of the knowledge that we currently understand of our own immune systems, and has allowed use to consider new approaches to treatment and prevention.

The delicate balance between the host and the virus appears to be a highly complicated affair, of which no one single component is central to the outcome of infection. It is also clear that the balance between the host and virus is not static, but rather in a constantly dynamic equilibrium.

11. APPENDIX

11.1. CHEMICALS

Chemical	Company	Cat No.
α-^{32}P labelled dCTP	PerkinElmer ICN	ADC32L ADC-2
Agar	OXOID	L11
Agarose	ICN	193983
Ampicillin	ICN	194526
Chloroform	Sigma	C2432
CsCl	ICN	160041
DTT	Sigma	D8255
EDTA	ICN	194822
Ethanol	Sigma	E7148
Glacial acetic acid	Sigma	A0808
Glutaraldehyde	Sigma	F1635
Glycogen	Roche	901 393
Guanidine thiocyanate	ICN	820991
HCl Hydrochloric acid	ICN	194054
Isoamyl-alcohol	Sigma	I0640
Kanamycin	ICN	194531
KCl	ICN	194844
KH_2PO_4	ICN	195453
$MgCl_2$	Sigma	M9272

Chemical	Company	Cat No.
$Na_2HPO_4.7H_2O$	ICN	191441
NaCl	ICN	152575
NaOH	Sigma	S0899
Nonfat dried milk	Diploma	935725
PEG 6000	ICN	195445
Phenol	ICN	802516
Proteinase K	Sigma	P6556
RMPI 1640	Sigma	R6504
Sarcosyl	Sigma	L5125
SDS	ICN	194831
Sodium acetate	ICN	194012
Sodium azide	Sigma	S8032
Sodium citrate	Sigma	S4641
Thymidine methyl ^{3}H	ICN	24067
Tris base	Sigma	T8524
Trypan Blue	Sigma	T5526
Tryptone	OXOID	L37
X-Gal	ICN	194811
Yeast Extract	OXOID	L21

11.2. SOLUTIONS

11.2.1.1.1. Bovine Lacto Transfer Technique Optimiser (BLOTTO)

2.5g Nonfat dried milk, and 0.01g Sodium azide dissolved in 20mL dH_2O. Stored at 4°C.

11.2.1.1.2. Calf Thymus (3mg/mL)

Calf thymus added to TE buffer (11.2.1.1.18, p.A2) to a concentration of 3mg/mL, solubilised by a heated magnetic stirrer. DNA was fragmented by sonication, aliquoted into 20mL volumes, stored at 4°C.

11.2.1.1.3. dH_2O

Tap water was treated in a Modulab LS reverse osmosis filter (LiquiPure, Warrendale, USA) until purified to a level where electrical resistance was 15-20MΩ. The purified water was then autoclaved for 20min at 121°C, and stored at RT until required.

11.2.1.1.4. DTT

3.1g Sodium DiThioThreitol (DTT) added to 15mL of Sodium Acetate (10mM pH 8.0), dissolved, then made up to 20mL with Sodium Acetate (10mM pH 8.0). Filter sterilised and stored at –20°C.

11.2.1.1.5. EDTA (0.5M pH 8.0)

18.61g EDTA added to 80mL of dH_2O, dissolved, pH adjusted to 8.0 (with NaOH pellets ~2grams), then made up to 100mL with dH_2O. Autoclaved for 20min at 121°C, stored at RT.

11.2.1.1.6. Foetal Calf Serum

Foetal Calf Serum (FCS) was obtained from CSL laboratories. It was heat inactivated at 56°C for 40mins, then alloquoted and stored at –20°C until required.

11.2.1.1.7. Formalin (10%)

10mL of 100% Formalin (40% w/v Glutaraldehyde in water) was made up to 100mL with PBS (11.2.1.1.9, p.A2). Stored at RT for up to 1 week.

11.2.1.1.8. Heparin PBS

1mL of Heparin (100 IU/mL) was made up to 100mL with PBS (11.2.1.1.9 p.A2). This produced a solution containing 10 IU/mL.

11.2.1.1.9. Phosphate Buffered Saline (PBS)

Chemical	Stock solution 10x conc (g/L)	Working solution 1x conc (mM)
KCl	2.0	2.7
KH_2PO_4	2.0	1.4
$Na_2HPO_4.7H_2O$	11.5	4.3
NaCl	80.0	137.0

All chemicals were added to 700mL of dH_2O, dissolved, then the solution made up to 1L with dH_2O. Autoclaved for 20min at 121°C, stored at RT. 1xPBS (pH ~7.3) was prepared by diluting 10xPBS 10-fold with dH_2O.

11.2.1.1.10. Sarcosyl (10% w/v)

10g Sarcosyl added to 80mL of dH_2O, dissolved, then made up to 100mL. Filter sterilised and stored at RT.

11.2.1.1.11. Sodium Acetate (3M pH 5.2)

40.81g NaAcetate.$3H_2O$ or 24.61g anhydrous NaAcetate added to 80mL of dH_2O, dissolved, pH adjusted to 5.2 (with Glacial Acetic acid), then made up to 100mL with dH_2O. Autoclaved for 20min at 121°C, stored at RT.

11.2.1.1.12. Sodium Dodecyl Sulphate (10% SDS)

Dissolve 100g SDS (also known as Sodium Lauryl Sulphate) in 900mL dH_2O, heat to 68°C, adjust pH to 7.2 with HCl, make up to 1L. Stored at RT.

11.2.1.1.13. Sodium Sodium Citrate (20xSSC)

Chemical	Stock solution (g/L)
NaCl	175.3
Sodium citrate	88.2

All chemicals were added to 700mL of dH_2O, dissolved, pH adjusted to 7.0, then made up to 1L with dH_2O. Autoclaved for 20min at 121°C, stored at RT. Required concentration of SSC made by diluting 20xSSC with dH_2O.

11.2.1.1.14. Sodium Sodium Citrate (2xSSC)

100mL of 20xSSC was made up to 1L with dH_2O. Stored at RT.

11.2.1.1.15. Sodium Hydroxide (1M NaOH)

40g NaOH pellets made up to 1L with dH_2O. Stored at RT.

11.2.1.1.16. TAE (50x)

242g Tris base added to 500mL dH_2O, dissolved, 57.1mL glacial acetic acid, and 100mL EDTA (pH8.0) added, then made up to 1L with dH_2O. Autoclaved for 20min at 121°C, stored at RT.

11.2.1.1.17. TAE (1x)

100mL of 50xTAE was made up to 5L with dH_2O. Stored at RT.

11.2.1.1.18. TE (pH 8.0)

TE (1mM EDTA, 10mM Tris, pH 8.0). 10mL Tris (1M, pH 8.0) and 2mL EDTA (0.5M pH 8.0) was added to 988mL autoclaved dH_2O. Stored at RT.

11.2.1.1.19. TE for PCR (pH 8.0)

TE (0.1mM EDTA, 10mM Tris, pH 8.0). 10mL Tris (1M, pH 8.0) and 200µL EDTA (0.5M pH 8.0) was added to 989.8mL autoclaved dH_2O. Stored at RT.

11.2.1.1.20. TELT

TELT solution comprised of 2.5M LiCl, 50mM Tris/HCl (pH 8.0), 62.5mM Na_2EDTA, and 4% (w/v) Triton X-100.

11.2.1.1.21. TNE

10mM Tris/HCl, 0.1M NaCl, and 5mM EDTA. Stored at RT.

11.2.1.1.22. Tris (1M pH 7.0 - 8.0)

121.1g Tris base added to 800mL of dH_2O, dissolved, pH adjusted as required (with concentrated HCl ~20-40mL), then made up to 1L with dH_2O. Autoclaved for 20min at 121°C, stored at RT.

11.2.1.1.23. X-Gal (40mg/mL)

400mg X-Gal added to 10mL dimethylformamide in a brown bottle. The solution was mixed until dissolved, wrapped in aluminium foil to protect from light, and stored at –20°C until required.

11.3. RAKBETA SCINTILLATION COUNTER

The LKB 1214 Rakbeta Counter was used to quantitatively determine the amount of ractioactivity in a given sample. Two forms of radioactive isotope were used throughout the experimental procedures: Tritium (^3H), and Phosphorus (^{32}P). Both required different programs to be specifically counted.

11.3.1.1. Tritium Program

The tritium program was used in the lymphoblastogenesis experiments in which ^3H radiolabelled thymidine was used. The program for the scintillation counter is given below (Table 78 p.A3).

11.3.1.2. Phosphorus Program

The phophorus program was used for DHBV dot blot hybridisation in which a ^{32}P radiolabelled deoxycytidine DNA probe was used. The program for the scintillation counter is given (Table 78 p.A3).

PARAMETER GROUP 02	PARAMETER GROUP 08
ID: 3H	ID: 32P
01 MODE 3	01 MODE 3
02 TIME 00060	02 TIME 00060
03 COUNTS 900000	03 COUNTS 900000
04 LCR 0000	04 LCR 0000
05 HCR 1	05 HCR 1
06 BG 1 0000	06 BG 1 0000
07 BG 2 0000	07 BG 2 0000
08 CH 1 008-110	08 CH 1 110-212
09 CH 2 000-000	09 CH 2 110-212
10 CH 3 100-135	10 CH 3 100-135
11 CH 4 135-184	11 CH 4 135-184
12 STD TIME 030	12 STD TIME 030
13 PRINT 01,02,04,06,08	13 PRINT 01,04,08
14 REP 01	14 REP 01
15 EFF1% RATIO 70.50 1.510 60.28 1.212 46.94 1.002 35.49 .844 27.11 .745 20.83 .649 16.64 .589 13.82 .558 12.23 .526 11.20 .502	15 EFF1% RATIO

Table 78. *Tritium and Phosphorus program for the scintillation counter.*
Parameter group 02: Tritium (^3H). Parameter group 08: Phosphorus (^{32}P).

11.4. CYCLE SEQUENCING

Cycle sequencing was performed using the Corbett Research GS-2000 (http://www.corbettresearch.com) and a cycle sequencing kit.

11.4.1. Corbett Research GS-2000

The Corbett Robotics Gel-Scan 2000 is a gel electrophoresis system for real-time DNA fragment analysis (Figure 67 p.A4).

Samples are loaded onto an Ultra-Thin vertical gel, a laser scans the base of the gel and detects DNA fluorescence. During the run a 2-dimensional image of the gel is built up on the screen. Ultra thin gels result in a dramatic decrease in run times over competitors systems, with no reduction in resolution.

Figure 67. *Photograph of the Corbett GS-2000.*

11.4.2. Thermo Sequenase cycle sequencing kit

The Amersham Life-Science Thermo Sequenase™ fluorescent-labelled primer cycle sequencing kit (Amersham, Buckinghamshire, England) is recommended for fluorescent dye primer sequencing of single stranded or double stranded DNA templates.

Thermo Sequenase is a new thermostable DNA polymerase specifically engineered for DNA sequencing. Amersham have used a recent discovery (Reeve and Fuller, 1995; Tabor and Richardson, 1995) to construct this exonuclease-free thermostable DNA polymerase. Like Sequenase™ T7 DNA polymerase, Thermo Sequenase generates uniform (and therefore easy to read) sequence band patterns. However, the thermostability of this enzyme also makes it suitable for cycle sequencing. Thermo Sequenase therefore combines accuracy comparable with Sequenase T7 DNA polymerase with the sensitivity of cycle sequencing. The contents of each pack are described in Table 79 (p.A5).

Reagent pack	Contents
A reagent	Tris-HCI (pH9.5), magnesium chloride, Tween™ 20, NonidetTM P-40, 2-mercaptoethanol, dATP, dCTP, dGTP, dTTP, ddATP, thermostable pyrophosphatase and Thermo Sequenase DNA polymerase.
C reagent	Tris-HCI (pH9.5), magnesium chloride, Tween™ 20, NonidetTM P-40, 2-mercaptoethanol, dATP, dCTP, dGTP, dTTP, ddCTP, thermostable pyrophosphatase and Thermo Sequenase DNA polymerase.
G reagent	Tris-HCI (pH9.5), magnesium chloride, Tween™ 20, NonidetTM P-40, 2-mercaptoethanol, dATP, dCTP, dGTP, dTTP, ddGTP, thermostable pyrophosphatase and Thermo Sequenase DNA polymerase.
T reagent	Tris-HCI (pH9.5), magnesium chloride, Tween™ 20, NonidetTM P-40, 2-mercaptoethanol, dATP, dCTP, dGTP, dTTP, ddTTP, thermostable pyrophosphatase and Thermo Sequenase DNA polymerase.

Table 79. *Contents of the cycle sequencing kit.*

The loading dye used for sequencing consisted of a denaturing agent to ensure that the DNA was run through the gel as single stranded products. The denaturing agent was Formamide, and the other components of the loading dye were EDTA, and methyl violet.

11.4.3. Cycle Sequencing Optimisation data

The various conditions tested for cycle sequencing optimisation are represented by some of the gels run on the GS-2000. The ranges of conditions tested for optimisation are tabulated (Table 80 p.A5).

Condition	Values tested
Type / amount of template	
PCR fragment	10, 25, 50, 75, 100, 200, and 500 ng/µL
Plasmid product	0.25, 0.5, 0.75, 1, 2, 4, 6, and 8 µg/µL
Labelled primer concentration	0.5, 1, 2.5, 5, 7.5, 10, 15, and 20 pmol/µL
Number of reaction cycles	10, 15, 20, 25, 30, 35, and 40 cycles
Annealing / Extension temperature	50, 55, 58, 60, 62, 64, 68, and 70°C
Amount of sample loaded on the gel	0.25, 0.5, 1, 2, 2.5, 4, 5, 6, and 8 µL

Table 80. *Range of values tested during optimisation of the Sequencing reactions.*

The final optimised reaction conditions are described in Optimised Cycle Sequencing protocol (Section 2.3.5.1, p.92).

Examples of the sequencing gels used to determine the optimal conditions are provided in Figure 68 - Figure 71 (p.A6-A9).

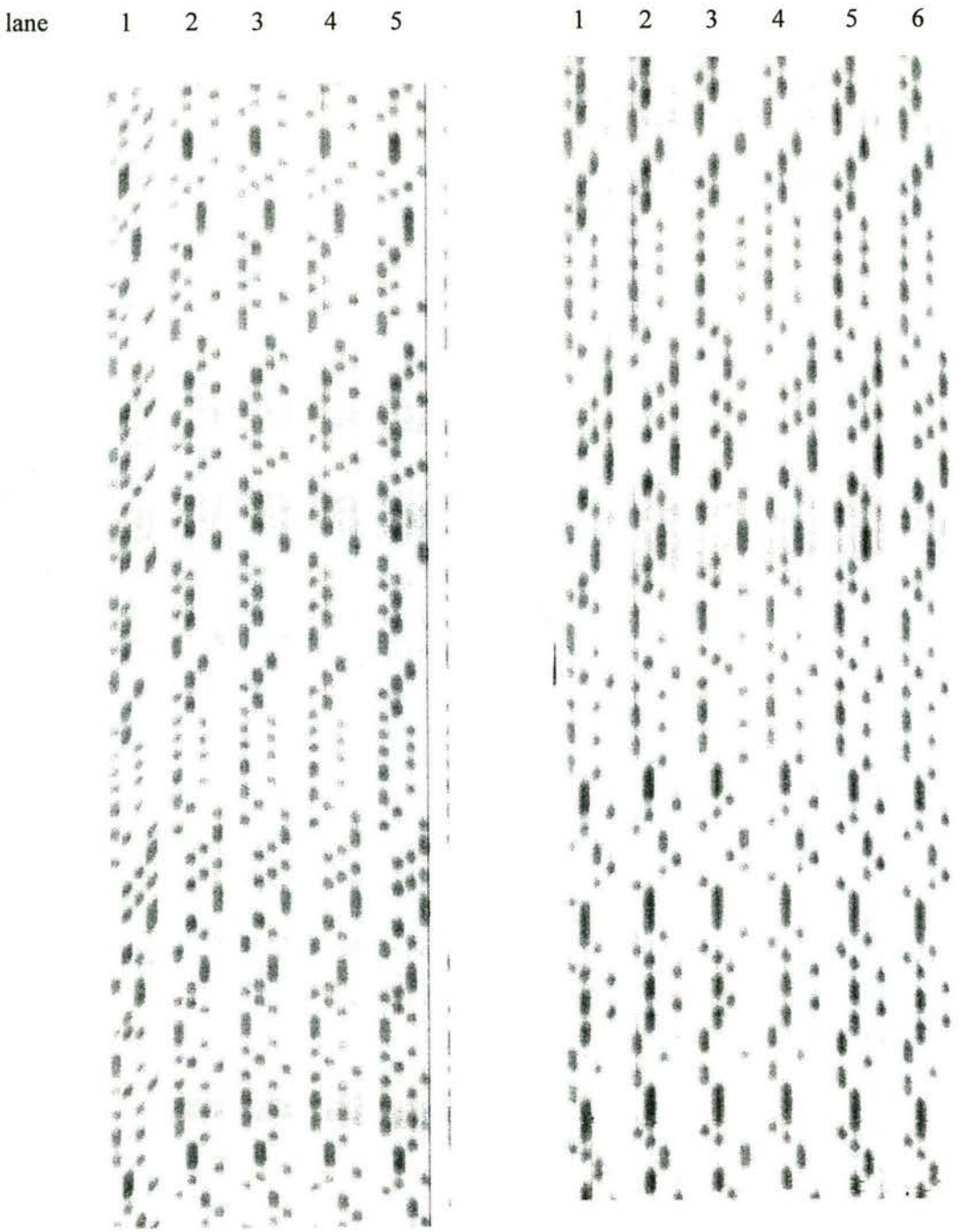


Figure 68. *Partial sequencing gels: Initial comparison of PCR and plasmid templates.*
Conditions for these gels were 5ng/μL primer, 30 cycles, 60°C anneal/extend, and 2μL loaded onto each gel. All gels are loaded with sequencing reactions for A, C, G, and T from left to right.

(a) All five reactions are identical; 500ng/μL PCR product

(b) All six reactions are identical; 1μg/μL plasmid

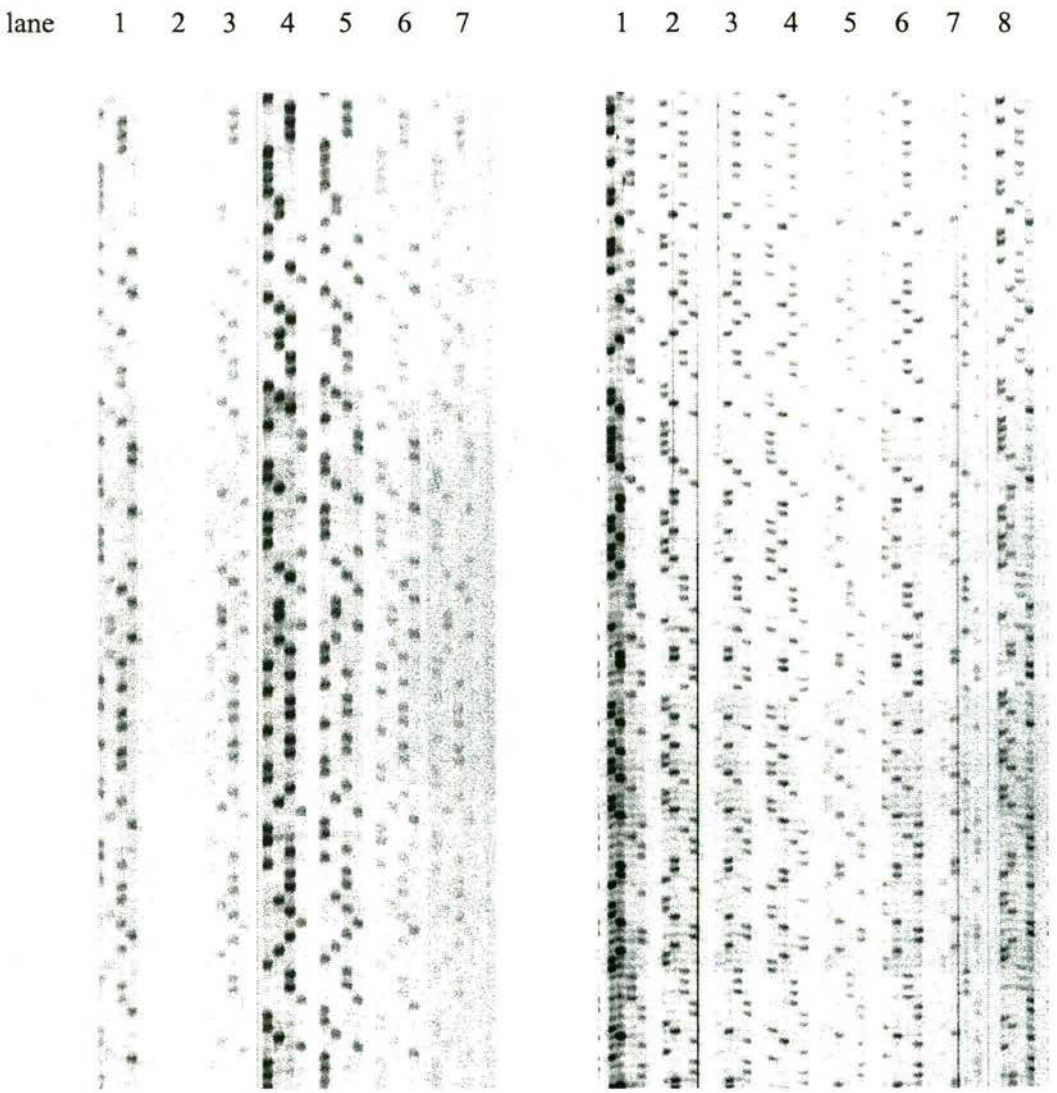


Figure 69. *Partial sequencing gels: Effect of purification and Anneal / Extension temperature.*

Conditions for these gels were 1μg/μL plasmid, 5ng/μL primer, 30 cycles, and 60°C anneal/extend (gel a). All gels are loaded with sequencing reactions for A, C, G, and T from left to right.

(a) lanes 1-3: non-purified sequencing reaction (5, 1, and 2 μL loaded) lanes 4-7: Ethanol purified sequencing reaction (5, 2, 1, and 0.5 μL loaded)

(b) 58°C, 60°C, 62°C, 64°C anneal/extend temperature (2 and 1 μL loaded)

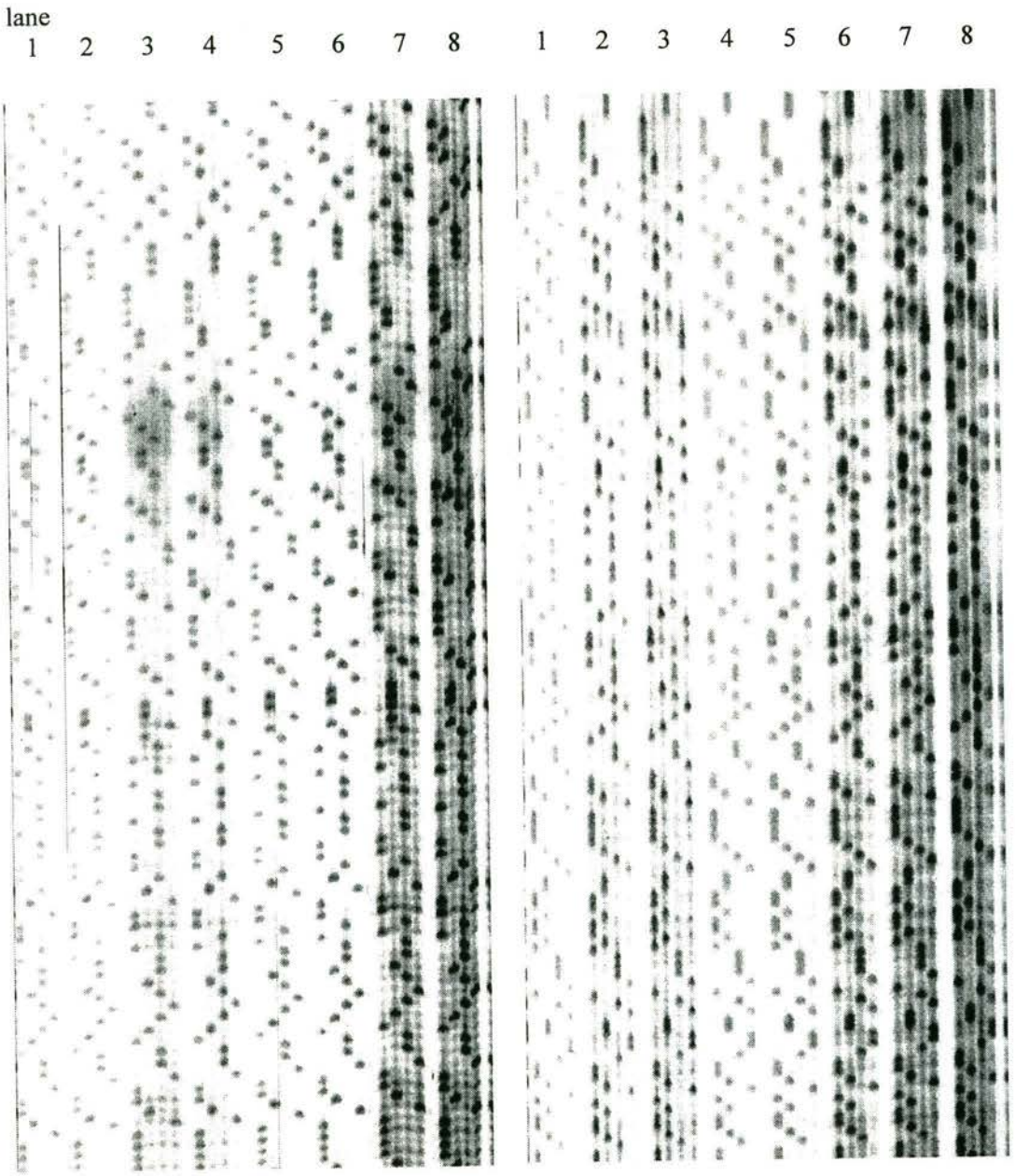


Figure 70. *Partial sequencing gels: Amount of primer and number of cycles.*

Conditions for these gels were 1μg/μL plasmid (gel a), 5ng/μL primer (gel b), 30 cycles (gel a), and 60°C anneal/extend. All gels are loaded with sequencing reactions for A, C, G, and T from left to right.

(a) 0.5, 1, 2.5, 5, 7.5, 10, 15, and 20 ng/μL primer

(b) lanes 1-2: 20 cycles (1 and 2 μL loaded), lanes 3-4: 25 cycles (1 and 2 μL loaded), lanes 5-6: 30 cycles, 1μg/μL plasmid (1 and 2 μL loaded),), lanes 7-8: 30 cycles, 2μg/μL plasmid (1 and 2 μL loaded)

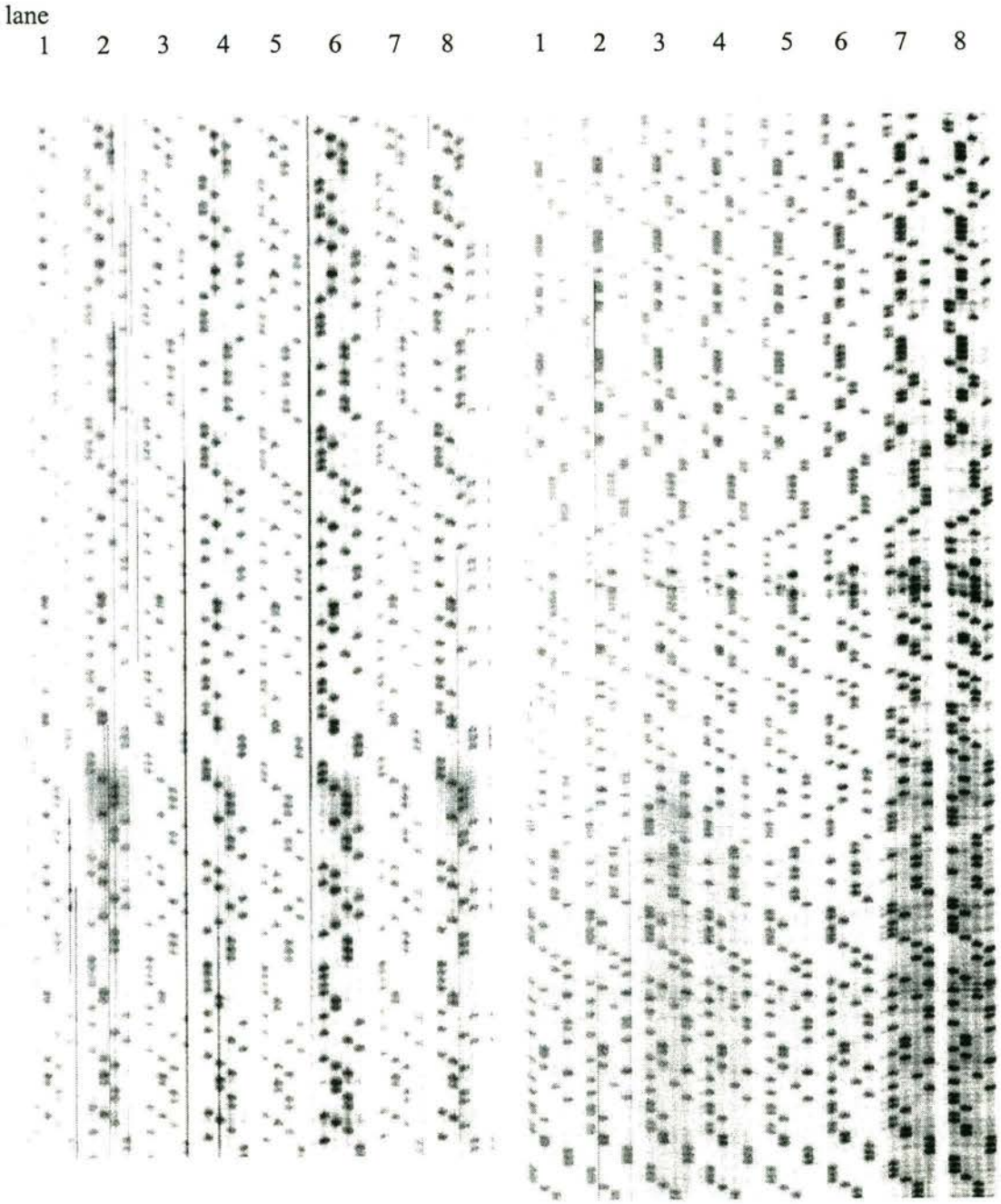


Figure 71. *Partial sequencing gels: Amount of PCR product and amount loaded onto the gel.*

Conditions for these gels were 200ng/μL PCR product (gel 2), 5ng/μL primer, 30 cycles, and 60°C anneal/extend. All gels are loaded with sequencing reactions for A, C, G, and T from left to right.

(a) 50, 100, 500, and 200 ng/μL PCR product (1 and 2 μL loaded)

(b) 0.25, 0.5, 0.75, 1, 2, 4, 6, and 8 μL loaded

11.5. DNA SEQUENCING OF THE PERSISTENCE - CLEARANCE MODEL EXPERIMENT

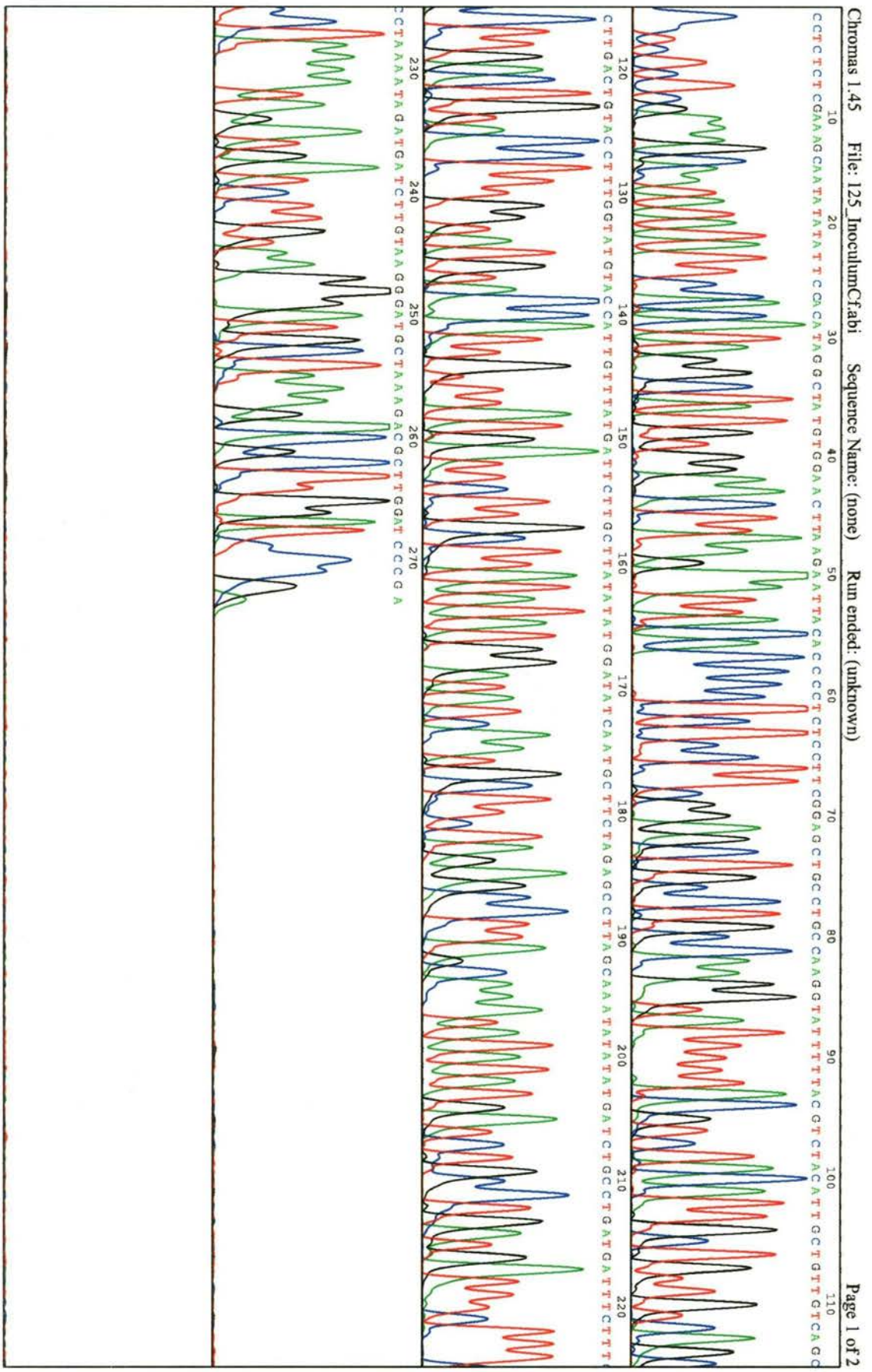
This section is the appendix for Chapter 4: DNA Sequencing of the Persistence - Clearance model experiment.

11.5.1. Examples of the edited sequence data output

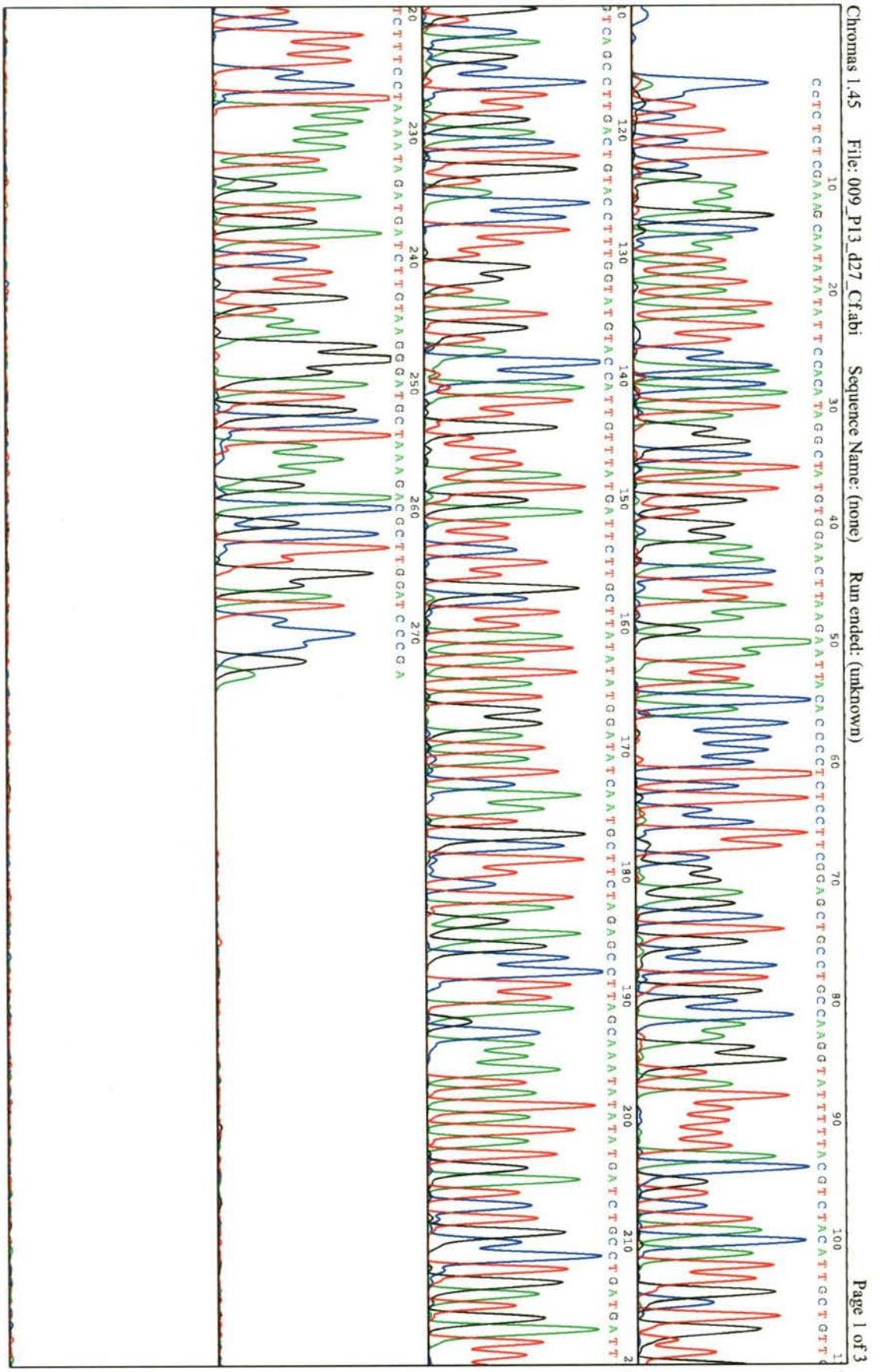
Examples of the edited sequence data output of the Persistence – Clearance model experiment are demonstrated.

11.5.2. Multiple Sequence Alignments

The automated or computer estimated sequence was manually checked (and altered if necessary) before being aligned using PileUp or ClustalW (Appendix 11.6.1, p.A42). After alignment, it was again manually checked (and altered if necessary).

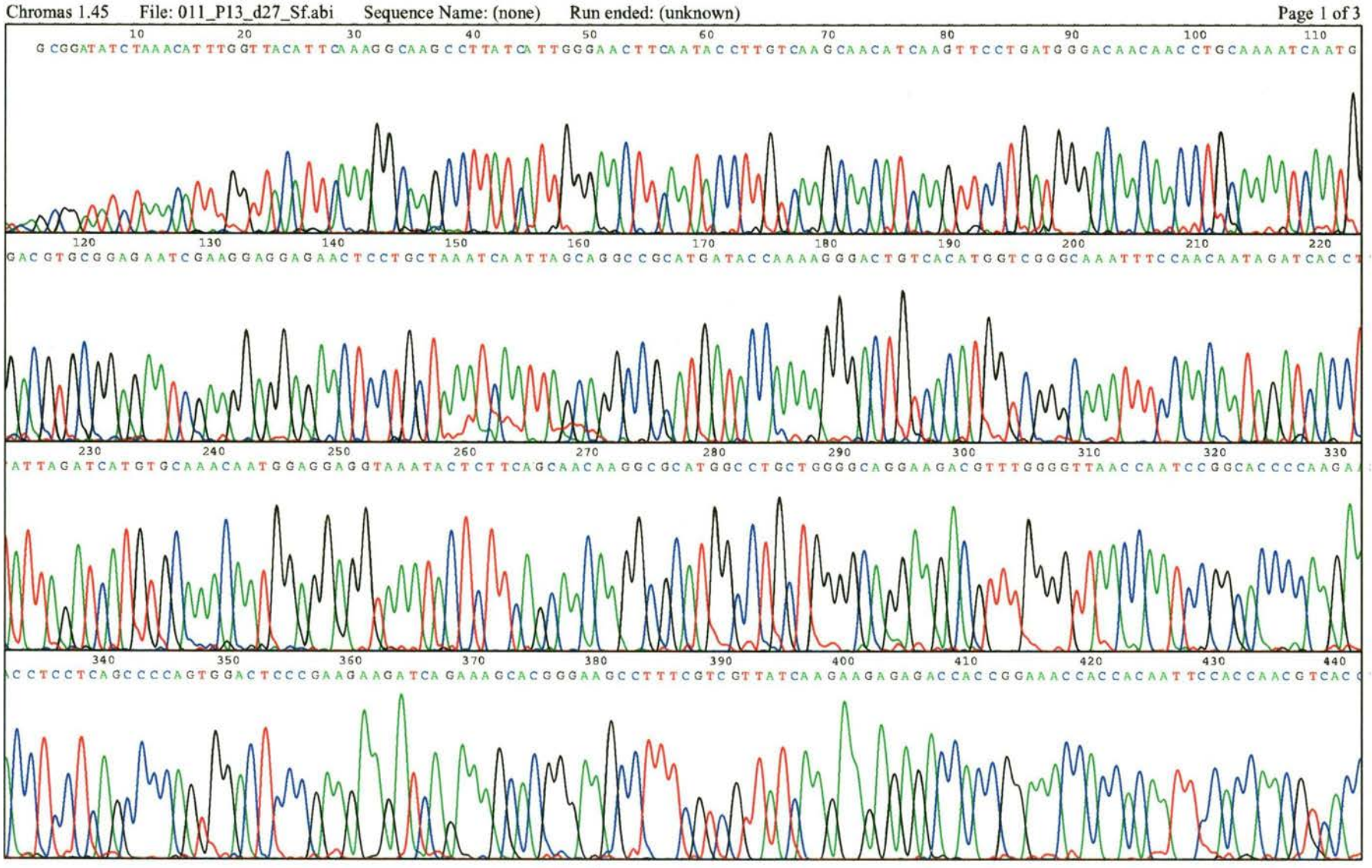


Edited sequence data output for the Core forward region of the inoculum.

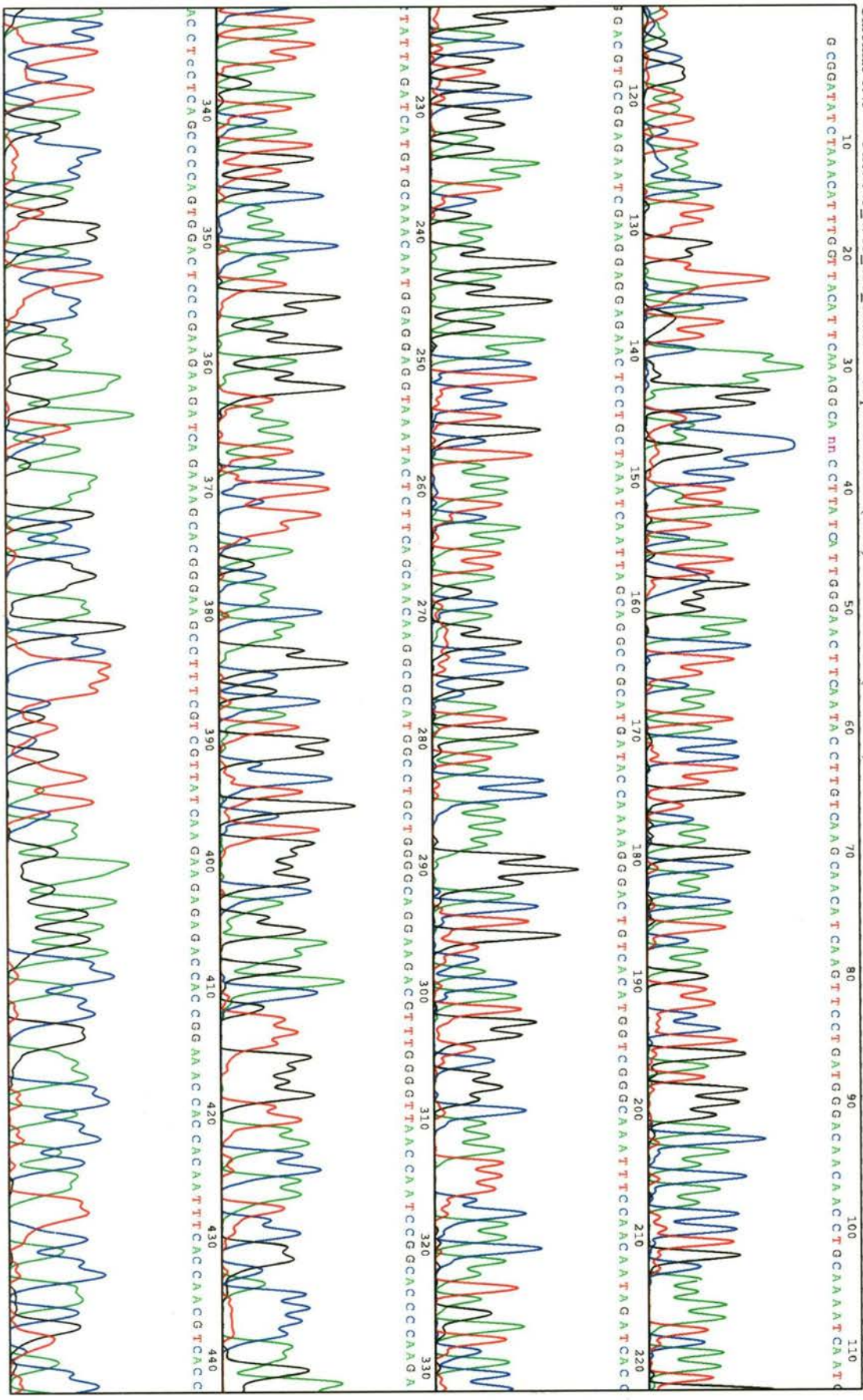


Edited sequence data output for the Core forward region of duck P13 on day 27.

Edited sequence data output for the Surface forward region of the inoculum.

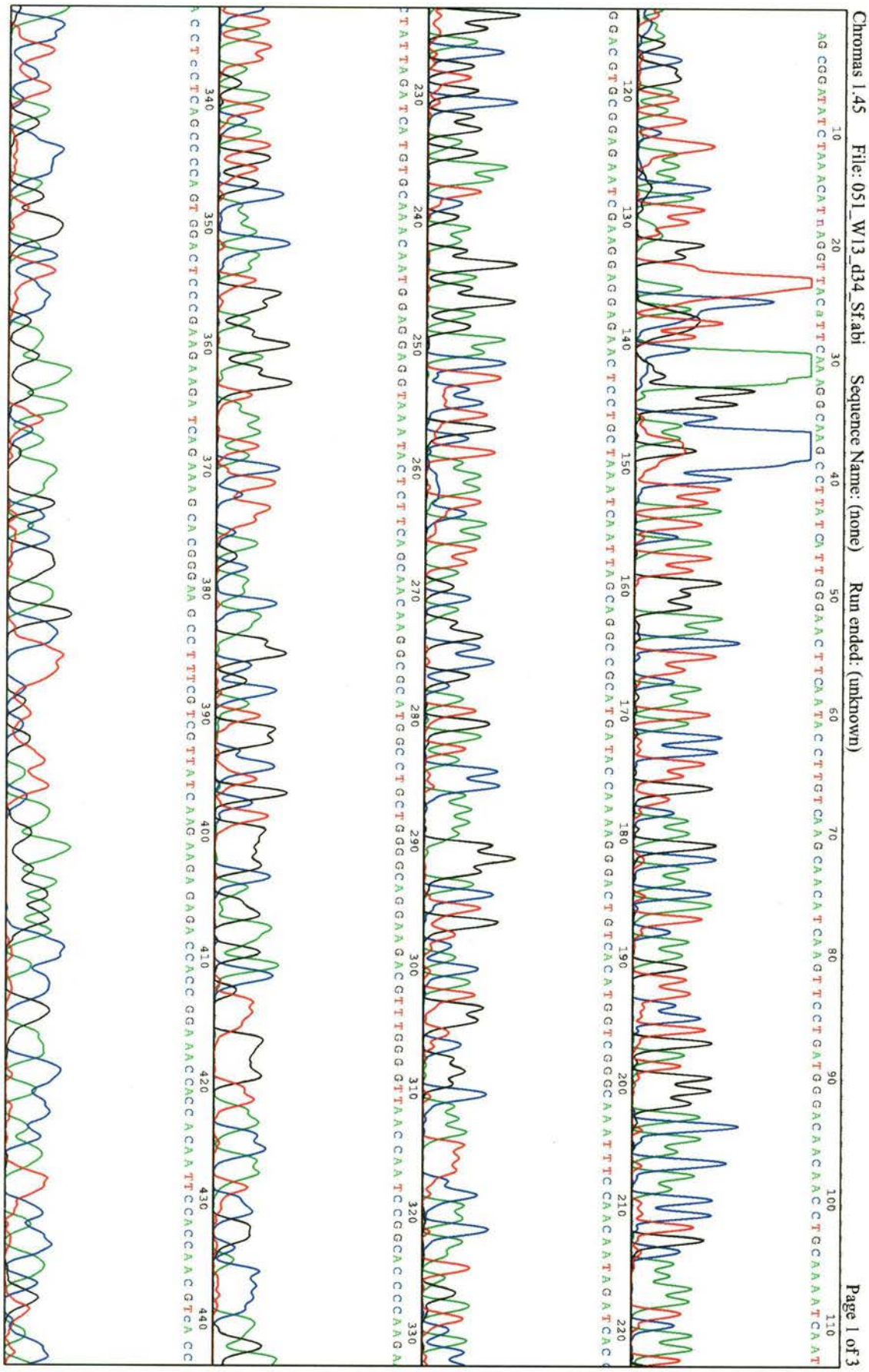


Edited sequence data output for the Surface forward region of duck P13 on day 27.



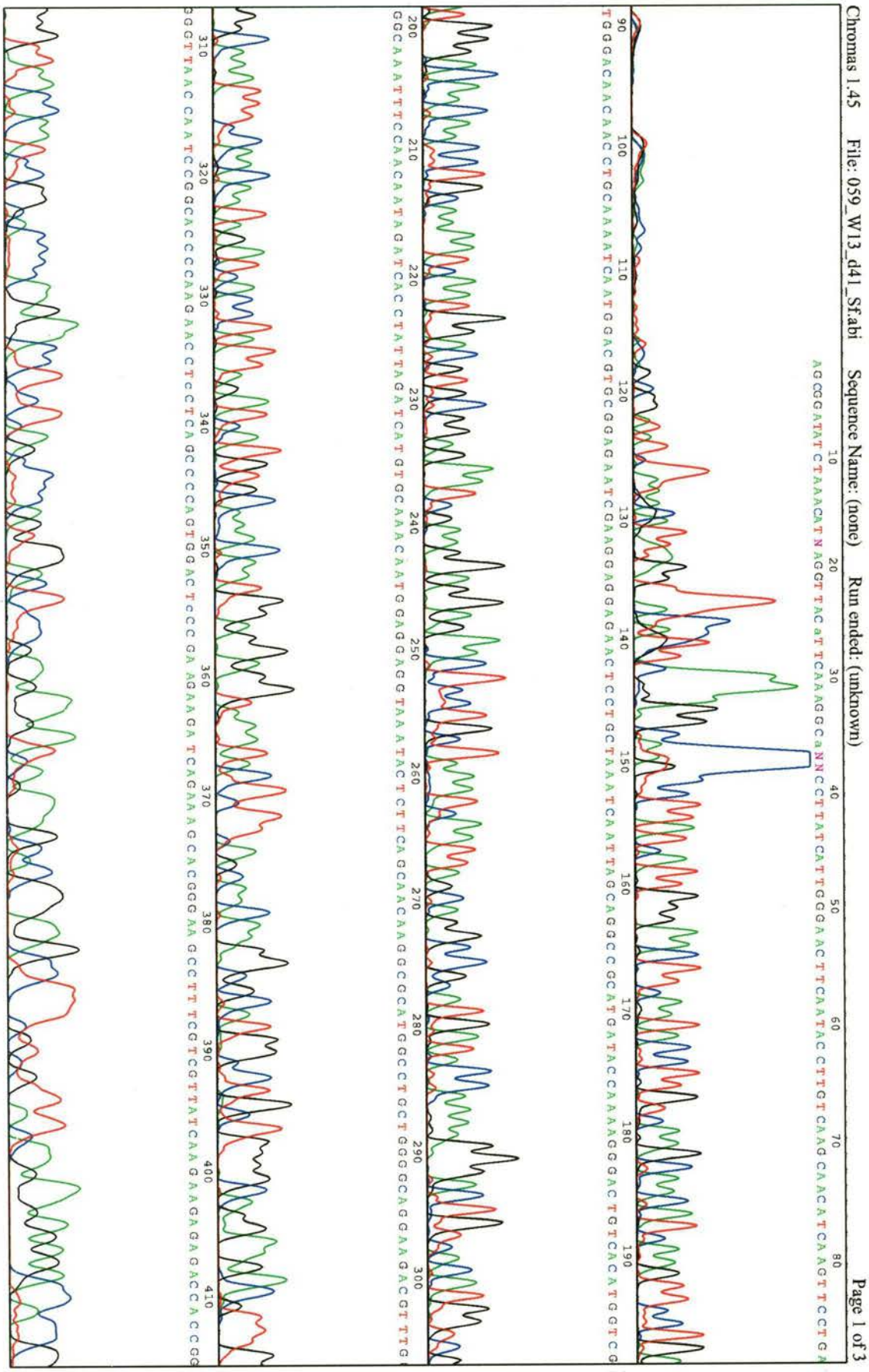
Edited sequence data output for the Surface forward region of duck W13 on day 20.

Edited sequence data output for the Surface forward region of duck W13 on day 29.



Edited sequence data output for the Surface forward region of duck W13 on day 34.

Edited sequence data output for the Surface forward region of duck W13 on day 39.

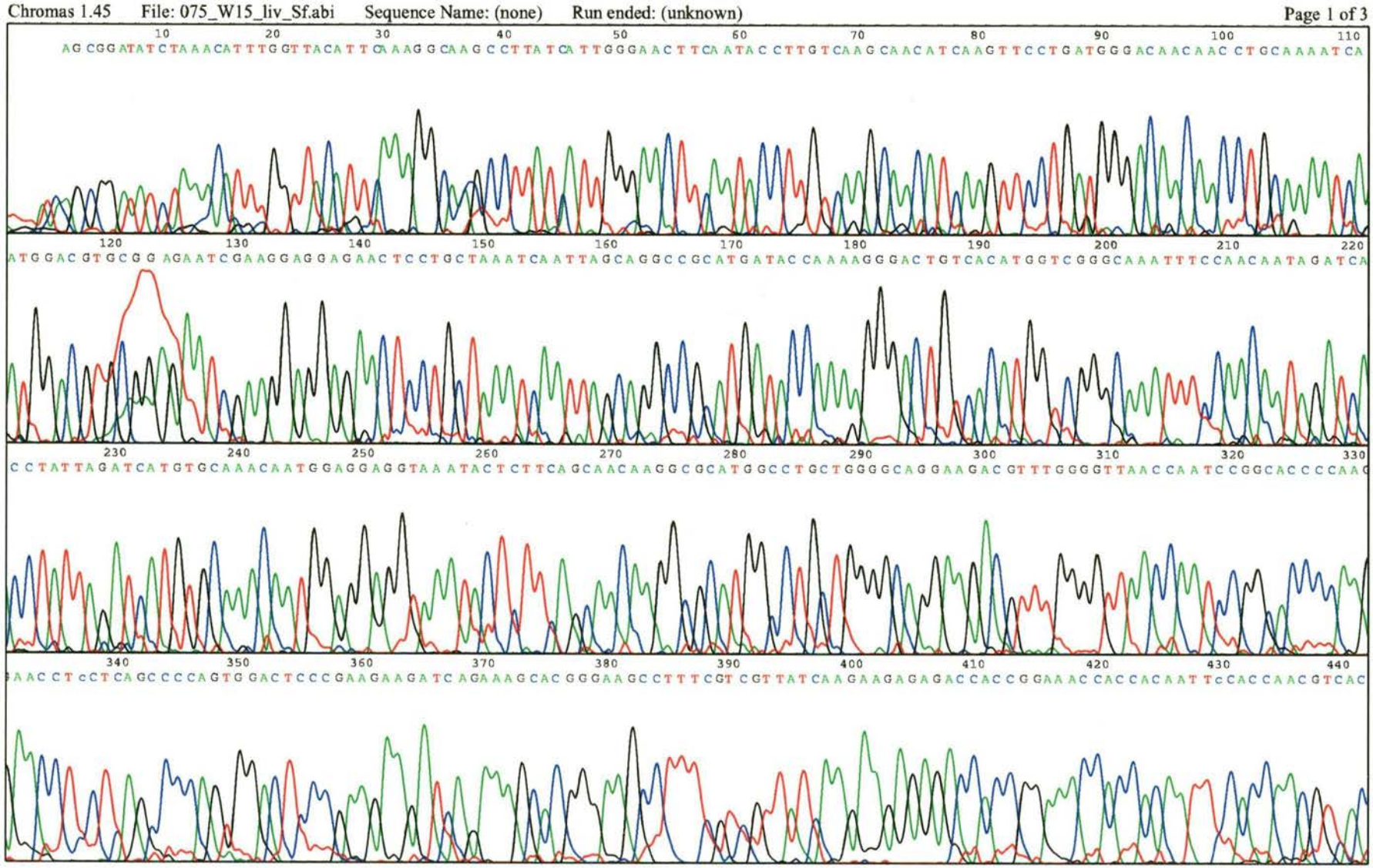


Edited sequence data output for the Surface forward region of duck W13 on day 41.

Edited sequence data output for the Surface forward region of duck W13 in the liver (day 43)

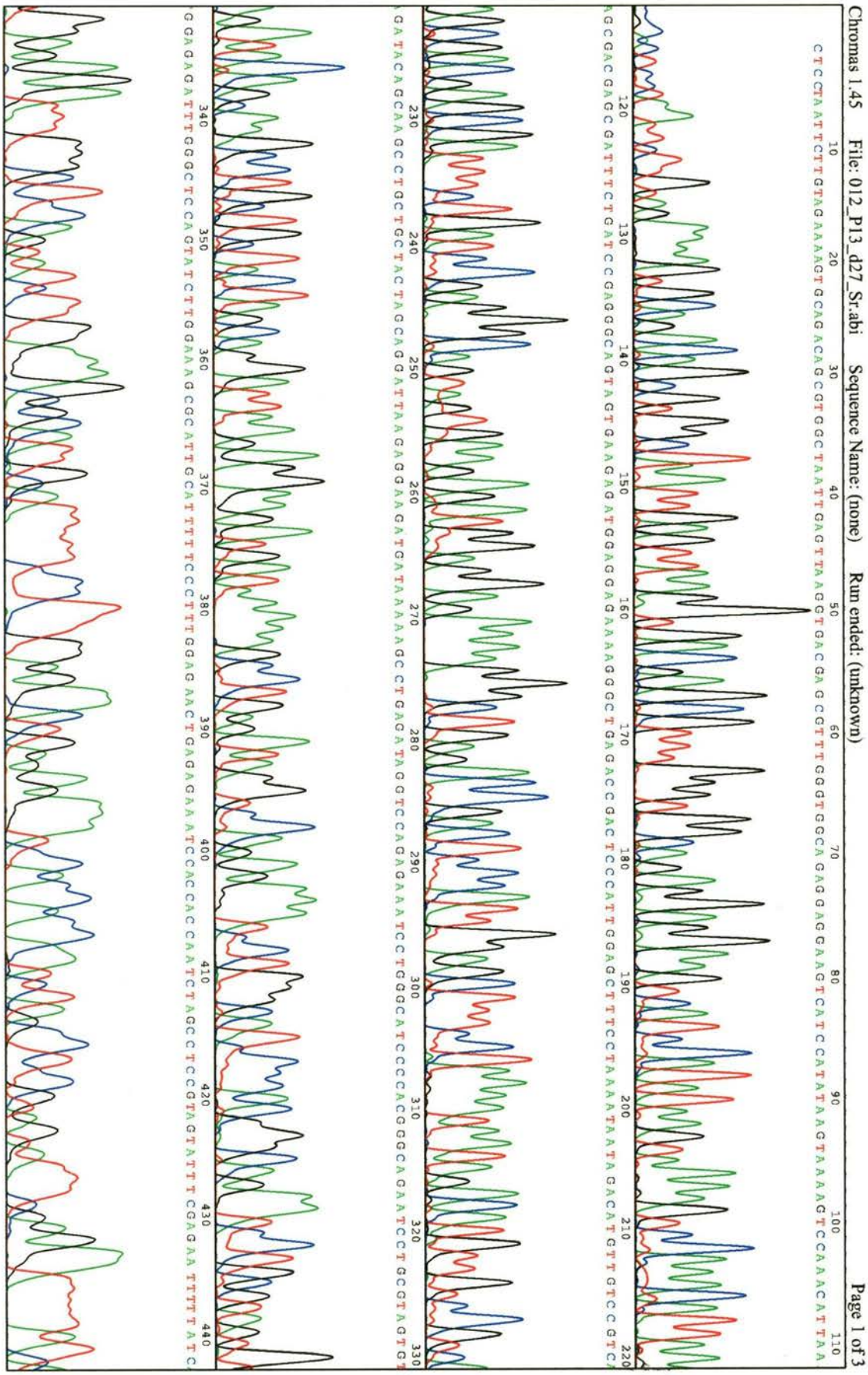
Edited sequence data output for the Surface forward region of duck W15 on day 13.

Edited sequence data output for the Surface forward region of duck W15 on day 18.



Edited sequence data output for the Surface forward region of duck W15 in the liver (day 43)

Edited sequence data output for the Surface reverse region of the inoculum.



Edited sequence data output for the Surface reverse region of duck P13 on day 27.

```
                    *      2420         *      2440         *      2460         *      2480         *      2500
adhbv_f     : ACAATTGTACTTTGTTCCGAGTAAATATAATCCTGCTGACGGCCCATCCAGGCACAAACCGCCTGATTGGACGGCTCTTACATACACCCCTCTCTCGAAA : 2500
inoculumcf  : ----------------------------------------------------------------------------------------------CCTCTCTCGAAA :   12
p13_d11_cf  : ----------------------------------------------------------------------------------------------CTCTCTCGAAA :   11
p13_d27_cf  : ----------------------------------------------------------------------------------------------CCTCTCTCGAAA :   12
p13_d43_cf  : ----------------------------------------------------------------------------------------------CTCTCTCGAAA :   11
p13_liv_cf  : ----------------------------------------------------------------------------------------------CTCTCTCGAAA :   11
p14_d11_cf  : ----------------------------------------------------------------------------------------------CTCTCTCGAAA :   11
p14_d27_cf  : ----------------------------------------------------------------------------------------------TCTCTCGAAA :   10
p14_liv_cf  : ----------------------------------------------------------------------------------------------CTCTCTCGAAA :   11
w13_d20_cf  : ----------------------------------------------------------------------------------------------CTCTCTCGAAA :   11
w13_d29_cf  : ----------------------------------------------------------------------------------------------TCTCTCGAAA :   10
w13_d41_cf  : ----------------------------------------------------------------------------------------------CTCTCTCGAAA :   11
w13_liv_cf  : ----------------------------------------------------------------------------------------------CCTCTNTCGAAA :   12
w15_d13_cf  : ----------------------------------------------------------------------------------------------CTCTCTCGAAA :   11
w15_d18_cf  : ----------------------------------------------------------------------------------------------CCTCTCTCGAAA :   12
b26_d15_cf  : ----------------------------------------------------------------------------------------------CTCTCTCGAAA :   11
b26_d25_cf  : ----------------------------------------------------------------------------------------------CTCTCTCGAAA :   11
b26_liv_cf  : ----------------------------------------------------------------------------------------------CTCTCTCGAAA :   11
b35_d15_cf  : ----------------------------------------------------------------------------------------------CTCTCTCGAAA :   11
b35_d25_cf  : ------------------------------------------------------------------------------------------------CTCTCGAAA :    9
b35_liv_cf  : ----------------------------------------------------------------------------------------------CCTCTCTCGAAA :   12
b37_liv_cf  : ----------------------------------------------------------------------------------------------CTCTCTCGAAA :   11
                                                                                                             ctCTCTCGAAA
```

Multiple Sequence Alignment of the forward Core region.

adhbv: Australian Duck Hepatitis B Virus (GenBank DHV6350, AJ006350); inoculum: starting inoculum; all others ducknumber_dayofsample.

```
                    *      2520          *      2540          *      2560          *      2580          *      2600
adhbv_f     : GCAATATATATTCCACATAGGCTATGTGGAACTTAAGAATTACACCCCTCTCCTTCGGAGCTGCCTGCCAAGGTATTTTTACGTCTACATTGCTGTTGTC : 2600
inoculumcf  : GCAATATATATTCCACATAGGCTATGTGGAACTTAAGAATTACACCCCTCTCCTTCGGAGCTGCCTGCCAAGGTATTTTTACGTCTACATTGCTGTTGTC :  112
p13_d11_cf  : GCAATATATATTCCACATAGGCTATGTGGAACTTAAGAATTACACCCCTCTCCTTCGGAGCTGCCTGCCAAGGTATTTTTACGTNTACATTGCTGNTGNC :  111
p13_d27_cf  : GCAATATATATTCCACATAGGCTATGTGGAACTTAAGAATTACACCCCTCTCCTTCGGAGCTGCCTGCCAAGGTATTTTTACGTCTACATTGCTGTTGTC :  112
p13_d43_cf  : GCAATATATATTCCACATAGGCTATGTGGAACTTAAGAATTACACCCCTCTCCTTCGGAGCTGCCTGCCAAGGTATTTTTACGTCTACATTGCTGTTGTC :  111
p13_liv_cf  : GCAATATATATTCCACATAGGCTATGTGGAACTTAAGAATTACACCCCTCTCCTTCGGAGCTGCCTGCCAAGGTATTTTTACGTCTACATTGCTGTTGTC :  111
p14_d11_cf  : GCAATATATATTCCACATAGGCTATGTGGAACTTAAGAATTACACCCCTCTCCTTCGGAGCTGCCTGCCAAGGTATTTTTACGTCTACATTGCTGTTGTC :  111
p14_d27_cf  : GCAATATNNATTCCACATAGGCTATGTGGAACTTAAGAATTACACCCCTCTCCTTCGGAGCTGCCTGCCAAGGTATTTTTACGTCTACATTGCTGTTGTC :  110
p14_liv_cf  : GCAATATATATTNCACATANGCTANGTGGANCTTAAGAATTACACCCCTCTCCTTCGGAGCTGCCTGCCAAGGTATTTTTACGTCTACATTGCTGTTGTC :  111
w13_d20_cf  : GCAATATATATTCCACATAGGCTATGTGGAACTTAAGAATTACACCCCTCTCCTTCGGAGCTGCCTGCCAAGGTATTTTTACGTCTACATTGCTGTTGTC :  111
w13_d29_cf  : GCAATATATATTNCACATANGCTANGTGGANCTTAAGAATTACACCCCTCTCCTTCGGAGCTGCCTGCCAAGGTATTTTTACGTCTACATTGCTGTTGTC :  110
w13_d41_cf  : GCAATATATATTCCACATAGGCTATGTGGAACTTAAGAATTACACCCCTCTCCTTCGGAGCTGCCTGCCAAGGTATTTTTACGTCTACATTGCTGTTGTC :  111
w13_liv_cf  : GCAATATANATTCCACATAGGCTATGTGGAACTTAAGAATTACACCCCTCTCCTTCGGAGCTGCCTGCCAAGGTATTTTTACGTCTACATTGCTGTTGTC :  112
w15_d13_cf  : GCAATATATATTNCACATANGCTATGTGGNNCTTAAGAATTACACCCCTCTCCTTCGGAGCTGCCTGCCAAGGTATTTTTACGTCTACATTGCTGTTGTC :  111
w15_d18_cf  : GCAATNTANATTNCACATANNCTATGTGGAACTTAAGAATTACACCCCTCTCCTTCGGAGCTGCCTGCCAAGGTATTTTTACGTCTACATTGCTGTTGTC :  112
b26_d15_cf  : GCAATATATATTCCACATAGGCTATGTGGAACTTAAGAATTACACCCCTCTCCTTCGGAGCTGCCTGCCAAGGTATTTTTACGTCTACATTGCTGTTGTC :  111
b26_d25_cf  : GCAATATATATTCCACATAGGCTATGTGGAACTTAAGAATTACACCCCTCTCCTTCGGAGCTGCCTGCCAAGGTATTTTTACGTCTACATTGCTGNTGTC :  111
b26_liv_cf  : GCAATATATATTCCACATAGGCTATGTGGAACTTAAGAATTACACCCCTCTCCTTCGGAGCTGCCTGCCAAGGTATTTTTACGTCTACATTGCTGTTGTC :  111
b35_d15_cf  : GCAATATATATTCCACATAGGCTATGTGGAACTTAAGAATTACACCCCTCTCCTTCGGAGCTGCCTGCCAAGGTATTTTTACGTCTACATTGCTGTTGTC :  111
b35_d25_cf  : GCAATATATATTCCACATAGGCTATGNGGAACTTAAGAANTACACCCCTCTNCTTCGGAGCTGCCTGCCAAGGTATTNTTACGNCTACANTGCTGNTGTC :  109
b35_liv_cf  : GCAATATATATTCCACATAGGCTATGTGGAACTTAAGAATTACACCCCTCTCCTTCGGAGCTGCCTGCCAAGGTATTTTTACGTCTACATTGCTGTTGTC :  112
b37_liv_cf  : GCAATATATATTCCACATAGGCTATGTGGAACTTAAGAATTACACCCCTCTCCTTCGGAGCTGCCTGCCAAGGTATTTTTACGTCTACATTGCTGTTGTC :  111
              GCAATATATATTCCACATAGGCTATGTGGAACTTAAGAATTACACCCCTCTCCTTCGGAGCTGCCTGCCAAGGTATTTTTACGTCTACATTGCTGTTGTC
```

continued - *Multiple Sequence Alignment of the forward Core region.*

adhbv: Australian Duck Hepatitis B Virus (GenBank DHV6350, AJ006350); inoculum: starting inoculum; all others ducknumber_dayofsample.

```
                    *      2620          *      2640          *      2660          *      2680          *      2700
adhbv_f     : AGCCTTGACTGTACCTTTGGTATGTACCATTGTTTATGATTCTTGCTTATATATGGATATCAATGCTTCTAGAGCCTTAGCAAATATATATGATCTGCCT : 2700
inoculumcf  : AGCCTTGACTGTACCTTTGGTATGTACCATTGTTTATGATTCTTGCTTATATATGGATATCAATGCTTCTAGAGCCTTAGCAAATATATATGATCTGCCT :  212
p13_d11_cf  : AGCCTTGACTGNACCTTNGGNATGTACCATTGNNNATGATTCTTGCTTATATATGGATATNAATGCTTCTAGAGCCTTAGCAAATATATATGATCTGCCT :  211
p13_d27_cf  : AGCCTTGACTGTACCTTTGGTATGTACCATTGTTTATGATTCTTGCTTATATATGGATATCAATGCTTCTAGAGCCTTAGCAAATATATATGATCTGCCT :  212
p13_d43_cf  : AGCCTTGACTGTACCTTTGGTATGTACCATTGTTTATGATTCTTGCTTATATATGGATATCAATGCTTCTAGAGCCTTAGCAAATATATATGATCTGCCT :  211
p13_liv_cf  : AGCCTTGACTGTACCTTTGGTATGTACCATTGTTTATGATTCTTGCTTATATATGGATATCAATGCTTCTAGAGCCTTAGCAAATATATATGATCTGCCT :  211
p14_d11_cf  : AGCCTTGACTGTACCTTTGGTATGTACCATTGTTTATGATTCTTGCTTATATATGGATATCAATGCTTCTAGAGCCTTAGCAAATATATATGATCTGCCT :  211
p14_d27_cf  : AGCCTTGACTGTACCTTTGGTATGTACCATTGTTTATGATTCTTGCTTATATATGGATATCAATGCTTCTAGAGCCTTAGCAAATATATATGATCTGCCT :  210
p14_liv_cf  : AGCCTTGACTGTACCTTTGGTATGTACCATTGTTTATGATTCTTGCTTATATATGGATATCAATGCTTCTAGAGCCTTAGCAAATATATATGATCTGCCT :  211
w13_d20_cf  : AGCCTTGACTGTACCTTTGGTATGTACCATTGTTTATGATTCTTGCTTATATATGGATATCAATGCTTCTAGAGCCTTAGCAAATATATATGATCTGCCT :  211
w13_d29_cf  : AGCCTTGACTGTACCTTTGGTATGTACCATTGTTTATGATTCTTGCTTATATATGGATATCAATGCTTCTAGAGCCTTAGCAAATATATATGATCTGCCT :  210
w13_d41_cf  : AGCCTTGACTGTACCTTTGGTATGTACCATTGTTTATGATTCTTGCTTATATATGGATATCAATGCTTCTAGAGCCTTAGCAAATATATATGATCTGCCT :  211
w13_liv_cf  : AGCCTTGACTGTACCTTTGGTATGTACCATTGTTTATGATTCTTGCTTATATATGGATATCAATGCTTCTAGAGCCTTAGCAAATATATATGATCTGCCT :  212
w15_d13_cf  : AGCCTTGACTGTACCTTTGGTATGTACCATTGNTTATNATTCTTGNTTATATATGGATATCAATGCTTCTAGAGCCTTAGCAAATATATATGATCTGCCT :  211
w15_d18_cf  : AGCCTTGACTGTACCTTTGGTATGTACCATTGTTTATGATTCTTGCTTATATATGGATATCAATGCTTCTAGAGCCTTAGCAAATATATATGATCTGCCT :  212
b26_d15_cf  : AGCCTTGACTGTACCTTTGGTATGTACCATTGTTTATGATTCTTGCTTATATATGGATATCAATGCTTCTAGAGCCTTAGCAAATATATATGATCTGCCT :  211
b26_d25_cf  : AGCCTTGACTGTACCTTTGGTATGTACCATTGTTTATGATTCTTGCTTATATATGGATATCAATGCTTCTAGAGCCTTAGCAAATATATATGATCTGCCT :  211
b26_liv_cf  : AGCCTTGACTGTACCTTTGGTATGTACCATTGTTTATGATTCTTGCTTATATATGGATATCAATGCTTCTAGAGCCTTAGCAAATATATATGATCTGCCT :  211
b35_d15_cf  : AGCCTTGACTGTACCTTTGGTATGTACCATTGTTTATGATTCTTGCTTATATATGGATATCAATGCTTCTAGAGCCTTAGCAAATATATATGATCTGCCT :  211
b35_d25_cf  : AGCCTTGACTGNACCTNTGNTNTGTACNNTTGTTTATGATTCTTGCTTATATATGGATATCAATGCTTCTAGAGCCTTAGCAAATATATATGATCTGCCT :  209
b35_liv_cf  : AGCCTTGACTGTACCTTTGGTATGTACCATTGTTTATGATTCTTGCTTATATATGGATATCAATGCTTCTAGAGCCTTAGCAAATATATATGATCTGCCT :  212
b37_liv_cf  : AGCCTTGACTGTACCTTTGGTATGNNCCATTGNTTATGATTCTTGCTTATATATGGATATCAATGCTTCTAGAGCCTTAGCAAATATATATGATCTGCCT :  211
              AGCCTTGACTGTACCTTTGGTATGTACCATTGTTTATGATTCTTGCTTATATATGGATATCAATGCTTCTAGAGCCTTAGCAAATATATATGATCTGCCT
```

continued - *Multiple Sequence Alignment of the forward Core region.*

adhbv: Australian Duck Hepatitis B Virus (GenBank DHV6350, AJ006350); inoculum: starting inoculum; all others ducknumber_dayofsample.

```
                    *      2720          *      2740          *      2760          *      2780          *      2800
adhbv_f     : GATGATTTCTTTCCTAAAATAGATGATCTTGTAAGGGATGCTAAAGACGCTTTAGAACCTTATTGGAAATCTGATTCAATAAAGAAACATGTTTTAATTG : 2800
inoculumcf  : GATGATTTCTTTCCTAAAATAGATGATCTTGTAAGGGATGCTAAAGACGCTTG-GATCCCGA------------------------------------ :  273
p13_d11_cf  : GATGATNTNTTTCCTAANATNNATGATCTTGNAAGGGATGCTAAAGACGCTTG-GATCCCGA------------------------------------ :  272
p13_d27_cf  : GATGATTTCTTTCCTAAAATAGATGATCTTGTAAGGGATGCTAAAGACGCTTG-GATCCCGA------------------------------------ :  273
p13_d43_cf  : GATGATTTCTTTCCTAAAATAGATGATCTTGTAAGGGATGCTAAAGACGCTTG-GATCCCGA------------------------------------ :  272
p13_liv_cf  : GATGATTTCTTTCCTAAAATAGATGATCTTGTAAGGGATGCTAAAGACGCTTG-GATCCCGA------------------------------------ :  272
p14_d11_cf  : GATGATTTCTTTCCTAAAATAGATGATCTTGTAAGGGATGCTAAAGACGCTTG-GATCCCGA------------------------------------ :  272
p14_d27_cf  : GATGATTTCTTTCCTAAAATAGATGATCTTGTAAGGGATGCTAAAGACGCTTG-GATCCCGA------------------------------------ :  271
p14_liv_cf  : GATGATTTCTTTCCTAAAATAGATGATCTTGTAAGGGATGCTAAAGACGCTTG-GATCCCGA------------------------------------ :  272
w13_d20_cf  : GATGATTTCTTTCCTAAAATAGATGATCTTGTAAGGGATGCTAAAGACGCTTG-GATCCCGA------------------------------------ :  272
w13_d29_cf  : GATGATTTCTTTCCTAAAATAGATGATCTTGTAAGGGATGCTAAAGACGCTTG-GATCCCGA------------------------------------ :  271
w13_d41_cf  : GATGATTTCTTTCCTAAAATAGATGATCTTGTAAGGGATGCTAAAGACGCTTG-GATCCCGA------------------------------------ :  272
w13_liv_cf  : GATGATTTCTTTCCTAAAATAGATGATCTTGTAAGGGATGCTAAAGACGCTTG-GATCCCGA------------------------------------ :  273
w15_d13_cf  : GATGATTTCTTTCCTAAAATAGATGATCTTGTAAGGGATGCTAAAGACGCTTG-GATCCCG------------------------------------- :  271
w15_d18_cf  : GATGATTTCTTTCCTAAAATAGATGATCTTGTAAGGGATGCTAAAGACGCTTG-GATCCCGA------------------------------------ :  273
b26_d15_cf  : GATGATTTCTTTCCTAAAATAGATGATCTTGTAAGGGATGCTAAAGACGCTTG-GATCCCGA------------------------------------ :  272
b26_d25_cf  : GATGATTTCTTTCCTAAAATAGATGATCTTGTAAGGGATGCTAAAGACGCTTG-GATCCCGA------------------------------------ :  272
b26_liv_cf  : GATGATTTCTTTCCTAAAATAGATGATCTTGTAAGGGATGCTAAAGACGCTTG-GATCCCGA------------------------------------ :  272
b35_d15_cf  : GATGATTTCTTTCCTAAAATAGATGATCTTGTAAGGGATGCTAAAGACGCTTG-GATCCCGA------------------------------------ :  272
b35_d25_cf  : GATGATTTCTTTCCTAAAATNGANGATCTTGTAAGGGATGCTAAAGACGCTTG-GATCCCG------------------------------------- :  269
b35_liv_cf  : GATGATTTCTTTCCTAAAATAGATGATCTTGTAAGGGATGCTAAAGACGCTTG-GATCCCGA------------------------------------ :  273
b37_liv_cf  : GATGATNTCTTTCCTAAAATAGATGATCTTGTAAGGGATGCTAAAGACGCTTG-GATCCCGA------------------------------------ :  272
              GATGATTTCTTTCCTAAAATAGATGATCTTGTAAGGGATGCTAAAGACGCTTg GAtCCcga
```

continued - *Multiple Sequence Alignment of the forward Core region.*

adhbv: Australian Duck Hepatitis B Virus (GenBank DHV6350, AJ006350); inoculum: starting inoculum; all others ducknumber_dayofsample.

Multiple Sequence Alignment of the forward Surface region.

adhbv: Australian Duck Hepatitis B Virus (GenBank DHV6350, AJ006350); inoculum: starting inoculum; all others ducknumber_dayofsample.

```
                    *       820         *       840         *       860         *       880         *       900
adhbv_f     : ATGGGACAACAACCTGCAAAATCAATGGACGTGCGGAGAATCGAAGGAGGAGAACTCCTGCTAAATCAATTAGCAGGCCGCATGATACCAAAAGGGACTG :  900
inoculumsf  : ATGGGACAACAACCTGCAAAATCAATGGACGTGCGGAGAATCGAAGGAGGAGAACTCCTGCTAAATCAATTAGCAGGCCGCATGATACCAAAAGGGACTG :  186
p13_d06_sf  : ATGGGACAACAACCTGCAAAATCAATGGACGTGCGGAGAATCGAAGGAGGAGAACTCCTGCTAAATCAATTAGCAGGCCGCATGATACCAAAAGGGACTG :  186
p13_d11_sf  : ATGGGACAACAACCTGCAAAATCAATGGACGTGCGGAGAATCGAAGGAGGAGAACTCCTGCTAAATCAATTAGCAGGCCGCATGATACCAAAAGGGACTG :  186
p13_d27_sf  : ATGGGACAACAACCTGCAAAATCAATGGACGTGCGGAGAATCGAAGGAGGAGAACTCCTGCTAAATCAATTAGCAGGCCGCATGATACCAAAAGGGACTG :  186
p13_d43_sf  : ATGGGACAACAACCTGCAAAATCAATGGACGTGCGGAGAATCGAAGGAGGAGAACTCCTGCTAAATCAATTAGCAGGCCGCATGATACCAAAAGGGACTG :  186
p13_liv_sf  : ATGGGACAACAACCTGCAAAATCAATGGACGTGCGGAGAATCGAAGGAGGAGAACTCCTGCTAAATCAATTAGCAGGCCGCATGATACCAAAAGGGACTG :  186
p14_d06_sf  : ATNGGNCAACANCCTNNAAAATNAATGGNNNNGCNGNNAATNGANGGNGGNAAACTCNTNNTNNNTCAATTNNNNGGCNGNNNGANACCNANAGGNNNTN :  180
p14_d11_sf  : ATGGGACAACAACCTGCAAAATCAATGGACGTGCGGAGAATCGAAGGAGGAGAACTNNTGCTAAATCAATTAGCAGGCCGCATGATACCAAAAGGGACTG :  175
p14_d27_sf  : ATGGGACAACAACCTGCAAAATCAATGGACGTGCGGAGAATCGAAGGAGGAGAACTCCTGCTAAATCAATTAGCAGGCCGCATGATACCAAAAGGGACTG :  186
p14_d43_sf  : ATGGGACAACAACCTGCAAAATCAATGGACGTGCGGAGAATCGAAGGAGGAGAACTCCTGCTAAATCAATTAGCAGGCCGCATGATACCAAAAGGGACTG :  170
p14_liv_sf  : ATGGGACAACAACCTGCAAAATCAATGGACGTGCGGAGAATCGAAGGAGGAGAACTCCTGCTAAATCAATTAGCAGGCCGCATGATACCAAAAGGGACTG :  187
w13_d20_sf  : ATGGGACAACAACCTGCAAAATCAATGGACGTGCGGAGAATCGAAGGAGGAGAACTCCTGCTAAATCAATTAGCAGGCCGCATGATACCAAAAGGGACTG :  186
w13_d29_sf  : ATGGGACAACAACCTGCAAAATCAATGGACGTGCGGAGAATCGAAGGAGGAGAACTCCTGCTAAATCAATTAGCAGGCCGCATGATACCAAAAGGGACTG :  186
w13_d34_sf  : ATGGGACAACAACCTGCAAAATCAATGGACGTGCGGAGAATCGAAGGAGGAGAACTCCTGCTAAATCAATTAGCAGGCCGCATGATACCAAAAGGGACTG :  187
w13_d39_sf  : ATGGGACAACAACCTGCAAAATCAATGGACGTGCGGAGAATCGAAGGAGGAGAACTCCTGCTAAATCAATTAGCAGGCCGCATGATACCAAAAGGGACTG :  187
w13_d41_sf  : ATGGGACAACAACCTGCAAAATCAATGGACGTGCGGAGAATCGAAGGAGGAGAACTCCTGCTAAATCAATTAGCAGGCCGCATGATACCAAAAGGGACTG :  187
w13_liv_sf  : ATGGGACAACAACCTGCAAAATCAATGGACGTGCGGAGAATCGAAGGAGGAGAACTCCTGCTAAATCAATTAGCAGGCCGCATGATACCAAAAGGGACTG :  187
w15_d13_sf  : ATGGGACAACAACCTGCAAAATCAATGGACGTGCGGAGAATCGAAGGAGGAGAACTCCTGCTAAATCAATTAGCAGGCCGCATGATACCAAAAGGGACTG :  187
w15_d18_sf  : ATGGGACAACAACCTGCAAAATCAATGGACGTGCGGAGAATCGAAGGAGGAGAACTCCTGCTAAATCAATTAGCAGGCCGCATGATACCAAAAGGGACTG :  186
w15_liv_sf  : ATGGGACAACAACCTGCAAAATCAATGGACGTGCGGAGAATCGAAGGAGGAGAACTCCTGCTAAATCAATTAGCAGGCCGCATGATACCAAAAGGGACTG :  187
b26_d15_sf  : ATGGGACAACAACCTGCAAAATCAATGGACGTGCGGAGAATCGAAGGAGGAGAACTCCTGCTAAATCAATTAGCAGGCCGCATGATACCAAAAGGGACTG :  186
b26_d25_sf  : ATGGGACAACAACCTGCAAAATCAATGGACGTGCGGAGAATCGAAGGAGGAGAACTCCTGCTAAATCAATTAGCAGGCCGNATGATACCAAAAGGGACTG :  185
b26_d27_sf  : ATGGGACAACAACCTGCAAAATCAATGGACGTGCGGAGAATNGAANGAGGAGAACTCCTGCTAAATCAATTAGCAGGCCGCATGATACCAAAAGGGACTG :  173
b26_d36_sf  : ATGGGACAACANCNTGNAAAATCAATGGACNTGCGGNGAATCGAAGGAGGAGAACTCCTGCTNANTCAATTAGCAGGCCGNATGATACCAAAAGGGACTG :  187
b26_liv_sf  : ATGGGACAACAACCTGCAAAATCAATGGACGTGCGGAGAATCGAAGGAGGAGAACTCCTGCTAAATCAATTAGCAGGCCGCATGATACCAAAAGGGACTG :  185
b35_d15_sf  : ATGGGACAACAACCTGCAAAATCAATGGACGTGCGGAGAATCGAAGGAGGAGAACTCNTGCTAANTNAATTAGCAGGCCGNATGATACCAAAAGGGACTG :  185
b35_d25_sf  : ATGGGACAACAACCTGCAAAATCAATGGACGTGCGGAGAATCGAAGGAGGAGAACTCCTGCTAAATCAATTAGCAGGCCGCATGATACCAAAAGGGACTG :  185
b35_d27_sf  : ATGGGACAACANCCTGCAAAATCAATGGACGTGCGGAGAATCGAAGGAGGAGAACTCCTGCTAANTNAATTANNNGGCCGNATGATACCAAAAGGGACTG :  185
b35_d36_sf  : ATGGGACAACAACCTGCAAAATCAATGGACGTGCGGAGAATCGAAGGAGGAGAACTCCTGCTAAATNAATTAGCAGGCCGNATGATACCAAAAGGGACTG :  186
b35_liv_sf  : ATGGGACAACAACCTGCAAAATCAATGGACGTGCGGAGAATCGAAGGAGGAGAACTCCTGCTAAATCAATTAGCAGGCCGCATGATACCAAAAGGGACTG :  186
b37_d36_sf  : ATGGGACANCNACCTGCAAAATCAATGGACGTGCGGAGAATCNNAGGAGGNNAACTCCNGCTNNNN-NATTNNNNNGCCGCNTGATNNCNNAAGGNNCTG :  166
b37_liv_sf  : ATGGGACAACANCCTGCAAAATCAATGGACGTGNNNNNNNATCGANGGNGGAGAACTCCTGCTNAATCAATTANCAGGCCGNATGATNCCAAAAGGNACTG :  186
              ATGGGACAACAACCTGCAAAATCAATGGACGTGCGGAGAATCGAAGGAGGAgAACTCCTGCTAAATcAATTAGCAGGCCGCATGATACCAAAAGGGACTG
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continued - *Multiple Sequence Alignment of the forward Surface region.*

adhbv: Australian Duck Hepatitis B Virus (GenBank DHV6350, AJ006350); inoculum: starting inoculum; all others ducknumber_dayofsample.

```
                    *       920         *       940         *       960         *       980         *      1000
adhbv_f     : TCACATGGTCGGGCAAATTTCCAACAATAGATCACCTATTAGATCATGTGCAAACAATGGAGGAGGTAAATACTCTTCAGCAACAAGGCGCATGGCCTGC : 1000
inoculumsf  : TCACATGGTCGGGCAAATTTCCAACAATAGATCACCTATTAGATCATGTGCAAACAATGGAGGAGGTAAATACTCTTCAGCAACAAGGCGCATGGCCTGC :  286
p13_d06_sf  : TCACATGGTCGGGCAAATTTCCAACAATAGATCACCTATTAGATCATGTGCAAACAATGGAGGAGGTAAATACTCTTCAGCAACAAGGCGCATGGCCTGC :  286
p13_d11_sf  : TCACATGGTCGGGCAAATTTCCAACAATAGATCACCTATTAGATCATGTGCAAACAATGGAGGAGGTAAATACTCTTCAGCAACAAGGCGCATGGCCTGC :  286
p13_d27_sf  : TCACATGGTCGGGCAAATTTCCAACAATAGATCACCTATTAGATCATGTGCAAACAATGGAGGAGGTAAATACTCTTCAGCAACAAGGCGCATGGCCTGC :  286
p13_d43_sf  : TCACATGGTCGGGCAAATTTCCAACAATAGATCACCTATTAGATCATGTGCAAACAATGGAGGAGGTAAATACTCTTCAGCAACAAGGCGCATGGCCTGC :  286
p13_liv_sf  : TCACATGGTCGGGCAAATTTCCAACAATAGATCACCTATTAGATCATGTGCAAACAATGGAGGAGGTAAATACTCTTCAGCAACAAGGCGCATGGCCTGC :  286
p14_d06_sf  : TTAGNCGGANCCACNCNNTT-CAACAACAGATCACCTATTNGATNATGTGCNNACAATGGGGGNGGTNTATACTNTTCCT-NNCAAGGCGCATGGNCTGC :  278
p14_d11_sf  : TNACATGGTCGGGCAAATTTCCAACAATAGATCACCTATTAGATCATGTGCAAACAATGGAGGAGGTAAATACTCTTCAGCAACAAGGCGCATGGNCTGC :  275
p14_d27_sf  : TCACATGGTCGGGCAAATTTCCAACAATAGATCACCTATTAGATCATGTGCAAACAATGGAGGAGGTAAATACTCTTNAGCAACAAGGCGCATGGCCTGC :  286
p14_d43_sf  : TCACATGGTCGGGCAAATTTCCAACAATAGATCACCTATTAGATCATGTGCAAACAATGGAGGAGGTAAATACTCTTCAGCAACAAGGCGCATGGCCTGC :  270
p14_liv_sf  : TCACATGGTCGGGCAAATTTCCAACAATAGATCACCTATTAGATCATGTGCAAACAATGGAGGAGGTAAATACTCTTCAGCAACAAGGCGCATGGCCTGC :  287
w13_d20_sf  : TCACATGGTCGGGCAAATTTCCAACAATAGATCACCTATTAGATCATGTGCAAACAATGGAGGAGGTAAATACTCTTCAGCAACAAGGCGCATGGCCTGC :  286
w13_d29_sf  : TCACATGGTCGGGCAAATTTCCAACAATAGATCACCTATTAGATCATGTGCAAACAATGGAGGAGGTAAATACTCTTCAGCAACAAGGCGCATGGCCTGC :  286
w13_d34_sf  : TCACATGGTCGGGCAAATTTCCAACAATAGATCACCTATTAGATCATGTGCAAACAATGGAGGAGGTAAATACTCTTCAGCAACAAGGCGCATGGCCTGC :  287
w13_d39_sf  : TCACATGGTCGGGCAAATTTCCAACAATAGATCACCTATTAGATCATGTGCAAACAATGGAGGAGGTAAATACTCTTCAGCAACAAGGCGCATGGCCTGC :  287
w13_d41_sf  : TCACATGGTCGGGCAAATTTCCAACAATAGATCACCTATTAGATCATGTGCAAACAATGGAGGAGGTAAATACTCTTCAGCAACAAGGCGCATGGCCTGC :  287
w13_liv_sf  : TCACATGGTCGGGCAAATTTCCAACAATAGATCACCTATTAGATCATGTGCAAACAATGGAGGAGGTAAATACTCTTCAGCAACAAGGCGCATGGCCTGC :  287
w15_d13_sf  : TCACATGGTCGGGCAAATTTCCAACAATAGATCACCTATTAGATCATGTGCAAACAATGGAGGAGGTAAATACTCTTCAGCAACAAGGCGCATGGCCTGC :  287
w15_d18_sf  : TCACATGGTCGGGCAAATTTCCAACAATAGATCACCTATTAGATCATGTGCAAACAATGGAGGAGGTAAATACTCTTCAGCAACAAGGCGCATGGCCTGC :  286
w15_liv_sf  : TCACATGGTCGGGCAAATTTCCAACAATAGATCACCTATTAGATCATGTGCAAACAATGGAGGAGGTAAATACTCTTCAGCAACAAGGCGCATGGCCTGC :  287
b26_d15_sf  : TCACATGGTCGGGCAAATTTCCAACAATAGATCACCTATTAGATCATGTGCAAACAATGGAGGAGGTAAATACTCTTCAGCAACAAGGCGCATGGCCTGC :  286
b26_d25_sf  : TCACATGGTCGGGCAAATTTCCAACAATAGATCACCTATTAGATCATGTGCAAACAATGGAGGAGGTAAATACTCTTCAGCAACAAGGCGCATGGCCTGC :  285
b26_d27_sf  : TCACATGGTCGGGCAAATTTCCAACAATAGATCACCTATTAGATCATGTGCAAACAATGGAGGAGGTAAATACTCTTCAGCAACAAGGCGCATGGCCTGC :  273
b26_d36_sf  : TTACATGGTCNGGCANATTTCCAACAATAGATCACCTATTAGATCATGTGCAAACAATGGAGGAGGTAAATACTCTTCAGCAACAAGGCGCATGGCCTGC :  287
b26_liv_sf  : TCACATGGTCGGGCAAATTTCCAACAATAGATCACCTATTAGATCATGTGCAAACAATGGAGGAGGTAAATACTCTTCAGCAACAAGGCGCATGGCCTGC :  285
b35_d15_sf  : TCACATGGTCGGGCAAATTTCCAACAATAGATCACCTATTAGATCATGTGCAAACAATGGAGGAGGTAAATACTCTTCAGCAACAAGGCGCATGGCCTGC :  285
b35_d25_sf  : TCACATGGTCGGGCAAATTTCCAACAATAGATCACCTATTAGATCATGTGCAAACAATGGAGGAGGTAAATACTCTTCAGCAACAAGGCGCATGGCCTGC :  285
b35_d27_sf  : TCACATGGNCGGGCANATTTCCAACAATAGATCACCTATTAGATCATGTGCAAACAATGGAGGAGGTAAATACTCTTCAGCAACAAGGCGCATGGCCTGC :  285
b35_d36_sf  : TCACATGGTCGGGCAAATTTCCAACAATAGATCACCTATTAGATCATGTGCAAACAATGGAGGAGGTAAATACTCTTCAGCAACAAGGCGCATGGCCTGC :  286
b35_liv_sf  : TCACATGGTCGGGCAAATTTCCAACAATAGATCACCTATTAGATCATGTGCAAACAATGGAGGAGGTAAATACTCTTCAGCAACAAGGCGCATGGCCTGC :  286
b37_d36_sf  : TCCCATGGNCGGTCNAATTTCCAACAATAGATNACNTATTAGANCATGTGCAAGCTGNGGATGCTGTNAATGCTCTTCAG-AACANGGCGCATGGCCTGC :  265
b37_liv_sf  : TCACATGGTCGGGCANATTTNCAACAATAGATCACCTATTAGATCATGTGCAAACAATGGAGGAGGTAAATACTCTTCAGCAACAAGGCGCATGGCCTGC :  286
              TcacAtGGtCgggCAaATTTcCAACAAtAGATCACCTATTAGATCATGTGCAAaCaaTGGagGagGTAaATaCTCTTCagcAACAAGGCGCATGGCCTGC
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continued - *Multiple Sequence Alignment of the forward Surface region.*

adhbv: Australian Duck Hepatitis B Virus (GenBank DHV6350, AJ006350); inoculum: starting inoculum; all others ducknumber_dayofsample.

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                    *      1020          *      1040          *      1060          *      1080          *      1100
adhbv_f     : TGGGGCAGGAAGACGTTTGGGGTTAACCAATCCGGCACCCCAAGAACCTCCTCAGCCCCAGTGGACTCCCGAAGAAGATCAGAAAGCACGGGAAGCCTTT : 1100
inoculumsf  : TGGGGCAGGAAGACGTTTGGGGTTAACCAATCCGGCACCCCAAGAACCTCCTCAGCCCCAGTGGACTCCCGAAGAAGATCAGAAAGCACGGGAAGCCTTT :  386
p13_d06_sf  : TGGGGCAGGAAGACGTTTGGGGTTAACCAATCCGGCACCCCAAGAACCTCCTCAGCCCCAGTGGACTCCCGAAGAAGATCAGAAAGCACGGGAAGCCTTT :  386
p13_d11_sf  : TGGGGCAGGAAGACGTTTGGGGTTAACCAATCCGGCACCCCAAGAACCTCCTCAGCCCCAGTGGACTCCCGAAGAAGATCAGAAAGCACGGGAAGCCTTT :  386
p13_d27_sf  : TGGGGCAGGAAGACGTTTGGGGTTAACCAATCCGGCACCCCAAGAACCTCCTCAGCCCCAGTGGACTCCCGAAGAAGATCAGAAAGCACGGGAAGCCTTT :  386
p13_d43_sf  : TGGGGCAGGAAGACGTTTGGGGTTAACCAATCCGGCACCCCAAGAACCTCCTCAGCCCCAGTGGACTCCCGAAGAAGATCAGAAAGCACGGGAAGCCTTT :  386
p13_liv_sf  : TGGGGCAGGAAGACGTTTGGGGTTAACCAATCCGGCACCCCAAGAACCTCCTCAGCCCCAGTGGACTCCCGAAGAAGATCAGAAAGCACGGGAAGCCTTT :  386
p14_d06_sf  : TGGGGCAGGAAGACGTTTGGGGTTTACCAATNCGGNACCCCAANAACCTNCTTNNCCCCAGTNGNCTCCCGNNGAAGATTNTAAAGCACGGGAAGCCTTT :  378
p14_d11_sf  : TGGGGCAGGAAGACGTTTGGGGTTAACCAATCCGGCACCCCAAGAACCTTCTTAG-CCCAGTGGACTCCCGAAGAAGATCAGAAAGCACGGGAAGCCTTT :  374
p14_d27_sf  : TGGGGCAGGAAGACGTTTGGGGTTAACCAATCCGGCACCCCAAGAACCTNCTNAGCCCCAGTGGACTCCCGAAGAAGATCAGAAAGCACGGGAAGCCTTT :  386
p14_d43_sf  : TGGGGCAGGAAGACGTTTGGGGTTAACCAATCCGGCACCCCAAGAACCTNCTNAGCCCCAGTGGACTTCCGAAGAAGATNANAAANCACGGGAAGCCTTT :  370
p14_liv_sf  : TGGGGCAGGAAGACGTTTGGGGTTAACCAATCCGGCACCCCAAGAACCTCCTCAGCCCCAGTGGACTCCCGAAGAAGATCAGAAAGCACGGGAAGCCTTT :  387
w13_d20_sf  : TGGGGCAGGAAGACGTTTGGGGTTAACCAATCCGGCACCCCAAGAACCTCCTCAGCCCCAGTGGACTCCCGAAGAAGATCAGAAAGCACGGGAAGCCTTT :  386
w13_d29_sf  : TGGGGCAGGAAGACGTTTGGGGTTAACCAATCCGGCACCCCAAGAACCTCCTCAGCCCCAGTGGACTTCCGAAGAAGATCAGAAAGCACGGGAAGCCTTT :  386
w13_d34_sf  : TGGGGCAGGAAGACGTTTGGGGTTAACCAATCCGGCACCCCAAGAACCTCCTCAGCCCCAGTGGACTCCCGAAGAAGATCAGAAAGCACGGGAAGCCTTT :  387
w13_d39_sf  : TGGGGCAGGAAGACGTTTGGGGTTAACCAATCCGGCACCCCAAGAACCTCCTCAGCCCCAGTGGACTCCCGAAGAAGATCAGAAAGCACGGGAAGCCTTT :  387
w13_d41_sf  : TGGGGCAGGAAGACGTTTGGGGTTAACCAATCCGGCACCCCAAGAACCTCCTCAGCCCCAGTGGACTCCCGAAGAAGATCAGAAAGCACGGGAAGCCTTT :  387
w13_liv_sf  : TGGGGCAGGAAGACGTTTGGGGTTAACCAATCCGGCACCCCAAGAACCTCCTCAGCCCCAGTGGACTCCCGAAGAAGATCAGAAAGCACGGGAAGCCTTT :  387
w15_d13_sf  : TGGGGCAGGAAGACGTTTGGGGTTAACCAATCCGGCACCCCAAGAACCTCCTCAGCCCCAGTGGACTCCCGAAGAAGATCAGAAAGCACGGGAAGCCTTT :  387
w15_d18_sf  : TGGGGCAGGAAGACGTTTGGGGTTAACCAATCCGGCACCCCAAGAACCTCCTCAGCCCCAGTGGACTCCCGAAGAAGATCAGAAAGCACGGGAAGCCTTT :  386
w15_liv_sf  : TGGGGCAGGAAGACGTTTGGGGTTAACCAATCCGGCACCCCAAGAACCTCCTCAGCCCCAGTGGACTCCCGAAGAAGATCAGAAAGCACGGGAAGCCTTT :  387
b26_d15_sf  : TGGGGCAGGAAGACGTTTGGGGTTAACCAATCCGGCACCCCAAGAACCTCCTCAGCCCCAGTGGACTCCCGAAGAAGATCAGAAAGCACGGGAAGCCTTT :  386
b26_d25_sf  : TGGGGCAGGAAGACGTTTGGGGTTAACCAATCCGGCACCCCAAGAACCTCCTCAGCCCCAGTGGACTCCCGAAGAAGATCAGAAAGCACGGGAAGCCTTT :  385
b26_d27_sf  : TGGGGCAGGAAGACGTTTGGGGTTAACCAATCCGGCACCCCAAGAACCTNCTCAGCCCCAGTGGACTCCCGAAGAAGATCAGAAAGCACGGGAAGCCTTT :  373
b26_d36_sf  : TGGGGCAGGAAGACGTTTGGGGTTAACCAATCCGGCACCCCAAGAACCTCCTCAGCCCCAGTGGACTCCCGAAGAAGATCANAAAGCACGGGAAGCCTTT :  387
b26_liv_sf  : TGGGGCAGGAAGACGTTTGGGGTTAACCAATCCGGCACCCCAAGAACCTCCTCAGCCCCAGTGGACTCCCGAAGAAGATCAGAAAGCACGGGAAGCCTTT :  385
b35_d15_sf  : TGGGGCAGGAAGACGTTTGGGGTTAACCAATCCGGCACCCCAAGAACCTCCTCAGCCCCAGTGGACTCCCGAAGAAGATCAGAAAGCACGGGAAGCCTTT :  385
b35_d25_sf  : TGGGGCAGGAAGACGTTTGGGGTTAACCAATCCGGCACCCCAAGAACCTCCTCAGCCCCAGTGGACTCCCGAAGAAGATCAGAAAGCACGGGAAGCCTTT :  385
b35_d27_sf  : TGGGGCAGGAAGACGTTTGGGGTTAACCAATCCGGCACCCCAAGAACCTNCTCAGNCCCAGTGGACTCCCGAAGAAGATCAGAAAGCACGGGAAGCCTTT :  385
b35_d36_sf  : TGGGGCANGAAGACGTTTGGGGTTAACCAATCCGGCACCCCAAGAACCTCCTCAGCCCCAGTGGACTCCCGAAGAAGATCAAAAAGCACGGGAAGCCTTT :  386
b35_liv_sf  : TGGGGCAGGAAGACGTTTGGGGTTAACCAATCCGGCACCCCAAGAACCTCCTCAGCCCCAGTGGACTCCCGAAGAAGATCAGAAAGCACGGGAAGCCTTT :  386
b37_d36_sf  : TGGGGCACGAAGANATTNNGGGNTTACCAATCNGNCACCNCATGAACCTTCTTAGCCCCANTGGACT-CCGAATAATATCAAAGAGCANGNGNAGNCTTT :  364
b37_liv_sf  : TGGGGCAGGAAGACGTTTGGGGTTAACCAATCCGGCACCCCAAGAACCTCCTCAGCCCCAGTGGACTCCCGAAGAAGATCAGAAAGCACGGGAAGCCTTT :  386
              TGGGGCAgGAAGACgTTTGGGGTTaACCAATCCGGCACCCCAaGAACCTcCTcAGcCCCAGTGGACTcCCGAAgAAgATcAgAaAGCACGGGAAGCCTTT
```

continued - *Multiple Sequence Alignment of the forward Surface region.*

adhbv: Australian Duck Hepatitis B Virus (GenBank DHV6350, AJ006350); inoculum: starting inoculum; all others ducknumber_dayofsample.

```
                        *      1120          *      1140          *      1160          *      1180          *      1200
adhbv_f     : CGTCGTTATCAAGAAGAGAGACCACCGGAAACCACCACAATTCCACCAACGTCACCAACTCCGTGGAAACTACAACCAGGGGACGATCCCCTACTCGAGA : 1200
inoculumsf  : CGTCGTTATCAAGAAGAGAGACCACCGGAAACCACCACAATTCCACCAACGTCACCAACTCCGTGGAAACTACAACCAGGGGACGATCCCCTACTCGAGA :  486
p13_d06_sf  : CGTCGTTATCAAGAAGAGAGACC-CCGGAAACCACCACAATTTCACCAACGTCACCAACTTCGTGGAAACTACAACCAGGGGACGATTCCCTACTCGAGA :  485
p13_d11_sf  : CGTCGTTATCAAGAAGAGAGACCACCGGAAACCACCACAATTCCACCAACGTCACCAACTTCGTGGAAACTACAACCAGGGGACGAT-CCCTACTCGAGA :  485
p13_d27_sf  : CGTCGTTATCAAGAAGAGAGACCACCGGAAACCACCACAATTCCACCAACGTCACCAACTCCGTGGAAACTACAACCAGGGGACGATCCCCTACTCGAGA :  486
p13_d43_sf  : CGTCGTTATCAAGAAGAGAGACC-CCGGAAACCACCACAATTTCACCAACGTCACCAACTTCGTGGAAACTACAACCAGGGGACGATCCCCTACTCGAGA :  485
p13_liv_sf  : CGTCGTTATCAAGAAGAGAGACCACCGGAAACCACCACAATTTCACCAACGTCACCAACTNCGTGGAAACTACAACCAGGGGACGATCCCCTACTNGAGA :  486
p14_d06_sf  : TTTNNTTTTAAAGAANAGAGACC-CCCGGNACCACCACAATTCCACCAACGT-ACCAACTTCGNGGNAACTNCNACCNAGGGGCGATCCCCTTNTNNNGN :  476
p14_d11_sf  : CGTCCGTTNCAAGAAGAGAGACCACCGGAAACCACCACAATTTCACCCACGTTACCAACTTCGTGGAAACTACACCNAGGGGACGATCCCCTACTCNAGA :  474
p14_d27_sf  : CGTCGTTATCAAGAAGAGAGACC-CCGGAAACC-CCACAATTTC-CCAACGTNACCAACTTCGTGGAAACTACAACCAGGGGACGAT-CCCTACTNGAGA :  482
p14_d43_sf  : CGTCGTTATCAAGAAGAGAGACC-CCGGAAACCCCCCACAATTTCACCAACGTCACCAACTTCGTGGAAACTACAACCAGGGGACGAT-NCCTACTNGAGA :  468
p14_liv_sf  : CGTCGTTATCAAGAAGAGAGACCACCGGAAACCACCACAATTTCACCAACGTCACCAACTTCGTGGAAACTACAACCAGGGGACGATCCCCTACTCGAGA :  487
w13_d20_sf  : CGTCGTTATCAAGAAGAGAGACCACCGGAAACCACCACAATTTCACCAACGTCACCAACTTCGTGGAAACTACAACCAGGGGACGATCCCCTACTCGAGA :  486
w13_d29_sf  : CGTCGTTATCAAGAAGAGAGACCACCGGAAACCACCACAATTNCACCAACGTCACCAACTCCGTGGAAACTACAACCAGGGGACGATCCCCTACTCGAGA :  486
w13_d34_sf  : CGTCGTTATCAAGAAGAGAGACCACCGGAAACCACCACAATTCCACCAACGTCACCAACTNCGTGGAAACTACAACCAGGGGACGATCCCCTACTCGAGA :  487
w13_d39_sf  : CGTCGTTATCAAGAAGAGAGACCACCGGAAACCACCACAATTNCACCAACGTCACCAACTCCGTGGAAACTACAACCAGGGGACGATCCCCTACTCGAGA :  487
w13_d41_sf  : CGTCGTTATCAAGAAGAGAGACCACCGGAAACCACCACAATTNCACCAACGTCACCAACTTCGTGGAAACTACAACCAGGGGACGATCCCCTACTCGAGA :  487
w13_liv_sf  : CGTCGTTATCAAGAAGAGAGACCACCGGAAACCACCACAATTTCACCAACGTNACCAACTTCGTGGAAACTACAACCAGGGGACGATCCCCTACTCGAGA :  487
w15_d13_sf  : CGTCGTTATCAAGAAGAGAGACCACCGGAAACCACCACAATTTCACCAACGTCACCAACTCCGTGGAAACTACAACCAGGGGACGATCCCCTACTCGAGA :  487
w15_d18_sf  : CGTCGTTATCAAGAAGAGAGACCACCGGAAACCACCACAATTNCACCAACGTCACCAACTNCGTGGAAACTACAACCAGGGGACGATCCCCTACTCGAGA :  486
w15_liv_sf  : CGTCGTTATCAAGAAGAGAGACCACCGGAAACCACCACAATTCCACCAACGTCACCAACTCCGTGGAAACTACAACCAGGGGACGATCCCCTACTCGAGA :  487
b26_d15_sf  : CGTCGTTATCAAGAAGAGAGACCACCGGAAACCACCACAATTTCACCAACGTNACCAACTTCGTGGAAACTACAACCAGGGGACGAT-CCCTACTNGAGA :  485
b26_d25_sf  : CGTCGTTATCAAGAAGAGAGACCACCGGAAACCACCACAATTTCACCAACGTCACCAACTTCGTGGAAACTACAACCAGGGGACGATCCCCTACTNGAGA :  485
b26_d27_sf  : T-TNGTTATCAAGAAGAGAGACCACCGGAAACCACC-CAATTTCACCAACGTGACCAACTTCGTGGAAACTACAACCAGGGGACGAT-CCCTACTCGAGA :  470
b26_d36_sf  : NGTNGTTATCAAGAAGAGAGACC-CCGGAAACCACCACAATTTCACCAACGTGACCAACTTCGTGGAAACTACAACCAGGGGACGATCCCCTACTNGAGA :  486
b26_liv_sf  : CGTCGTTATCAAGAAGAGAGACCACCGGAAACCACCACAATTTCACCAACGTCACCAACTTCGTGGAAACTACAACCAGGGGACGAT-CCCTACTCGAGA :  484
b35_d15_sf  : CGTCGTTATCAAGAAGAGAGACCACCGGAAACCACCACAATTCCACCAACGTCACCAACTTCGTGGAAACTACAACCAGGGGACGATCCCCTACTCGAGA :  485
b35_d25_sf  : CGTCGTTATCAAGAAGAGAGACCACCGGAAACCACCACAATTCCACCAACGTCACCAACTCCGTGGAAACTACAACCAGGGGACGATCCCCTACTCGAGA :  485
b35_d27_sf  : CGNCGNTATCAAGAAGAGAGACCACCGGAAACCACCACAATTNCACCAACGN-ACCAACTTCGTGGAAACTACAACCAGGGGACGATCCCCTACTCGAGA :  484
b35_d36_sf  : NGTCGTTATCAAGAAGAGAGACCACCGGAAACCACCACAATTTCACCAACGTGACCAACTTCGTGGAAACTACAACCAGGGGACGATNCCCTACTCGAGA :  486
b35_liv_sf  : CGTCGTTATCAAGAAGAGAGACCACCGGAAACCACCACAATTCCACCAACGTCACCAACTTCGTGGAAACTACAACCAGGGGACGATCCCCTACTCGAGA :  486
b37_d36_sf  : TTNGGNTATCNGANAGAGAGACCACCGCAAACCACCACNATTTCNCCTACGNAGACNANTTCNNGGAANANACAAGCCTNNNACGAATCCCTTNTGCNAA :  464
b37_liv_sf  : CGTNGTTATCAAGAAGAGAGACCACCGGAAACCACCACAATTCCACCAACGTCACCAACTTCGTGGAAACTACAACCAGGGGACGATCCCCTACTNGAGA :  486
              cgTcgtTaTcAagAAGAGAGACCaCCggaAACCaCCaCAATT CaCCaACGTcacCAACT CGTGGAAAcTACAacCagGGGaCGAt CCCTaCTcgAgA
```

continued - *Multiple Sequence Alignment of the forward Surface region.*

adhbv: Australian Duck Hepatitis B Virus (GenBank DHV6350, AJ006350); inoculum: starting inoculum; all others ducknumber_dayofsample.

```
                    *      1220          *      1240          *      1260          *      1280          *      1300
adhbv_f     : ACAAA-TCTCT-GCTCGAG-ACTCATCCTCTTTAC-CAGAA-TCCGGAGCC-GGCC-GTGCCT-GTGATAAAG-ACTC-CTCCCCT-CAAGAA-GAAGAA : 1288
inoculumsf  : ACAAA-TCTCT-GCTCGAG-ACTCATCCTCTTTAC-CAGAA-TCCGGAGCC-GGCC-GTGCCT-GTGATAAAG-ACTA-CTCCCCT-CAAGAA-GAAGAA :  574
p13_d06_sf  : ACAAA-TCTCT-GCTCGAG-ACTCATTCTTCTTAC-CAGAA-TCCGGAGCC-CGCC-CTGCCTTGTGATAAAA-ACTA-CTTCCCTTAAAGAA-GAAGAA :  575
p13_d11_sf  : ACAAA-TCTCT-GCTCGAG-ACTCATTCT-CTTTC-CAGAA-TCCGGAGCC-GGCC-GTGCCT-GTGATAAAG-ACT--CTTCCCTTAA-GAA-GAAGAA :  571
p13_d27_sf  : ACAAA-TCTCT-GCTCGAG-ACTCATCCTCTTTAC-CAGAA-TCCGGAGCC-GGCC-GTGCCT-GTGATAAAG-ACTA-CTCCCCT-CAAGAA-GAAGAA :  574
p13_d43_sf  : ACAAA-TCTCT-GCTCGAG-ACTCATCCT-CTTAC-CAGAA-TCCGGAGCC-GGCC-GTGCCTTGTGATAAAG-ACTA-CTTCCCTTCA-GAA-GAAGAA :  573
p13_liv_sf  : ACAAA-TCTCT-GTTCGAG-ACTNATCCTCTTTAC-CAGAAATCCGGAGCC-GGCCCGTGCCTTGTGATAAAG-ACTA-CTNCC-T-CAAGAAAGAANAA :  577
p14_d06_sf  : ACNAAATCTTTTGGTTTNGGAATATTCTTTTTTAN-CAGAATTCCGGAGNC-GGCC-GTGCCTTGTGATAAAAANTTN--NNCCCTCCAAGAAAAAANAA :  571
p14_d11_sf  : ACAAAATCTTTTGTTNGAG-ACTTATTCTNTTTAC-CAGAATTCCGGAGCC-GGCC-GTGCCTTGTGATAAAG-ACTNCCTCCCCTTCAAGAA-GAANAA :  568
p14_d27_sf  : ACAAA-TCTNT-GNTNGAG-ACTCATC-T-NTTTC-CAGAA-TCCGGAGCC-GGCC-GTGCCT-GTGATAAAA-ACTA-CTCCC-TTAA-GAA-AAAAAA :  567
p14_d43_sf  : ACAAATNTTTT--GTNGAG-ACTNATTCTTTTTAC-CAGAA-TNCGGAGNC-GGNCCGTGCCTTGTGATAAAAAACTA-CTCCCCT-NAAGAA-NAANAA :  559
p14_liv_sf  : ACAAA-TCTNT-GNTCGAG-ACTCATTCT-NTTTC-CAGAA-TTCGGAGCC-GGCC-GTGCCT-GTGATAAAAGACTA-CTTCCCTTAANAAN-AAAAAA :  576
w13_d20_sf  : ACAAA-TCTCT-GCTCGAG-ACTCATCCTCTTTAC-CAGAA-TCCGGAGCC-GGCC-GTGCCT-GTGATAAAG-ACTA-CTCCCCT-CAAGAA-GAAGAA :  574
w13_d29_sf  : ACAAA-TCTCT-GCTCGAG-ACTCATNCTCTTTAC-CAGAA-TCCGGAGCC-GGCC-GTGCCT-GTGATAAAG-ACTA-CTCCCNT-CAAGAA-GAAGAA :  574
w13_d34_sf  : ACAAA-TCTCT-GCTCGAG-ACTCATCCTCTTTAC-CAGAA-TCCGGAGCC-GGCC-GTGCCT-GTGATAAAG-ACTA-CTCCCCT-CAAGAA-GAAGAA :  575
w13_d39_sf  : ACAAA-TCTCT-GCTCGAG-ACTCATCCTCTTTAC-CAGAA-TCCGGAGCC-GGCC-GTGCCT-GTGATAAAG-ACTA-CTCCCCT-CAAGAA-GAAGAA :  575
w13_d41_sf  : ACAAA-TCTCT-GCTCGAG-ACTCATCCTCTTTAC-CAGAA-TCCGGAGCC-GGCC-GTGCCT-GTGATAAAG-ACTA-CTCCCCT-CAAGAA-GAAGAA :  575
w13_liv_sf  : ACAAA-TCTCT-GCTCGAG-ACTCATCCTCTTTAC-CAGAA-TCCGGAGCC-GGCC-GTGCCT-GTGATAAAG-ACTA-CTNCCCTTCAAGAA-GAAGAA :  576
w15_d13_sf  : ACAAA-TCTCT-GCTCGAG-ACTCATCCTCTTTAC-CAGAA-TCCGGAGCC-GGCC-GTGCCT-GTGATAAAG-ACTA-CTNCCCT-CAAGAA-GAAGAA :  575
w15_d18_sf  : ACAAA-TCTCT-GCTCGAG-ACTCATNCTCTTTAC-CAGAA-TCCGGAGCC-GGCC-GTGCCT-GTGATAAAG-ACTA-CTCCCCT-CAAGAA-GAAGAA :  574
w15_liv_sf  : ACAAA-TCTCT-GCTCGAG-ACTCATCCTCTTTAC-CAGAA-TCCGGAGCC-GGCC-GTGCCT-GTGATAAAG-ACTA-CTCCCCT-CAAGAA-GAAGAA :  575
b26_d15_sf  : ACAAA-TCTNT-GGTCGAG-ACTCATCCT-CTTTC-CAGAA-TC-GGAGCC-GGCC-GTGCCT-GTGATAAAN-ACTA-CTCCCCTNAA-AAA-AAANAA :  571
b26_d25_sf  : ACAAA-TCTNT-GCTCGAG-ACTCATCCTNTTTAC-CAGAATCCCGGAGCC-GGCC-GTGCCTTGTGATAAAG-ACTA-CTCCC-T-CAAGAA-GAAGAA :  574
b26_d27_sf  : ACAAAATTTCT-GCTCGAGGACTCATCCTCTTTAC-CAGAA-TTCGGAGCCGGGCC-GTGCCT-TTGATAAAAGACTA-CTCCCCT-CAAGAA-GAAGAA :  562
b26_d36_sf  : ACAAA-TNTNT-GCTCGAG-ACTCATNCTNTTTAC-CAGAA-TNCGGAGCC-GGGCCGTGCCT-GTGATAAAN-ACTA-CTTCCCT-CAAGAA-NAAGAA :  575
b26_liv_sf  : ACAAAATCTCT-GCTCGAG-ACTCATNCTCTTTAC-CAGAAATCCGGAGCCCGGNCCGTGCCT-GTGATAAAA-ACTA-CTTCCCTTAAAAAAAAAANAA :  578
b35_d15_sf  : ACAAA-TCTCT-GCTCGAG-ACTCATCCTNTTTAC-CAGAA-TCCGGAGCC-GGCC-GTGCCT-GTGATAAAA-ACTA-CTCCCCTCAAAGAA-GAANAA :  574
b35_d25_sf  : ACAAA-TCTCT-GCTCGAG-ACTCATCCTCTTTAC-CAGAA-TCCGGAGCC-GGCC-GTGCCT-GTGATAAAG-ACTA-CTNCCCT-CAAGAA-GAAGAA :  573
b35_d27_sf  : ACAAA-TCTCT-GCTCGAG-ACTCATCCTNTTTAC-CAGAA-TCCGGAGGC-GGGCCGTGCCTTGTGATAAAG-ACTA-CTNCCCT-CAAGAA-GAAGAA :  574
b35_d36_sf  : ACAAA-TCTCT-GCTCGAGGACTCATCCTCTTTTC-CAGAA-TCCGGAGGC-CGGCCGTGCCT-GTGATAAAN-ACTA-CTCCCCT-CAAGAAGAAAGAA :  577
b35_liv_sf  : ACAAA-TCTCT-GCTCGAGGACTCATCCTCTTTAC-CAGAA-TCCGGAGCC-GGNC-GTGCCTTGTGATAAAG-ACTA-CTNCCCT-CAAGAA-GAAGAA :  576
b37_d36_sf  : NCTAA-TATTT-GCNCGAG-AGTTNCCCNCNTTNCNCNTAATTCTTGAGGCGGGGC-GGGCCTTGATANAAAAGACNA-CTCCCCT-CCGGANTAATGAA :  558
b37_liv_sf  : ACAAA-TNTTTTGNTCGAG-ACTCATCCTNTTTAC-CAGAA-TCNNGGAGC-CGGCCGGGCCT-GTGATAAAG-ACTA-CTTCCCT-CAAGAA-GAANAA :  576
              ACaAA TcTcT gcTcgAG AcTcatccT tTTaC CAgAA tccgGagcC gGcC gtGCCT gtgATAAA  AcTa cTcCCcT caagAA gAagAA
```

continued - *Multiple Sequence Alignment of the forward Surface region.*

adhbv: Australian Duck Hepatitis B Virus (GenBank DHV6350, AJ006350); inoculum: starting inoculum; all others ducknumber_dayofsample.

```
                    *      1220          *      1240          *      1260          *      1280          *      1300
adhbv_r     : GATGGCGTTGTTTTGTCAAAGTTTATGCGCACTCCTAATTCTTGTAGAAAAGTGCAGACAGCGTGGCTAATTGAGTTAAGGTGACGAGCGTTTGGGTGGC : 1300
inoculumsr  : ------------------------GCGCACTCCTAATTCTTGTAGAAAAGTGCAGACAGCGTGGCTAATTGAGTTAAGGTGACGAGCGTTTGGGTGGC :   74
p13_d06_sr  : ------------------------GCGCACTCCTAATTCTTGTAGAAAAGTGCAGACAGCGTGGCTAATTGAGTTAAGGTGACGAGCGTTTGGGTGGC :   74
p13_d11_sr  : ------------------------GCGCACTCCTAATTCTTGTAGAAAAGTGCAGACAGCGTGGCTAATTGAGTTAAGGTGACGAGCGTTTGGGTGGC :   74
p13_d27_sr  : ----------------------------CTCCTAATTCTTGTAGAAAAGTGCAGACAGCGTGGCTAATTGAGTTAAGGTGACGAGCGTTTGGGTGGC :   69
p13_d43_sr  : ----------------------------CACTCCTAATTCTTGTAGAAAAGTGCAGACAGCGTGGCTAATTGAGTTAAGGTGACGAGCGTTTGGGTGGC :   71
p13_liv_sr  : ----------------------------------AATTCTTGTAGAAAAGTGCAGACAGCGTGGCTAATTGAGTTNNGGTGACGAGCGTTTGGGTGGC :   64
p14_d06_sr  : ----------------------------------CTAANNCTNGNANAAANNNGCNGACAGNNNNGCTAANNGAGNTANNGTGACGAGCGTTTGNGNGGC :   66
p14_d11_sr  : --------------------------GCACTCCTAATTCTTGTAGAAAAGTGCAGACAGCGTGNCTAATNGAGTTAAGGTGACGAGCGTTTGGGTGGC :   72
p14_d27_sr  : ------------------------GCGCACTCCTAATTCTTGTAGAAAAGTGCAGACAGCGTGGCTAATTGAGTTAAGGTGACGAGCGTTTGGGTGGC :   74
p14_d43_sr  : ------------------------GCGCACTCCTAATTCTTGTAGAAAAGTGCAGACAGCGTGGCTAATTGAGTTAAGGTGACGAGCGTTTGGGTGGC :   74
p14_liv_sr  : ----------------------------------ATTCTTGTAGAAAAGTNCAGACAGCGTNGCTAATNGAGTTNNGGTGACGAGCGTTTGGGTGGC :   63
w13_d20_sr  : ------------------------GCGCACTCCTAATTCTTGTAGAAAAGTGCNGACAGCGTGGCTAATTGAGTTAAGGTGACGAGCGTTTGGGTGGC :   74
w13_d29_sr  : ------------------------GCGCACTCCTAATTCTTGTAGAAAAGTGCAGACAGCGTNGCTAATTGAGTTANGGTGACGAGCGTTTGGGTGGC :   74
w13_d34_sr  : ------------------------GCGCACTCCTAATTCTTGTAGAAAAGTGCAGACAGCGTGGCTAATTGAGTTNNGGTGACGAGCGTTTGGGTGGC :   74
w13_d39_sr  : ------------------------GCGCACTCCTAATTCTTGTAGAAAAGTGCAGACAGCGTNGCTAANTGAGTTANGGTGACGAGCGTTTGGGTGGC :   74
w13_d41_sr  : --------------------------CGCACTCCTAATTCTTGTAGAAAAGTNCAGACAGCGTNGCTAATTGAGTTNAGGTGACGAGCGTTTGGGTGGC :   73
w13_liv_sr  : ------------------------GCGCACTCCTAATTCTTGTAGAAAAGTNCNGACANCGTGNCTNATTGAGTTAAGGTGACGAGCGTTTGGGTGGC :   74
w15_d13_sr  : --------------------------GCGACTCCTAATTCTTGTAGAAAAGTNCAGACAGCGTNGCTAATTGAGTTNAGGTGACGAGCGTTTGGGTGGC :   73
w15_d18_sr  : ------------------------GCGCACTCCTAATTCTTGTAGAAAAGTGCAGACAGCGTGGCTAATTGAGTTAAGGTGACGAGCGTTTGGGTGGC :   74
w15_liv_sr  : ------------------------GCGCACTCCTAATTCTTGTAGAAAAGTGCAGACAGCGTGGCTAATTGAGTTAAGGTGACGAGCGTTTGGGTGGC :   74
b26_d15_sr  : --------------------------CNCACTCCTAATTCTTGTAGAAAAGTGCAGACAGCGTGGCTAATTGAGTTAAGGTGACGAGCGTTTGGGTGGC :   73
b26_d25_sr  : --------------------------CNCACTCCTAATTCTTGTAGAAAAGTGCAGACAGCGTGGCTAATTGAGTTAAGGTGACGAGCGTTTGGGTGGC :   73
b26_d27_sr  : ------------------------GCGCACTCCTAATTCTTGTAGAAAAGTGCAGACAGCGTGGCTAATTGAGTTAAGGTGACGAGCGTTTGGGTGGC :   74
b26_d36_sr  : ----------------------------CACTNCTAATTCTTGNAGNAAAGTGCAGACAGCGTGGCTAATTGAGTTAAGGTGACNAGCGTTTGGGNGGC :   71
b26_liv_sr  : ------------------------ATGCGCACTCCTAATTCTTGTAGAAAAGTGCAGACAGCGTGGCTAATTGAGTTAAGGTGACGAGCGTTTGGGTGGC :   76
b35_d15_sr  : --------------------------CGC-CTCCTAATTCTTGTAGAAAAGTGCAGACAGCGTGGCTAATTGAGTTAAGGTGACGAGCGTTTGGGTGGC :   72
b35_d25_sr  : ------------------------GCGC-CTCCTAATTCTTGTAGAAAAGTGCAGACAGCGTGGCTAATTGAGTTAAGGTGACGAGCGTTTGGGTGGC :   73
b35_d27_sr  : --------------------------CGC-CTCCTAATTCTTGTAGAAAAGTGCAGACAGCGTGGCTAATTGAGTTAAGGTGACGAGCGTTTGGGTGGC :   72
b35_d36_sr  : ----------------------------CACTCCTAATTCTTGTAGAAAAGTGCAGACAGCGTGGCTAATTGAGTTAAGGTGACGAGCGTTTGGGTGGC :   71
b35_liv_sr  : ---------------------------------------------GTAGAAAAGTGCAGACAGCGTGGCTAATTGAGTTAAGGTGACGAGCGTTTGGGTGGC :   57
b37_d36_sr  : ------------------------GCGCACTCCTAATTCTTGTAGAAAAGTGCAGACAGCGTGGCTAATTGAGTTAAGGTGACGAGCGTTTGGGTGGC :   74
b37_liv_sr  : --------------------------CGCACTNCTAATTCTTGNAGAAAAGTGCAGACAGCGNGGCTAATTGAGTTAAGGTGACNAGCGTTTGGGTGGC :   73
                                          c ctcctaattcttGTAGAAAAGTGCAGACAGCGTGGCTAATTGAGTTAAGGTGACGAGCGTTTGGGTGGC
```

Multiple Sequence Alignment of the reverse Surface region.

adhbv: Australian Duck Hepatitis B Virus (GenBank DHV6350, AJ006350); inoculum: starting inoculum; all others ducknumber_dayofsample.

```
                        *      1320          *      1340          *      1360          *      1380          *      1400
adhbv_r     : AGAGGAGGAAGTCATCCATATAAGTAAAAGTCCAAACATTAAAGCGACGAGCGATTTCTGATCCGAGGGCAGTAGTGAAGAGATGGAGGAGAAAAGGGCT : 1400
inoculumsr  : AGAGGAGGAAGTCATCCATATAAGTAAAAGTCCAAACATTAAAGCGACGAGCGATTTCTGATCCGAGGGCAGTAGTGAAGAGATGGAGGAGAAAAGGGCT :  174
p13_d06_sr  : AGAGGAGGAAGTCATCCATATAAGTAAAAGTCCAAACATTAAAGCGACGAGCGATTTCTGATCCGAGGGCAGTAGTGAAGAGATGGAGGAGAAAAGGGCT :  174
p13_d11_sr  : AGAGGAGGAAGTCATCCATATAAGTAAAAGTCCAAACATTAAAGCGACGAGCGATTTCTGATCCGAGGGCAGTAGTGAAGAGATGGAGGAGAAAAGGGCT :  174
p13_d27_sr  : AGAGGAGGAAGTCATCCATATAAGTAAAAGTCCAAACATTAAAGCGACGAGCGATTTCTGATCCGAGGGCAGTAGTGAAGAGATGGAGGAGAAAAGGGCT :  169
p13_d43_sr  : AGAGGAGGAAGTCATCCATATAAGTAAAAGTCCAAACATTAAAGCGACGAGCGATTTCTGATCCGAGGGCAGTAGTGAAGAGATGGAGGAGAAAAGGGCT :  171
p13_liv_sr  : AGAGGAGGAAGTCATCCATATAAGTAAAAGTCCAAACATTAAAGCGACGAGCGATTTCTGATCCGAGGGCAGTAGTGAAGAGATGGAGGAGAAAAGGGCT :  164
p14_d06_sr  : CNAGGANGANGNANCCCATATAGGTGAAAGTCCANTNNTNTTTTTNACGNGCGAACCNTGNNNNGNNGGCNGNNNTNGAGAANAGGANNACNAACGGNCN :  166
p14_d11_sr  : AGAGGAGGAAGTCATCCATATAAGTAAAAGTCCAAACATTAAAGCGACGAGCGATTTCTGATCCGAGGGCAGTAGTGAAGAGATGGAGGAGAAAAGGGCT :  172
p14_d27_sr  : AGAGGAGGAAGTCATCCATATAAGTAAAAGTCCAAACATTAAAGCGACGAGCGATTTCTGATCCGAGGGCAGTAGTGAAGAGATGGAGGAGAAAAGGGCT :  174
p14_d43_sr  : AGAGGAGGAAGTCATCCATATAAGTAAAAGTCCAAACATTAAAGCGACGAGCGATTTCTGATCCGAGGGCAGTAGTGAAGAGATGGAGGAGAAAAGGGCT :  174
p14_liv_sr  : AGAGGAGGAAGTCATCCATATAAGTAAAAGTCCAAACATTAAAGCGACGAGCGATTTCTGATCCGAGGGCAGTAGTGAAGAGATGGAGGAGAAAAGGGCT :  163
w13_d20_sr  : AGAGGAGGAAGTCATCCATATAAGTAAAAGTCCAAACATTAAAGCGACGAGCGATTTCTGATCCGAGGGCAGTAGTGAAGAGATGGAGGAGAAAAGGGCT :  174
w13_d29_sr  : AGAGGAGGAAGTCATCCATATAAGTAAAAGTCCAAACATTAAAGCGACGAGCGATTTCTGATCCGAGGGCAGTAGTGAAGAGATGGAGGAGAAAAGGGCT :  174
w13_d34_sr  : AGAGGAGGAAGTCATCCATATAAGTAAAAGTCCAAACATTAAAGCGACGAGCGATTTCTGATCCGAGGGCAGTAGTGAAGAGATGGAGGAGAAAAGGGCT :  174
w13_d39_sr  : AGAGGAGGAAGTCATCCATATAAGTAAAAGTCCAAACATTAAAGCGACGAGCGATTTCTGATCCGAGGGCAGTAGTGAAGAGATGGAGGAGAAAAGGGCT :  174
w13_d41_sr  : AGAGGAGGAAGTCATCCATATAAGTAAAAGTCCAAACATTAAAGCGACGAGCGATTTCTGATCCGAGGGCAGTAGTGAAGAGATGGAGGAGAAAAGGGCT :  173
w13_liv_sr  : AGAGGAGGAAGTCATCCATATAAGTAAAAGTCCAAACATTAAAGCGACGAGCGATTTCTGATCCGAGGGCAGTANTNAAGAGATGGAGGAGAAAAGGGCT :  174
w15_d13_sr  : AGAGGAGGAAGTCATCCATATAAGTAAAAGTCCAAACATTAAAGCGACGAGCGATTTCTGATCCGAGGGCAGTAGTGAAGAGATGGAGGAGAAAAGGGCT :  173
w15_d18_sr  : AGAGGAGGAAGTCATCCATATAAGTAAAAGTCCAAACATTAAAGCGACGAGCGATTTNTGATCCGAGGGCAGTAGTGAAGAGATGGAGGAGAAAAGGGCT :  174
w15_liv_sr  : AGAGGAGGAAGTCATCCATATAAGTAAAAGTCCAAACATTNTAGCGACGAGCGATTTCTGATCCGAGGGCAGTAGTGAAGAGATGGAGGAGAAAAGGGCT :  174
b26_d15_sr  : AGAGGAGGAAGTCATCCATATAAGTAAAAGTCCAAACATTAAAGCGACGAGCGATTTCTGATCCGAGGGCAGTAGTGAAGAGATGGAGGAGAAAAGGGCT :  173
b26_d25_sr  : AGAGGAGGAAGTCATCCATATAAGTAAAAGTCCAAACATTAAAGCGACGAGCGATTTNTGATCCGAGGGCAGTAGTGAAGAGATGGAGGAGAAAAGGGCT :  173
b26_d27_sr  : AGAGGAGGAAGTCATCCATATAAGTAAAAGTCCAAACATTAAAGCGACGAGCGATTTCTGATCCGAGGGCAGTAGTGAAGAGATGGAGGAGAAAAGGGCT :  174
b26_d36_sr  : NGAGGAGGAAGTCATCCATATAAGTNAAAGTCCAAACATTAAAGCGACGAGCGATTTTTGATNCGAGGGCAGTCNTGAAGAGATGGAGGAGAAAAGGGCT :  171
b26_liv_sr  : AGAGGAGGAAGTCATCCATATAAGTAAAAGTCCAAACATTAAAGCGACGAGCGATTTCTGATCCGAGGGCAGTAGTGAAGAGATGGAGGAGAAAAGGGCT :  176
b35_d15_sr  : AGAGGAGGAAGTCATCCATATAAGTAAAAGTCCAAACATTAAAGCGACGAGCGATTTCTGATCCGAGGGCAGTAGTGAAGAGATGGAGGAGAAAAGGGCT :  172
b35_d25_sr  : AGAGGAGGAAGTCATCCATATAAGTAAAAGTCCAAACATTAAAGCGACGAGCGATTTCTGATCCGAGGGCAGTANTGAAGAGATGGAGGAGAAAAGGGCT :  173
b35_d27_sr  : AGAGGAGGAAGTCATCCATATAAGTAAAAGTCCAAACATTAAAGCGACGAGCGATTTCTGATCCGAGGGCAGTANTNNAGAGATGGAGGAGAAAAGGGCT :  172
b35_d36_sr  : AGAGGAGGAAGTCATCCATATAAGTAAAAGTCCAAACATTAAAGCGACGAGCGATTTCTGATCCGAGGGCAGTAGTGAAGAGATGGAGGAGAAAAGGGCT :  171
b35_liv_sr  : AGAGGAGGAAGTCATCCATATAAGTAAAAGTCCAAACATTAAAGCGACGAGCGATTTCTGATCCGAGGGCAGTAGTGAAGAGATGGAGGAGAAAAGGGCT :  157
b37_d36_sr  : AGAGGAGGAAGTCATCCATATAAGTAAAAGTCCAAACATTAAAGCGACGAGCGATTTCTGATCCGNGGGCAGTNNTGAAGAGNTGGAGGAGAAAAGGGCT :  174
b37_liv_sr  : NNAGGAGGANGTCATCCATATAAGTNANAGTCCAAACNTTAAANNGACGAGCGANNNNTGANNCGAGGGCAGNNNTGAAGAGATGGAGGANAAAAGGGCT :  173
              aGAGGAGGAAGTcAtCCATATAaGTaAAAGTCCAAaCATTaaagcGACGAGCGAtttcTGATCCGAGGGCAGTaGTGaAGAgAtGGAGGAgAAAaGGGCT
```

continued - *Multiple Sequence Alignment of the reverse Surface region.*

adhbv: Australian Duck Hepatitis B Virus (GenBank DHV6350, AJ006350); inoculum: starting inoculum; all others ducknumber_dayofsample.

```
                    *      1420          *      1440          *      1460          *      1480          *      1500
adhbv_r     : GAGACCGACTCCCATTGGAGCTTTCCTAAAATAATAGACATGTTGTCCGTCAGATACAGCAAGCCTGCTGTTACTAGCAGGATTAAGAGGAAGATGATAA : 1500
inoculumsr  : GAGACCGACTCCCATTGGAGCTTTCCTAAAATAATAGACATGTTGTCCGTCAGATACAGCAAGCCTGCTGCTACTAGCAGGATTAAGAGGAAGATGATAA :  274
p13_d06_sr  : GAGACCGACTCCCATTGGAGCTTTCCTAAAATAATAGACATGTTGTCCGTCAGATACAGCAAGCCTGCTGCTACTAGCAGGATTAAGAGGAAGATGATAA :  274
p13_d11_sr  : GAGACCGACTCCCATTGGAGCTTTCCTAAAATAATAGACATGTTGTCCGTCAGATACAGCAAGCCTGCTGCTACTAGCAGGATTAAGAGGAAGATGATAA :  274
p13_d27_sr  : GAGACCGACTCCCATTGGAGCTTTCCTAAAATAATAGACATGTTGTCCGTCAGATACAGCAAGCCTGCTGCTACTAGCAGGATTAAGAGGAAGATGATAA :  269
p13_d43_sr  : GAGACCGACTCCCATTGGAGCTTTCCTAAAATAATAGACATGTTGTCCGTCAGATACAGCAAGCCTGCTGCTACTAGCAGGATTAAGAGGAAGATGATAA :  271
p13_liv_sr  : GAGACCGACTCCCATTGGAGCTTTCCTAAAATAATAGACATGTTGTCCGTCAGATACAGCAAGCCTGCTGCTACTAGCAGGATTAAGAGGAAGATGATAA :  264
p14_d06_sr  : GNGACCGNCCCCCANAGGAGCNNTTCNAAAANAATCCACATGTTGTCTNANAGATACAGCAAGCCNGGTGCTACTAGCAGGATTAAGAGGAAGATGATAA :  266
p14_d11_sr  : GAGACCGACTCCCATTGGAGCTTTCCTAAAATAATAGACATGTTGTCCGTCAGATACAGCAAGCCTGCTGCTACTAGCAGGATTAAGAGGAAGATGATAA :  272
p14_d27_sr  : GAGACCGACTCCCATTGGAGCTTTCCTAAAATAATAGACATGTTGTCCGTCAGATACAGCAAGCCTGCTGCTACTAGCAGGATTAAGAGGAAGATGATAA :  274
p14_d43_sr  : GAGACCGACTCCCATTGGAGCTTTCCTAAAATAATAGACATGTTGTCCGTCAGATACAGCAAGCCTGCTGCTACTAGCAGGATTAAGAGGAAGATGATAA :  274
p14_liv_sr  : GAGACCGACTCCCATTGGAGCTTTCCTAAAATAATAGACATGTTGTCCGTCAGATACAGCAAGCCTGCTGCTACTAGCAGGATTAAGAGGAAGATGATAA :  263
w13_d20_sr  : GAGACCGACTCCCATTGGAGCTTTCCTAAAATAATAGACATGTTGTCCGTCAGATACAGCAAGCCTGCTGCTACTAGCAGGATTAAGAGGAAGATGATAA :  274
w13_d29_sr  : GAGACCGACTCCCATTGGAGCTTTCCTAAAATAATAGACATGTTGTCCGTCAGATACAGCAAGCCTGCTGCTACTAGCAGGATTAAGAGGAAGATGATAA :  274
w13_d34_sr  : GAGACCGACTCCCATTGGAGCTTTCCTAAAATAATAGACATGTTGTCCGTCAGATACAGCAAGCCTGCTGCTACTAGCAGGATTAAGAGGAAGATGATAA :  274
w13_d39_sr  : GAGACCGACTCCCATTGGAGCTTTCCTAAAATAATAGACATGTTGTCCGTCAGATACAGCAAGCCTGCTGCTACTAGCAGGATTAAGAGGAAGATGATAA :  274
w13_d41_sr  : GAGACCGACTCCCATTGGAGCTTTCCTAAAATAATAGACATGTTGTCCGTCAGATACAGCAAGCCTGCTGCTACTAGCAGGATTAAGAGGAAGATGATAA :  273
w13_liv_sr  : GAGACCGACTCCCATTGGAGCTTTCCTAAAATAATAGACATGTTGTCCGTCAGATACAGCAAGCCTGCTGCTACTAGCAGGATTAAGAGGAAGATGATAA :  274
w15_d13_sr  : GAGACCGACTCCCATTGGAGCTTTCCTAAAATAATAGACATGTTGTCCGTCAGATACAGCAAGCCTGCTGCTACTAGCAGGATTAAGAGGAAGATGATAA :  273
w15_d18_sr  : GAGACCGACTCCCATTGGAGCTTTCCTAAAATAATAGACATGTTGTCCGTCAGATACAGCAAGCCTGCTGCTACTAGCAGGATTAAGAGGAAGATGATAA :  274
w15_liv_sr  : GAGACCGACTCCCATTGGAGCTTTCCTAAAATAATAGACATGTTGTCCGTCAGATACAGCAAGCCTGCTGCTACTAGCAGGATTAAGAGGAAGATGATAA :  274
b26_d15_sr  : GAGACCGACTCCCATTGGAGCTTTCCTAAAATAATAGACATGTTGTCCGTCAGATACAGCAAGCCTGCTGCTACTAGCAGGATTAAGAGGAAGATGATAA :  273
b26_d25_sr  : GAGACCGACTCCCATTGGAGCTTTCCTAAAATAATAGACATGTTGTCCGTCAGATACAGCAAGCCTGCTGCTACTAGCAGGATTAAGAGGAAGATGATAA :  273
b26_d27_sr  : GAGACCGACTCCCATTGGAGCTTTCCTAAAATAATAGACATGTTGTCCGTCAGATACAGCAAGCCTGCTGCTACTAGCAGGATTAAGAGGAAGATGATAA :  274
b26_d36_sr  : GANACCGACTCCCATTGGAGCTTTCCTAAAATAATAGACATGTTGTCCGTCAGATACAGCAAGCCTGCTGCTACTAGCAGGATTAAGAGGAAGATGATAA :  271
b26_liv_sr  : GAGACCGACTCCCATTGGAGCTTTCCTAAAATAATAGACATGTTGTCCGTCAGATACAGCAAGCCTGCTGCTACTAGCAGGATTAAGAGGAAGATGATAA :  276
b35_d15_sr  : GAGACCGACTCCCATTGGAGCTTTCCTAAAATAATAGACATGTTGTCCGTCAGATACAGCAAGCCTGCTGCTACTAGCAGGATTAAGAGGAAGATGATAA :  272
b35_d25_sr  : GAGACCGACTCCCATTGGAGCTTTCCTAAAATAATAGACATGTTGTCCGTCAGATACAGCAAGCCTGCTGCTACTAGCAGGATTAAGAGGAAGATGATAA :  273
b35_d27_sr  : GAGACCGACTCCCATTGGAGCTTTCCTAAAATAATAGACATGTTGTCCGTCAGATACAGCAAGCCTGCTGCTACTAGCAGGATTAAGAGGAAGATGATAA :  272
b35_d36_sr  : GAGACCGACTCCCATTGGAGCTTTCCTAAAATAATAGACATGTTGTCCGTCAGATACAGCAAGCCTGCTGCTACTAGCAGGATTAAGAGGAAGATGATAA :  271
b35_liv_sr  : GAGACCGACTCCCATTGGAGCTTTCCTAAAATAATAGACATGTTGTCCGTCAGATACAGCAAGCCTGCTGCTACTAGCAGGATTAAGAGGAAGATGATAA :  257
b37_d36_sr  : GAGACCGACTCCCATTGGAGCTTTCCTAAAATAATAGACATGTTGTCCGTCAGATACAGNAAGCCTGCTGCTACTAGCAGGATTAAGAGGAAGATGATNA :  274
b37_liv_sr  : GANACCGACTCCCATTGGAGCTTTNCTAAAATAATAGACATGTTGTCCGTCAGATACAGCAAGCCTGCTGCTACTAGCAGGATTAAGAGGAAGATGATAA :  273
              GAGACCGACtCCCATtGGAGCTTTcCTAAAATAATagACATGTTGTCcGtCAGATACAGCAAGCCTGcTGcTACTAGCAGGATTAAGAGGAAGATGATAA
```

continued - *Multiple Sequence Alignment of the reverse Surface region.*

adhbv: Australian Duck Hepatitis B Virus (GenBank DHV6350, AJ006350); inoculum: starting inoculum; all others ducknumber_dayofsample.

```
                    *      1520         *      1540         *      1560         *      1580         *      1600
adhbv_r     : AAAGCCTGAGATAGGTCCAGAGAAATCCTGGGCATCCCCACGGGCAGAATCCTGCGTAGTGTGGAGAGATTTGGGCTCCAGTATCTTGGAAAGCGCATTG : 1600
inoculumsr  : AAAGCCTGAGATAGGTCCAGAGAAATCCTGGGCATCCCCACGGGCAGAATCCTGCGTAGTGTGGAGAGATTTGGGCTCCAGTATCTTGGAAAGCGCATTG :  374
p13_d06_sr  : AAAGCCTGAGATAGGTCCAGAGAAATCCTGGGCATCCCCACGGGCAGAATCCTGCGTAGTGTGGAGAGATTTGGGCTCCAGTATCTTGGAAAGCGCATTG :  374
p13_d11_sr  : AAAGCCTGAGATAGGTCCAGAGAAATCCTGGGCATCCCCACGGGCAGAATCCTGCGTAGTGTGGAGAGATTTGGGCTCCAGTATCTTGGAAAGCGCATTG :  374
p13_d27_sr  : AAAGCCTGAGATAGGTCCAGAGAAATCCTGGGCATCCCCACGGGCAGAATCCTGCGTAGTGTGGAGAGATTTGGGCTCCAGTATCTTGGAAAGCGCATTG :  369
p13_d43_sr  : AAAGCCTGAGATAGGTCCAGAGAAATCCTGGGCATCCCCACGGGCAGAATCCTGCGTAGTGTGGAGAGATTTGGGCTCCAGTATCTTGGAAAGCGCATTG :  371
p13_liv_sr  : AAAGCCTGAGATAGGTCCAGAGAAATNCTGGGCATCCCCACGGGCAGAATCCTGCGTAGTGTGGAGAGATTTGGGCTC-AGTATCTTGGAAAGCGCATTG :  363
p14_d06_sr  : AAAGCCTGAGATAGG-CCAGAGAAATCCTGGGCATCCCCACGGGCAGAATCCTGCGTAGCGTGGAGAGATTTGGGCTCCAGTATCTTGGAAAGCGCATTG :  365
p14_d11_sr  : AAAGCCTGAGATAGGTCCAGAGAAATCCTGGGCATTCCCACGGGCAGAATCCTGCGTAGTGTGGAGAGATTTGGGCTCCAGTATCTTGGAAAGCGCATTG :  372
p14_d27_sr  : AAAGCCTGAGATAGGTCCAGAGAAATCCTGGGCATCCCCACGGGCAGAATCCTGCGTAGTGTGGAGAGATTTGGGCTCCAGTATCTTGGAAAGCGCATTG :  374
p14_d43_sr  : AAAGCCTGAGATAGGTCCAGAGAAATCCTGGGCATCCCCACGGGCAGAATCCTGCGTAGTGTGGAGAGATTTGGGCTCCAGTATCTTGGAAAGCGCATTG :  374
p14_liv_sr  : AAAGCCTGAGATAGGTCCAGAGAAATCCTGGGCATCCCCACGGGCAGAATCCTGCGTAGTGTGGAGAGATTTGGGCTCCAGTATCTTGGAAAGCGCATTG :  363
w13_d20_sr  : AAAGCCTGAGATAGGTCCAGAGAAATCCTGGGCATCCCCACGGGCAGAATCCTGCGTAGTGTGGAGAGATTTGGGCTCCAGTATCTTGGAAAGCGCATTG :  374
w13_d29_sr  : AAAGCCTGAGATAGGTCCAGAGAAATCCTGGGCATCCCCACGGGCAGAATCCTGCGTAGTGTGGAGAGATTTGGGCTCCAGTATCTTGGAAAGCGCATTG :  374
w13_d34_sr  : AAAGCCTGAGATAGGTCCAGAGAAATCCTGGGCATCCCCACGGGCAGAATCCTGCGTAGTGTGGAGAGATTTGGGCTCCAGTATCTTGGAAAGCGCATTG :  374
w13_d39_sr  : AAAGCCTGAGATAGGTCCAGAGAAATCCTGGGCATCCCCACGGGCAGAATCCTGCGTAGTGTGGAGAGATTTGGGCTCCAGTATCTTGGAAAGCGCATTG :  374
w13_d41_sr  : AAAGCCTGAGATAGGTCCAGAGAAATCCTGGGCATCCCCACGGGCAGAATCCTGCGTAGTGTGGAGAGATTTGGGCTCCAGTATCTTGGAAAGCGCATTG :  373
w13_liv_sr  : AAAGCCTGAGATAGGTCCAGAGAAATCCTGGGCATCCCCACGGGCAGAATNCTGCGTAGTGTGGAGAGATTTGGGCTCCAGTATCTTGGAAAGCGCATTG :  374
w15_d13_sr  : AAAGCCTGAGATAGGTCCAGAGAAATCCTGGGCATCCCCACGGGCAGAATCCTGCGTAGTGTGGAGAGATTTGGGCTCCAGTATCTTGGAAAGCGCATTG :  373
w15_d18_sr  : AAAGCCTGAGATAGGTCCAGAGAAATCCTGGGCATCCCCACGGGCAGAATCCTGCGTAGTGTGGAGAGATTTGGGCTCCAGTATCTTGGAAAGCGCATTG :  374
w15_liv_sr  : AAAGCCTGAGATAGGTCCAGAGAAATCCTGGGCATCCCCACGGGCAGAATCCTGCGTAGTGTGGAGAGATTTGGGCTCCAGTATCTTGGAAAGCGCATTG :  374
b26_d15_sr  : AAAGCCTGAGATAGGTCCAGAGAAATCCTGGGCATCCCCACGGGCAGAATCCTGCGTAGTGTGGAGAGATTTGGGCTCCAGTATCTTGGAAAGCGCATTG :  373
b26_d25_sr  : AAAGCNTGAGATAGGTCCAGAGAAATCNTGG-CATCCCCACGGGCAGAATCCTGCTTAGNGNGGANAGATTTGGN-TCCAGTNTNTTGGAAAGCGCATTG :  371
b26_d27_sr  : AAAGCCTGAGATAGGTCCAGAGAAATCCTGGGCATCCCCACGGGCAGAATCCTGCGTAGTGTGGAGAGATTTGGGCTCCAGTATCTTGGAAAGCGCATTG :  374
b26_d36_sr  : AAAGCCTGAGATAGGTCCAGAGAAATCCTGGGCATCCCCACGGGCAGAATCCTGCGTAGTGTGGAGAGATTTGGGCTCCAGTATCTTGGAAAGCGCATTG :  371
b26_liv_sr  : AAAGCCTGAGATAGGTCCAGAGAAATCCTGGGCATCCCCACGGGCAGAATCCTGCGTAGTGTGGAGAGATTTGGGCTCCAGTATCTTGGAAAGCGCATTG :  376
b35_d15_sr  : AAAGCCTGAGATAGGTCCAGAGAAATCCTGGGCATCCCCACGGGCAGAATCCTGCGTAGTGTGGAGAGATTTGGGCTCCAGTATCTTGGAAAGCGCATTG :  372
b35_d25_sr  : AAAGCCTGAGATAGGTCCAGAGAAATCCTGGGCATCCCCACGGGCAGAATCCTGCGTAGTGTGGAGAGATTTGGGCTCCAGTATCTTGGAAAGCGCATTG :  373
b35_d27_sr  : AAAGCCTGAGATAGGTCCAGAGAAATCCTGGGCATCCCCACGGGCAGAATCCTGCNTAGTGTGGAGAGATTTGGGCTCCAGTATCTTGGAAAGCGCATTG :  372
b35_d36_sr  : AAAGCCTGAGATAGGTCCAGAGAAATCCTGGGCATCCCCACGGGCAGAATCCTGCGTAGTGTGGAGAGATTTGGGCTCCAGTATCTTGGAAAGCGCATTG :  371
b35_liv_sr  : AAAGCCTGAGATAGGTCCAGAGAAATNCTGGGCATCCCCACGGGCANAATCCTGCGTAGTGTGGAGAGATTTGGGCTCCAGTATCTTGGAAAGCGCATTG :  357
b37_d36_sr  : AAAGCCTGAGATAGGTCCAGAGAAATCCTGGGCATCCCCACGGGCAGAATCCTGCGTAGTGTGGAGAGATTTGGGCTCCAGTATCTTGGAAAGCGCATTG :  374
b37_liv_sr  : AAAGCCTGAGATAGGTCCAGAGAAATCCTGGGCATCCCCACGGGCAGAATCCTGCGTAGTGTGGAGAGATTTGGGCTCCAGTATCTTGGAAAGCGCATTG :  373
              AAAGCCTGAGATAGGtCCAGAGAAATCCTGGgCATcCCCACGGGCAGAATCCTGCgTAGtGTGGAGAGATTTGGGcTCcAGTATCTTGGAAAGCGCATTG
```

continued - *Multiple Sequence Alignment of the reverse Surface region.*

adhbv: Australian Duck Hepatitis B Virus (GenBank DHV6350, AJ006350); inoculum: starting inoculum; all others ducknumber_dayofsample.

```
                    *      1620          *      1640          *      1660          *      1680          *      1700
adhbv_r     : CATTTTTCCCTTTGGAGAACTGAGAGAAATCCACCACCAATCTAGCCTCCGTAGTATTTCGAGAATTTTTATCAACAAGAAAAAGCCTACCAGTAATCC- : 1699
inoculumsr  : CATTTTTCCCTTTGGAGAACTGAGAGAAATCCACCACCAATCTAGCCTCCGTAGTATTTCGAGAATTTTTATCAACAAGAAAAAGCCTACCAGTAATCC- :  473
p13_d06_sr  : CATTTTTCCCTTTGGAGAACTGAGAGAAATCCACCACCAATCTAGCCTCCGTAGTATTTCGAGAATTTTTATCAACAAGAAAAAGCCTACCAGTAATCC- :  473
p13_d11_sr  : CATTTTTCCCTTTGGAGAACTGAGAGAAATCCACCACCAATCTAGCCTCCGTAGTATTTCGAGAATTTTTATCAACAAGAAAAAGCCTACCAGTAATCC- :  473
p13_d27_sr  : CATTTTTCCCTTTGGAGAACTGAGAGAAATCCACCACCAATCTAGCCTCCGTAGTATTTCGAGAATTTTTATCAACAAGAAAAAGCCTACCAGTAATCC- :  468
p13_d43_sr  : CATTTTTCCCTTTGGAGAACTGAGAGAAATCCACCACCAATCTAGCCTCCGTAGTATTTCGAGAATTTTTATCAACAAGAAAAAGCCTACCAGTAATCC- :  470
p13_liv_sr  : CATTTTTCC-TTTGGAGAACTGAGAGAAATCCCCACCAAATCTAGCCCCCGTAGTATTTCGAGAATTTTTATCAACAAGAAAAAGCCTACCAGTAATNCG :  462
p14_d06_sr  : GATTTTTCCCTTTGGAGAACTGNNAGAAATNCACCACCAATCTAGCCTCCGTAGTATTTCCAGAATTTTTATCAACAAGAAAAAGCCTACCNGTAATCC- :  464
p14_d11_sr  : CATTTTTCCTTTTGGAGAACTGAGAGAAATNCACCACCAATCTAGCCTCCGTAGTTTTTCGAGAATTTTTATCAACAAGAAAAAGCCTACCNGTAATCCC :  472
p14_d27_sr  : CATTTTTTCCTTTGGAGAACTGAGAGAAATCCACCACCAATCTAGCCTCCGTAGTATTTCGAGAATTTTTATCAACAAGAAAAAGCCTACCAGTAATCC- :  473
p14_d43_sr  : CATTTTTCCCTTTGGAGAACTGAGAGAAATCCACCACCAATCTAGCCTCCGTAGTATTTCGAGAATTTTTATCAACAAGAAAAAGCCTACCAGTAATCC- :  473
p14_liv_sr  : CATTTTTCCCTTTGGAGAACTGAGAGAAATCCACCACCAATCTAGCCTCCGTAGTATTTCGAGAATTTTTATCAACAAGAAAAAGCCTACCAGTAATCCG :  463
w13_d20_sr  : CATTTTTCCCTTTGGAGAACTGAGAGAAATCCACCACCAATCTAGCCTCCGTAGTATTTCGAGAATTTTTATCAACAAGAAAAAGCCTACCAGTAATCC- :  473
w13_d29_sr  : CATTTTTCCCTTTGGAGAACTGAGAGAAATCCACCACCAATCTAGCCTCCGTAGTATTTCGAGAATTTTTATCAACAAGAAAAAGCCTACCAGTAATCCC :  474
w13_d34_sr  : CATTTTTCCCTTTGGAGAACTGAGAGAAATCCACCACCAATCTAGCCTCCGTAGTATTTCGAGAATTTTTATCAACAAGAAAAAGCCTACCAGTAATCCC :  474
w13_d39_sr  : CATTTTTCCCTTTGGAGAACTGAGAGAAATCCACCACCAATCTAGCCTCCGTAGTATTTCGAGAATTTTTATCAACAAGAAAAAGCCTACCAGTAATNCG :  474
w13_d41_sr  : CATTTTTCCCTTTGGAGAACTGAGAGAAATCCACCACCAATCTAGCCTCCGTAGTATTTCGAGAATTTTTATCAACAAGAAAAAGCCTACCAGTAATCC- :  472
w13_liv_sr  : CATTTTTCC-TTTGGAGAACTGAGAGAAAT-CACCACCAATCTAGCCTCCNTAGTNTTTNGAGAATTTTTATNAACAAGAAAAAGCCTNCCAGTTATTCG :  472
w15_d13_sr  : CATTTTTCCCTTTGGAGAACTGAGAGAAATCCACCACCAATCTAGCCTTCGTAGTATTTCGAGAATTTTTATNAACAAGAAAAAGCCTACCAGTAATCCC :  473
w15_d18_sr  : CATTTTTCCCTTTGGAGAACTGAGAGAAATCCACCACCAATCTAGCCTCCGTAGTATTTCGAGAATTTTTATCAACAAGAAAAAGCCTACCAGTAATCC- :  473
w15_liv_sr  : CATTTTTCCCTTTGGAGAACTGAGAGAAATCCACCACCAATCTAGCCTCCGTAGTATTTCGAGAATTTTTATCAACAAGAAAAAGCCTACCAGTAATCC- :  473
b26_d15_sr  : CATTTTTCCCTTTGGAGAACTGAGAGAAATCCACCACCAATCTAGCCTCCGTAGTATTTCGAGAATTTTTATCAACAAGAAAAAGCCTACCAGTAATCC- :  472
b26_d25_sr  : CATTTTTCCCTTTGGAGAACTGAGAGAAATCCACC-CCAATCTAGCCTCCGTAGTATTTCGAGAATTTTTATNAACAAGAAAAAGCCTCCCAGTAATCC- :  469
b26_d27_sr  : CATTTTTCCCTTTGGAGAACTGAGAGAAATNCACCACCAATCTAGCCTTCGTAGTATTTCGAGAATTTTTATCAACAAGAAAA-GCCTACCANTAAT-CC :  472
b26_d36_sr  : CATTTTTCCCTTTGGAGAACTGAGAGAAATTCACCACCAATCTAGCCTCCGTAGTATTTCGAGAATTTTTATCAACAAGAAAAAGCTA-CCAGTAATCCG :  470
b26_liv_sr  : CATTTTTCCCTTTGGAGAACTGAGAGAAATCCACCACCAATCTAGCCTCCGTAGTATTTCGAGAATTTTTATCAACAAGAAAAAGCCTACCAGTAATNCC :  476
b35_d15_sr  : CATTTTTCCCTTTGGAGAACTGAGAGAAATCCACCACCAATCTAGCCTCCGTAGTATTTCGAGAATTTTTATCAACAAGAAAAAGCCTACCAGTAATCC- :  471
b35_d25_sr  : CATTTTTCCCTTTGGAGAACTGAGAGAAATCCACCACCAATCTAGCCTCCGTAGTATTTCGAGAATTTTTATCAACAAGAAAAAGCCTACCAGTAATCC- :  472
b35_d27_sr  : CATTTTTCCCTTTGGAGAACTGAGAGAAATCCACCACCAATCTAGCCTCCGTAGTATTTCGAGAATTTTTATCAACAAGAAAAAGCCTACCAGTAATNCC :  472
b35_d36_sr  : CATTTTTCCCTTTGGAGAACTGAGAGAAATCCACCACCAATCTAGCCTCCGTAGTATTTCGAGAATTTTTATCAACAAGAAAAAGCCTCCCAGTAATNC- :  470
b35_liv_sr  : NATTTTTCCCTTTGGAGAACTGAGAGAAATNCNCCACCAATCTAGCCTCCGNAGGATTTCGAGAATTTTTATTCACAAGAAAAAGCCTCCCAGTAATNCC :  457
b37_d36_sr  : CATTTTTCCCTTTGGAGAACTGAGAGAAATCCACCACCAATCTAGCCTCCGTAGTATTTCGAGAATTTTTATCAACAAGAAAAAGCCTACCAGTAATCC- :  473
b37_liv_sr  : CATTTTTCCCTTTGGAGAACTGAGAGAAATCCACCACCAATCTAGCCTCCGTAGTATTTCGAGAATTTTTATCAACAAGAAAAAGCCTACCAGTAATCC- :  472
              cATTTTTcCcTTTGGAGAACTGAGAGAAATcCaCcaCcAATCTAGCCtcCGTAGtaTTTCgAGAATTTTTATcaACAAGAAAAaGCctaCCAGTaATcC
```

continued - *Multiple Sequence Alignment of the reverse Surface region.*

adhbv: Australian Duck Hepatitis B Virus (GenBank DHV6350, AJ006350); inoculum: starting inoculum; all others ducknumber_dayofsample.

```
                    *      1720          *      1740          *      1760          *      1780          *      1800
adhbv_r     : GATTAGGCC-AGCTA-GTATTCCCCC-GAAGGTACCAGCCA-TTTTCTTCTTCTTGAGGGG-AGGA-GTCTTT-ATCA-CAGGCAC-GGCC-GGCT-CCG : 1788
inoculumsr  : GATTAGGCC-AGCTA-GTATTCCCCC-GAAGGTACCAGCCA-TTTTCTTCTTCTTGAGGGG-AGTA-GTCTTT-ATCA-CAGGCAC-GGCC-GGCT-CCG :  562
p13_d06_sr  : GATTAGGCC-AGCTA-GTATTCCCCC-GAAGGT-CCAGCCA-TTTTCTTCTTCTTGAGGGG-AGTA-GTCTTT-AT-A-CAGGCACCGGCC-GGCTTC-G :  561
p13_d11_sr  : GATTAGGCC-AGCTA-GTATTCCCCC-GAAGGT-CCAGCCA-TTTTCTTCTTCTTGAGGGG-AGTA-GTCTTT-ATCA-CAGGCACCGGCC-GGCTTCCG :  563
p13_d27_sr  : GATTAGGCC-AGCTA-GTATTCCCCC-GAAGGTACCAGCCA-TTTTCTTCTTCTTGAGGGG-AGTA-GTCTTT-ATCA-CAGGCACCGGCC-GGCT-CCG :  558
p13_d43_sr  : GATTAGGCC-AGCTA-GTATTCCCCC-GAAGGTACCAGCCA-TTTTCTTCTTCTTGAGGGG-AGTA-GTCTTT-ATCA-CAGGC-CCGGCC-GGCTTCCG :  560
p13_liv_sr  : -ATTAGGCC-AGCTTAGTATTTCCCCCGAAGGNACCAGCCCTTTTNTTNTTTNTTGAGGGGGAGNAAGTCTTTTATTACAAGGCCCNGGCC-GGNTTCCG :  559
p14_d06_sr  : GATTAGGCC-AGCTN-GGATTTCCCCCGAAGGNACCAGCCA-TTTTCTTCTTCTTGGGGGG-AGNA-GTCTTT-ATCA-CAGGCCCGGNCCGGCNTCCCG :  557
p14_d11_sr  : GANTAAGGCCAGCTAAGTANTTCCCCCGAANGNACCAGCCATTTTNTTTTTTTTTTGANGGGGAGNA-GTCTTT-ATTACAAGGCACCGGCCGGNTTCCGG :  570
p14_d27_sr  : GATTAGGCC-AGCTA-GTATTTCCCCCGAANGTACCAGCCA-TTTTNTTNTTNTTGAGGGGGAGTA-GTCTTT-ATTA-CAGGCCCCGGNCGGNTTNCGG :  567
p14_d43_sr  : CATTAGGCC-AGCTA-GTATTTCCCC-GAANGT-CCAGCCA-TTTTNTTNTTTNTTGGGGGGAGTA-GTCTTT--ATA-CAGGCCC--GGCCGGNTCCGG :  562
p14_liv_sr  : -ATTAGGCC-AGCTA-GTATTTCCCCCGAAGGTACCAGCCATTTTTTNTTTNTTTGAGGGG-AGTAAGTNTTTTATCACAAGGCCNCGGGCGGCTTTCGG :  559
w13_d20_sr  : GATTAGGCC-AGCTA-GTATTTCCCC-GAAGGT-CCAGCCA-TTTTNTTNTTNTTGAGGGG-AGTA-GTCTTT-ATCA-CAAGCACCGGNC-GGNTTC-N :  562
w13_d29_sr  : -ATTAGGCC-AGCTA-GTATT--TCCCGAAGGTACCAGCCA-TTTTNTTNTTNTTGAGGGG-AGTA-GTCTTT-ATCA-CAGGCACCGGCC-GGCTTCGG :  563
w13_d34_sr  : GATTAGGCC-AGCTA-GTATTTCCCC-GAAGGT-CCAGCCA-TTTTCTTNTTNTTGAGGGG-AGGA-GTCTTT-ATNA-CAAGCAC-GGGC-GGCTTC-G :  562
w13_d39_sr  : -ATTAGGGC-AGCTA-GTATT--CNCCGAAGGNACCAGCCA-TTTTNTTNTTTTTGAGGGG-AGNA-GTCTTT-ATTA-CAGGCACCGGCC-GGNTTCGG :  563
w13_d41_sr  : GANTAGGCC-AGCTA-GTATTTCCCC-GAAGGT-CCAGCCA-TTTTNTTNTTNTTTAGGGG-AGTA-GTCTTT-ATTA-CAAGCAC-NGGC-GGCTTC-G :  560
w13_liv_sr  : -ATTAGGCC-AGCTA-GTNTT--CCCCGAAGGTNCCAGCCA-TTTT-NTTTTTTTGAGGGG-AGTN-GTNTTTTATNACAAG-CACCGGCC-GGCTTCGG :  561
w15_d13_sr  : GATTAGGCC-AGCTA-GTATTTCCCC-GAAGGTACCAGCCA-TTTTNTTNTTTTTTGAGGGGGAGTA-GTCTTTTATNA-CAAGCCCCGGCCGGGTTCCGG :  567
w15_d18_sr  : GATTAGGCC-AGCTA-GTATTCCCCC-GAAGGTACCAGCCA-TTTTCTTNTTNTTGAGGGG-AGTA-GTCTTT-ATCA-CAGGCACCGGCC-GGCT-CCG :  563
w15_liv_sr  : GATTAGGCC-AGCTA-GTATTCCCCC-GAAGGT-CCAGCCA-TTTTCTTCTTCTTGAGGGG-AGTAGGTCTTT-ATCA-CAGGCAC-GGCC-GGCT-CCG :  562
b26_d15_sr  : GATTAGGCC-AGCTA-GTATTCCCCC-GAAGGT-CCAGCCA-TTTTCTTNTTNTTGAGGGG-AGTA-GTCTTT-ATNA-CAGGCAC-NGNC-GGCTTCCG :  561
b26_d25_sr  : GATTAGGCC-AGCTA-GTATTCCCCC-GAAGGTCCCAGCCA-TTTTNTTTTTTTTGAGGGG-AGNA-GTCTTT-ATTA-CAGGCCC-GGNCGGCTTNCGG :  560
b26_d27_sr  : GATTA-GGCCAGCTA-GTATTT-CCCCGAAGGTACCAGCCATTTTCTTNTTNTTTGAGGGG-AGNA-GTCTTT-ATTA-CAGGCACCGGCC-GGCTTCCG :  564
b26_d36_sr  : -ATTANGCC-AGCTT-GTATTTCCCCC--AANGTCCAGCCATTTTNTTTTTTTTTGAGGGGGNGGA-GGCTTT-ATTACAAGGCCCGGGCG-GGTTTCCG :  562
b26_liv_sr  : -ATTAGGCC-AGCTA-GTATTT-CCCCGAAGGTCCCAGCCATTTTCTTNTTTTTTGAGGGGAGGTA-GTCTTT-ATTACAAG-CACCGGC--GGCTNCCN :  567
b35_d15_sr  : GATTAGGCC-AGCTA-GTATTCCCCC-GAAGGTACCAGCCA-TTTTCTTCTTCTTGAGGGG-AGTA-GTCTTT-ATCA-CANGCACCGGCC-GGCTTCCG :  562
b35_d25_sr  : GATTAGGCC-AGCTA-GTATTCCCCC-GAAGGTACCAGCCA-TTTTCTTCTTCTTGAGGGG-AGTA-GTCTTT-ATCA-CAGGCACCGGCC-GGCT-TCG :  562
b35_d27_sr  : GATTAAGGCCAGNTA-GTATT--CCCCGAAGGT-CCAGCCATTTTCTTCTTNTT-GAGGGG-AGTA-GTCTTT-ATCA-CAGGCACCGGCC-GGCTTCCG :  562
b35_d36_sr  : GATTAGGCC-AGCTA-GTATTTCCCC-GAANGN-ACCACCA-TTTTCTTNTTNTTGANGGG-AGTA-GTCTTT-ATTA-CANGCAC-GGCC-GGCTCC-G :  558
b35_liv_sr  : -ATTANGCCCAGCTAAGTATTTCCCCCGAANGGACCAGNCATTTTNTTTTTTTTTGAGGGGGAGNA-GNCTTTTATTNCAAGGCACGGGCCCGGNTCCCG :  555
b37_d36_sr  : GATTAGGCC-AGCTA-GTATTTCCCC-GAAGGT-CCAGCCA-TTTTCTTCTTCTTGAGGGG-AGTA-GTCTTT-ATCA-CAGGCACGGGCC-GGCTCCCG :  563
b37_liv_sr  : GATTAGGCC-AGCTA-GTATTTCCCC-GAAGGTACCAGCCA-TTTTCTTCTTCTTGAGGGG-AGTA-GTCTTT-ATCA-CAGGCACCGGCC-GGCT-CCG :  562
               ATTAgGcC AGCTa GtATT ccCC gaAgGt cCagCCa TTTt Tt TT TtgaGGGG aGtA GtCTTT at A cAggC C gGcc GgcT c G
```

continued - *Multiple Sequence Alignment of the reverse Surface region.*

adhbv: Australian Duck Hepatitis B Virus (GenBank DHV6350, AJ006350); inoculum: starting inoculum; all others ducknumber_dayofsample.

11.6. ANGIS

The computational analysis of all sequence data was obtained using the Australian National Genomic Information Service (ANGIS) subscription service (http://www.angis.org.au) (Reisner, 1995). This service allows variously licensed computer software to be used by subscribers, of which the Department of Infectious Diseases and Immunology was a member. All analysis was performed via, 2D ANGIS, WebANGIS, or BioManager (previously BioNavigator) portals.

11.6.1. Multiple Sequence Analysis

Multiple sequence analysis is when sequence data from various samples is lined up to enable comparison with other samples. It was performed by obtaining the sequence data from samples and converting or uploading (depending on which portal was used, as BioManager was capable of automatically converting data when uploaded) via the portal, and setting up the program to analyse the data. The two programs that were used were PileUp (GCG), and ClustalW (Thompson *et al.*, 1994), they both produce very similar output, and were both used to average out any differences in the alignment of the data.

11.6.1.1. PileUp

PileUp was used under default conditions (Table 81, p.A42).

Options	
Gap creation penalty	8
Gap extension penalty	2
End gap penalty same as internal gap penalty	No
Choose default strand for gap insertion	No preference
Number of sequence symbols per line	50
Number of sequence symbols per block	10

Table 81. *Running conditions for the PileUp software.*

11.6.1.2. ClustalW

ClustalW was used under default conditions (Table 82, p.A42).

	Pairwise alignment options	Multiple alignment options
DNA weight matrix	IUB	IUB
Gap opening penalty	10	10
Gap extension penalty	0.1	0.05
Gap separation distance	8	8
End gap separation penalty	-	Yes

Table 82. *Alignment options for the ClustalW software.*

11.7. SEQUENCE OF DHBV

The sequence of DHBV as determined from cloned DNA by Alison Jilbert (Triyatni *et al.*, 2001). Obtained from GenBank and/or EMBL.

11.8. DNA VACCINE.

Statistical analysis from Chapter 8 is summarised in Table 83 (p.A43).

	DNAvacc1 group		Dv1 Control group			Fisher Exact	
	Resp	NonR	Resp	NonR		P	< 0.05
1-15	1	6	3	4	1-15	0.315	
7-14W-27	3	4	7	0	7-14W-27	0.070	
71-90	0	7	0	7	71-90	ns	
101-120	0	7	1	6	101-120	1.000	
229-248	0	7	2	5	229-248	0.462	
267-286	1	6	2	5	267-286	1.000	
307-326	0	7	1	6	307-326	1.000	
SMC PHA	7	0	7	0	SMC PHA	ns	
SMC LPS	6	1	5	2	SMC LPS	1.000	
PBMC PHA	7	0	7	0	PBMC PHA	ns	
PBMC LPS	7	0	7	0	PBMC LPS	ns	

Table 83. *Summary of the Statistical analysis of the DNAvacc1 experiment (significant P/N).*

Shade indicates a possible trend (P<0.10). ns: not significant.

11.9. LYMPHOBLASTOGENESIS ASSAY DATA

Contained in the following tables are the raw data used for statistical analysis. The duck numbers for the various groups have been summarised (Table 84 p.A43).

Group	Total	Duck numbers
Negative control	24	1A, 1B, 1C, 1D, 1E, 1F, 1G, 1H, 1I, 1J, 1K, 1L 2A, 2B, 2C, 2D, 2E, 2F, 2G, 2H, 2I P24P53 V2T, V2U
Protein vaccination	15	G51, G53, G63, G99, P63, W45 V2J, V2K, V2L, V2M, V2N, V2O, V2P, V2Q, V2S
Positive control	12	P72W48 V2R G531, G58, P631 G631, G72, G89 W105, W106, W107, W111
DNAvacc1	7	B67, B68, G57, G97, G98, W39, W133
Dv1 controls	7	G92, G93, G100, W42, W118, W120, W124
Bursectomy	7	W101, W109, W121, W130, W131, W132, W145
Thymectomy	13	W122, W125, W126, W147, W151, W152, W153, W156, W157, W160, W167, W168, W170

Table 84. *Summary of Duck Numbers for the various groups.*

Raw data for Negative control duck 1A

1A	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	61	38								>5000		>2.1		>2.1		<0.05	
Total 3H	132	87															
	R1	R2	R3	R4	R5	R6	Mean	SD									
1-15 [1]	75	46	77	58	44	59	60	14	-72		0.0		0.5		0.063		1-15 [1]
1-15 [10]	174	56	136	86	74	56	97	48	-35		0.5		0.7		0.372		1-15 [10]
1-15 [20]	126	42	154	85	69	82	93	40	-39		0.5		0.7		0.318		1-15 [20]
7-14W-27 [1]	33	77	140	46	50	40	64	40	-67		0.0		0.5		0.090		7-14W-27 [1]
7-14W-27 [10]	37	88	117	63	55	40	66	31	-65		0.1		0.5		0.095		7-14W-27 [10]
7-14W-27 [20]		34	104	48	52	42	56	28	-76		-0.1		0.4		0.077		7-14W-27 [20]
7-14R-27 [1]	51	75	52	90	45	54	61	17	-71		0.0		0.5		0.068		7-14R-27 [1]
7-14R-27 [10]	43	68	51	108	80	59	68	23	-64		0.1		0.5		0.100		7-14R-27 [10]
7-14R-27 [20]	42	82	64	109	73	57	71	23	-61		0.1		0.5		0.114		7-14R-27 [20]
22-41 [1]	91	112	91	169	106	78	108	32	-24		0.7		0.8		0.528		22-41 [1]
22-41 [10]	78	63	123	101	435	67	144	144	13		1.2		1.1		0.814		22-41 [10]
22-41 [20]	61	134	93	60	196	55	100	56	-32		0.5		0.8		0.424		22-41 [20]
37-56 [1]	54	63	79	69	93	169	88	42	-44		0.4		0.7		0.260		37-56 [1]
37-56 [10]	52	67	73	67	80	142	80	32	-52		0.3		0.6		0.179		37-56 [10]
37-56 [20]	65	59	76	77	91	231	100	65	-32		0.5		0.8		0.436		37-56 [20]
54-73 [1]	66	59	92	152	225	324	153	105	21		1.3		1.2		0.648		54-73 [1]
54-73 [10]	56	134	131	120	252	250	157	78	25		1.4		1.2		0.551		54-73 [10]
54-73 [20]	47	69	76	102	118	223	105	63	-26		0.6		0.8		0.519		54-73 [20]
71-90 [1]	44	55	52	79	105	203	89	60	-42		0.4		0.7		0.299		71-90 [1]
71-90 [10]	54	55	41	59	90	265	94	85	-38		0.5		0.7		0.388		71-90 [10]
71-90 [20]	87	65	49	66	53	78	66	14	-65		0.1		0.5		0.088		71-90 [20]
87-106 [1]	80	84	59	66	57	82	71	12	-61		0.1		0.5		0.111		87-106 [1]
87-106 [10]	77	75	77	76	53	123	80	23	-52		0.3		0.6		0.174		87-106 [10]
87-106 [20]	162	177	171	120	73	145	141	39	10		1.1		1.1		0.803		87-106 [20]
101-120 [1]	210	97	78	49	64	74	95	58	-36		0.5		0.7		0.366		101-120 [1]
101-120 [10]	113	104	73	54	56	82	80	24	-52		0.3		0.6		0.176		101-120 [10]
101-120 [20]	76	73	63	48	40	68	61	14	-71		0.0		0.5		0.067		101-120 [20]
116-130 [1]	150	148	114	82	57	98	108	37	-24		0.7		0.8		0.534		116-130 [1]
116-130 [10]	137	115	146	103	50	75	104	37	-27		0.6		0.8		0.471		116-130 [10]
116-130 [20]	76	89	77	73	56	70	73	11	-58		0.2		0.6		0.124		116-130 [20]
126-140 [1]	150	147	259	210	104	85	159	65	28		1.4		1.2		0.500		126-140 [1]
126-140 [10]		306	432	225	152	132	249	123	118		2.7	*	1.9		0.035	*	126-140 [10]
126-140 [20]	122	154	230	123	83	73	131	57	-1		1.0		1.0		0.982		126-140 [20]
136-150 [1]	61	98	122	81	79	53	82	25	-49		0.3		0.6		0.194		136-150 [1]
136-150 [10]	104	94	149	80	73	46	91	35	-41		0.4		0.7		0.285		136-150 [10]
136-150 [20]	52	117	329	501	94	127	203	175	72		2.0		1.5		0.241		136-150 [20]
146-160 [1]	93	143	178	408	120	106	174	118	43		1.6		1.3		0.384		146-160 [1]
146-160 [10]	113	111	193	383	97	128	171	109	39		1.6		1.3		0.412		146-160 [10]
146-160 [20]	603	240	165	413	193	215	305	170	173		3.5	*	2.3	*	0.008	*	146-160 [20]
156-170 [1]	230	217	364	369	253	280	285	66	154		3.2	*	2.2	*	0.001	*	156-170 [1]
156-170 [10]	84	68	205	78	147	128	118	53	-13		0.8		0.9		0.734		156-170 [10]
156-170 [20]	59	88	161	115	177	85	114	46	-18		0.7		0.9		0.647		156-170 [20]
166-180 [1]	67	53	101	199	205	443	178	145	46		1.7		1.4		0.396		166-180 [1]
166-180 [10]	66	65	110	168	197	535	190	177	58		1.8		1.4		0.340		166-180 [10]
166-180 [20]	71	76	108	239	181	493	194	160	63		1.9		1.5		0.278		166-180 [20]
176-195 [1]	111	138	210	272	201	323	209	79	77		2.1		1.6		0.081		176-195 [1]
176-195 [10]	215	210	264	199	155		208	39	77		2.1		1.6		0.079		176-195 [10]
176-195 [20]	138	85	148	170	186	219	158	46	26		1.4		1.2		0.504		176-195 [20]
191-210 [1]	78	81	111	97	230	100	116	57	-16		0.8		0.9		0.695		191-210 [1]
191-210 [10]	67	75	71	121	153	226	119	62	-13		0.8		0.9		0.749		191-210 [10]
191-210 [20]	133	103	117	230	219	107	151	58	20		1.3		1.1		0.625		191-210 [20]
210-229 [1]	242	93	118	143	129	108	139	53	7		1.1		1.1		0.858		210-229 [1]
210-229 [10]	402	185	117	111	142	91	175	116	43		1.6		1.3		0.378		210-229 [10]
210-229 [20]	314	245	311	248	264	204	264	42	133		2.9	*	2.0		0.003	*	210-229 [20]
229-248 [1]	215	105	125	85	88	58	113	55	-19		0.7		0.9		0.629		229-248 [1]
229-248 [10]	139	54	54	41	54	90	72	37	-60		0.2		0.5		0.127		229-248 [10]
229-248 [20]	245	96	85	77	86	144	122	65	-10		0.9		0.9		0.810		229-248 [20]
248-267 [1]	59	255	68	79	49	29	90	83	-42		0.4		0.7		0.337		248-267 [1]
248-267 [10]	68	298	45	49	39	40	90	102	-42		0.4		0.7		0.368		248-267 [10]
248-267 [20]	82	240	52	32	34	32	78	81	-53		0.2		0.6		0.223		248-267 [20]
267-286 [1]	411	339	205	139	61	60	202	146	71		2.0		1.5		0.201		267-286 [1]
267-286 [10]	284	407	633	339	315	175	359	154	227		4.2	*	2.7	*	0.001	*	267-286 [10]
267-286 [20]	160	242	223	82	136	58	150	74	18		1.3		1.1		0.659		267-286 [20]
287-306 [1]	27	70	352	184	164	78	146	117	14		1.2		1.1		0.773		287-306 [1]
287-306 [10]	27	50	225	196	246	70	136	97	4		1.1		1.0		0.929		287-306 [10]
287-306 [20]	58	88	163	294	39	75	119	95	-12		0.8		0.9		0.783		287-306 [20]
307-326 [1]	48	86	137	153	174	65	110	51	-21		0.7		0.8		0.588		307-326 [1]
307-326 [10]	65	136	207	111	634	212	227	207	96		2.4	*	1.7		0.166		307-326 [10]
307-326 [20]	37	91	183	306	303	110	172	113	40		1.6		1.3		0.407		307-326 [20]
sAg 10	58	54	94	62	136	88	82	31	-50		0.3		0.6		0.196		sAg 10
sAg 100	53	64	69	65	205	108	94	58	-38		0.5		0.7		0.347		sAg 100
N	24	56	40	42	35	47	40	11	-91		-0.3		0.3		0.022	*	N
N	94	147	103	32	87	27	81	45	-50		0.3		0.6		0.204		N
3H			161	351	68	80	165	131	33		1.5		1.3		0.558		3H
3H		69	86	169	163	87	114	47	-17		0.8		0.9		0.684		3H
3H																	3H
3H	60	247	114	59			120	89	-12		0.8		0.9		0.816		3H
	SMC																
N	105	70	56	41	279	209	126	96	-170		0.0		0.4		0.01	*	N
3H	307	414	172	222	415	249	296	101	0		1.0		1.0		1.00		3H
PHA - 1	765	592	756	776	640	665	699	77	403		3.4	*	2.4	*	0.00	*	PHA - 1
PHA - 5	2349	1767	2063	1334	1437	2933	1980	602	1684		10.9	*	6.7	*	0.00	*	PHA - 5
PHA - 10	5004	3469	4533	2909	2604	5186	3951	1105	3655		22.5	*	13.3	*	0.00	*	PHA - 10
LPS - 1	1093	951	989	764	846	1471	1019	249	722		5.3	*	3.4	*	0.00	*	LPS - 1
LPS - 5	1022	1046	910	1036	802	593	901	178	605		4.6	*	3.0	*	0.00	*	LPS - 5
LPS - 10	910	913	967	966	758	495	835	183	538		4.2	*	2.8	*	0.00	*	LPS - 10
LPS - 20	483	600	695	719	405	146	508	215	212		2.2	*	1.7		0.05		LPS - 20
LPS - 40	167	170	165	381	118	147	191	95	-105		0.4		0.6		0.09		LPS - 40
	PBMC																
N	166	139	165	131	76	34	118	53	-592		0.0		0.2		0.00	*	N
3H	441	966	825	809	819	403	710	231	0		1.0		1.0		1.00		3H
PHA - 1	899	3522	4489	1636	1225	2026	2299	1409	1589		3.7	*	3.2	*	0.02	*	PHA - 1
PHA - 5	1069	2404	2924	5275	4158	2260	3015	1493	2305		4.9	*	4.2	*	0.00	*	PHA - 5
PHA - 10	623	1426	1888	1580	1485	1207	1368	427	658		2.1	*	1.9		0.01	*	PHA - 10
LPS - 1	139	121	141	216	140	182	156	35	-554		0.1		0.2		0.00	*	LPS - 1
LPS - 5	133	161	107	121	131	74	121	29	-589		0.0		0.2		0.00	*	LPS - 5
LPS - 10	78	108	90	60	118	135	98	27	-612		0.0		0.1		0.00	*	LPS - 10
LPS - 20	95	117	112	49	64	32	78	35	-632		-0.1		0.1		0.00	*	LPS - 20
LPS - 40	156	105	104	56	117	96	105	32	-605		0.0		0.1		0.00	*	LPS - 40

Raw data for Negative control duck 1B

1B	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	230	183															
Total 3H	640	1146															
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1		<0.05	
1-15 [1]																	1-15 [1]
1-15 [10]																	1-15 [10]
1-15 [20]	1544	4064	1592	1218	868	872	1693	1203	1053		3.6	*	2.6	*	0.056		1-15 [20]
7-14W-27 [1]	395		1238	353	349	144	496	426	-145		0.6		0.8		0.785		7-14W-27 [1]
7-14W-27 [10]	145	3927	659	1278	2935	236	1530	1558	889		3.2	*	2.4	*	0.124		7-14W-27 [10]
7-14W-27 [20]	91	115	302	211	111	231	177	84	-464		-0.1		0.3		0.336		7-14W-27 [20]
7-14R-27 [1]	671	354	242	427	687	393	462	179	-178		0.6		0.7		0.710		7-14R-27 [1]
7-14R-27 [10]	297	4649		911	414	346	1323	1875	683		2.7	*	2.1		0.287		7-14R-27 [10]
7-14R-27 [20]	559	2123	2429	998	723	293	1187	879	547		2.3	*	1.9		0.286		7-14R-27 [20]
22-41 [1]	2011	705	4095	787	814	1745	1693	1299	1052		3.6	*	2.6	*	0.059		22-41 [1]
22-41 [10]	28801	3590	2133	2758	2975	15124	9230	10779	8590	*	21.9	*	14.4	*	0.000	*	22-41 [10]
22-41 [20]	1436	306	1696	3806	2155	187	1597	1333	957		3.3	*	2.5	*	0.086		22-41 [20]
37-56 [1]	196	193	495	414	515	201	335	156	-305		0.3		0.5		0.525		37-56 [1]
37-56 [10]	57	76	1171	874	786	76	506	496	-134		0.7		0.8		0.784		37-56 [10]
37-56 [20]	10325	2634	1779	1198	1248	280	2910	3714	2270		6.5	*	4.5	*	0.013	*	37-56 [20]
54-73 [1]	36459	3044	2848	11032	335	10367	10681	13352	10040	*	25.5	*	16.7	*	0.001	*	54-73 [1]
54-73 [10]	74	4365	1146	611	680	4444	1886	1980	1246		4.0	*	2.9	*	0.050	*	54-73 [10]
54-73 [20]	268	3017	1001	1385	975	17213	3976	6549	3336		9.1	*	6.2	*	0.020	*	54-73 [20]
71-90 [1]	91	179	787		793	43	378	379	-262		0.4		0.6		0.621		71-90 [1]
71-90 [10]	60	430	264	144	210	213	220	125	-420		0.0		0.3		0.383		71-90 [10]
71-90 [20]	162	273	177	113	193	154	178	53	-462		-0.1		0.3		0.337		71-90 [20]
87-106 [1]	7416	2831	408	929	1277	254	2186	2723	1545		4.8	*	3.4	*	0.037	*	87-106 [1]
87-106 [10]	7859	1015	1810	720	1753	9232	3731	3777	3091		8.5	*	5.8	*	0.001	*	87-106 [10]
87-106 [20]	530	1055	1354	2006	1677	637	1209	581	569		2.4	*	1.9		0.252		87-106 [20]
101-120 [1]	2264	2439	5593	405	1249	1001	2158	1851	1518		4.7	*	3.4	*	0.016	*	101-120 [1]
101-120 [10]	345	6279	2540	4121	242	44	2262	2544	1621		5.0	*	3.5	*	0.024	*	101-120 [10]
101-120 [20]	75	58	2583	238	180	99	539	1004	-102		0.8		0.8		0.844		101-120 [20]
116-130 [1]	287	506	423	1971	238	66	581	697	-59		0.9		0.9		0.906		116-130 [1]
116-130 [10]	345	395	7459	1009	443	48	1616	2879	976		3.4	*	2.5	*	0.192		116-130 [10]
116-130 [20]	2613	8685	1489	2306	811	7335	3873	3294	3233		8.9	*	6.0	*	0.000	*	116-130 [20]
126-140 [1]	1414	693	962	3757	392		1443	1347	803		3.0	*	2.3	*	0.176		126-140 [1]
126-140 [10]	4320	1507	878	804	293	3703	1917	1678	1277		4.1	*	3.0	*	0.034	*	126-140 [10]
126-140 [20]	699	661	1054	3274	1869	295	1309	1101	668		2.6	*	2.0		0.208		126-140 [20]
136-150 [1]	148	167	185	196	118	407	203	103	-437		-0.1		0.3		0.364		136-150 [1]
136-150 [10]	110	169	158	134	110	265	157	58	-483		-0.2		0.2		0.316		136-150 [10]
136-150 [20]	324	330	348	278	436	203	319	77	-321		0.2		0.5		0.503		136-150 [20]
146-160 [1]	11129	8921	940	843	499		4466	5136	3826		10.3	*	7.0	*	0.002	*	146-160 [1]
146-160 [10]	10437	3242	7487	1047	10776	109	5516	4694	4876		12.9	*	8.6	*	0.000	*	146-160 [10]
146-160 [20]	1602	1199	1130	1174	71	115	882	634	242		1.6		1.4		0.625		146-160 [20]
156-170 [1]	2717	848	9324	1097	224	135	2391	3522	1750		5.3	*	3.7	*	0.044	*	156-170 [1]
156-170 [10]	9239	2167	958	5779	7272	104	4253	3708	3613		9.8	*	6.6	*	0.000	*	156-170 [10]
156-170 [20]	123	13422	9844	209	60	224	3980	6035	3340		9.1	*	6.2	*	0.013	*	156-170 [20]
166-180 [1]	147	12	241	216	161	74	142	86	-499		-0.2		0.2		0.301		166-180 [1]
166-180 [10]	52	878	1133	2535	856	145	933	896	293		1.7		1.5		0.566		166-180 [10]
166-180 [20]	306	6243	8889	1043	265	202	2824	3779	2184		6.3	*	4.4	*	0.018	*	166-180 [20]
176-195 [1]	406	1149	2172	3205	1697	226	1476	1126	835		3.0	*	2.3	*	0.120		176-195 [1]
176-195 [10]	2339	2607	3083	3145	6454	70	2950	2055	2309		6.6	*	4.6	*	0.001	*	176-195 [10]
176-195 [20]	3346	2220	3582	1937	1499	77	2110	1284	1470		4.6	*	3.3	*	0.010	*	176-195 [20]
191-210 [1]	88	357	136	357	207	168	218	114	-422		0.0		0.3		0.381		191-210 [1]
191-210 [10]	186	354	133	177	194	159	200	78	-440		-0.1		0.3		0.361		191-210 [10]
191-210 [20]	155	219	121	32	162	127	136	62	-504		-0.2		0.2		0.296		191-210 [20]
210-229 [1]	374	1789	6799	7447	869	202	2913	3314	2273		6.5	*	4.5	*	0.008	*	210-229 [1]
210-229 [10]	455	2908	8831	3720	9840	251	4334	4115	3694		10.0	*	6.8	*	0.000	*	210-229 [10]
210-229 [20]		1148	388	5162	2069		2192	2096	1552		4.8	*	3.4	*	0.035	*	210-229 [20]
229-248 [1]	136	1737	2506	1731	583	126	1136	992	496		2.2	*	1.8		0.339		229-248 [1]
229-248 [10]	75	173	370	391	246	61	219	142	-421		0.0		0.3		0.382		229-248 [10]
229-248 [20]	230	250	251	112	111	96	175	76	-466		-0.1		0.3		0.334		229-248 [20]
248-267 [1]	311	264	366	192	22	181	222	121	-418		0.0		0.3		0.385		248-267 [1]
248-267 [10]	382		1277	2105	17	1999	1156	939	516		2.3	*	1.8		0.356		248-267 [10]
248-267 [20]	929	2428	1604	671	20	100	958	925	318		1.8		1.5		0.534		248-267 [20]
267-286 [1]	1343	1056	2927	2351	149	4480	2051	1540	1411		4.4	*	3.2	*	0.018	*	267-286 [1]
267-286 [10]	143	747	712	1182	202	7472	1743	2833	1103		3.7	*	2.7	*	0.138		267-286 [10]
267-286 [20]	129	4710	598	4810	374	2172	2132	2158	1492		4.6	*	3.3	*	0.025	*	267-286 [20]
287-306 [1]	532	375	387	459	310	182	374	121	-266		0.4		0.6		0.578		287-306 [1]
287-306 [10]	351	1401	1418	1535	152	215	845	668	205		1.5		1.3		0.679		287-306 [10]
287-306 [20]	207	715	2590	1743	1068	205	1088	936	448		2.1		1.7		0.384		287-306 [20]
307-326 [1]	498	745	627	2332	1450	181	972	787	332		1.8		1.5		0.510		307-326 [1]
307-326 [10]	339	887	1358	433	751	5159	1488	1835	847		3.1	*	2.3	*	0.163		307-326 [10]
307-326 [20]	3686	4175	9502	17749	4134	192	6573	6233	5932	*	15.5	*	10.3	*	0.000	*	307-326 [20]
sAg 10	254	162	872	1205	344	116	492	443	-148		0.6		0.8		0.760		sAg 10
sAg 100	185	135	158	131	152	110	145	26	-495		-0.2		0.2		0.304		sAg 100
N	80	258	139	111	88	231	151	76	-489		-0.2		0.2		0.310		N
N	109	353	717	352	230	94	309	229	-331		0.2		0.5		0.492		N
3H	66	2717	599	383	190	55	668	1025	28		1.1		1.0		0.957		3H
3H	179	254	447	133	197	108	220	122	-421		0.0		0.3		0.382		3H
3H	96	169	4851	405	2725	275	1420	1956	780		2.9	*	2.2	*	0.208		3H
3H	235	296	238	266	208	279	253	32	-387		0.1		0.4		0.420		3H
	SMC																
N	226	282	388	277	269	247	281	56	-420		0.0		0.4		0.00	*	N
3H	505	1051	904	737	532	480	701	237	0		1.0		1.0		1.00		3H
PHA - 1	5958	6953	7857	7870	7340	5760	6956	919	6255	*	15.9	*	9.9	*	0.00	*	PHA - 1
PHA - 5	10053	11424	11773	7542	13432	18247	12078	3608	11377	*	28.1	*	17.2	*	0.00	*	PHA - 5
PHA - 10	20427	19822	14824	17850	21880	20076	19146	2482	18445	*	44.9	*	27.3	*	0.00	*	PHA - 10
LPS - 1	718	867	839	1514	1576	725	1040	396	338		1.8		1.5		0.10		LPS - 1
LPS - 5	639	1352	1322	1213	1124	803	1075	291	374		1.9		1.5		0.03	*	LPS - 5
LPS - 10	666	1098	1672	1175	1070	370	1008	448	307		1.7		1.4		0.17		LPS - 10
LPS - 20	103	1293	1157	713	599	114	663	503	-38		0.9		0.9		0.87		LPS - 20
LPS - 40	48	51	163	47	73	63	74	45	-627		-0.5		0.1		0.00	*	LPS - 40
	PBMC																
N	24	45	217	208	56	29	97	91	-45		0.0		0.7		0.35		N
3H	70	137	220	218	80	124	141	65	0		1.0		1.0		1.00		3H
PHA - 1	8474	9389	5800	6563	7086	10360	7945	1757	7804	*	176.4	*	56.3	*	0.00	*	PHA - 1
PHA - 5	5071	4100	4760	2973	4423	4499	4304	730	4163		94.6	*	30.5	*	0.00	*	PHA - 5
PHA - 10	3162	3046	2626	1985	2768	2556	2690	418	2549		58.3	*	19.1	*	0.00	*	PHA - 10
LPS - 1	256	594	621	521	527	214	455	176	314		8.1	*	3.2	*	0.00	*	LPS - 1
LPS - 5	235	516	501	468	303	316	390	119	249		6.6	*	2.8	*	0.00	*	LPS - 5
LPS - 10	159	491	363	312	217	149	282	133	141		4.2	*	2.0		0.04	*	LPS - 10
LPS - 20	30	80	149	182	95	127	110	54	-31		0.3		0.8		0.39		LPS - 20
LPS - 40	21	28	67	148	173	210	108	80	-33		0.3		0.8		0.45		LPS - 40

Raw data for Negative control duck 1C

1C	Mean	SD							CPM-3H		S.I.		P/N		t-Test	
Total N	102	69								>5000		>2.1		>2.1		<0.05	
Total 3H	489	473															
	R1	R2	R3	R4	R5	R6	Mean	SD									
1-15 [1]	345	475	4308	1494	235		1371	1716	883		3.3	*	2.8	*	0.526		1-15 [1]
1-15 [10]	1722	1485	1119	1966	11		1260	765	772		3.0	*	2.6	*	0.254		1-15 [10]
1-15 [20]	558	3123	3594	3826	280		2276	1717	1787		5.6	*	4.7	*	0.227		1-15 [20]
7-14W-27 [1]	414	2305	5713	1055	1240		2145	2107	1657		5.3	*	4.4	*	0.344		7-14W-27 [1]
7-14W-27 [10]	1366	5365	1711	1834	2129		2481	1635	1992		6.2	*	5.1	*	0.168		7-14W-27 [10]
7-14W-27 [20]	53	2574	1717	952	102		1079	1080	591		2.5	*	2.2	*	0.507		7-14W-27 [20]
7-14R-27 [1]	146	127	351	89	74		157	112	-331		0.1		0.3		0.152		7-14R-27 [1]
7-14R-27 [10]	892	4570	2966	3586	149		2432	1856	1944		6.0	*	5.0	*	0.224		7-14R-27 [10]
7-14R-27 [20]	828	1645	3817	5529	2756		2915	1847	2426		7.3	*	6.0	*	0.142		7-14R-27 [20]
22-41 [1]	2396	4292	11326	12392	5742		7229	4406	6741	*	18.4	*	14.8	*	0.097		22-41 [1]
22-41 [10]	4899	4744	6348	5220	2521		4746	1393	4258		12.0	*	9.7	*	0.010	*	22-41 [10]
22-41 [20]	3217	9804	4089	4914	2981		5001	2792	4513		12.7	*	10.2	*	0.084		22-41 [20]
37-56 [1]	486	360	210	114			293	164	-196		0.5		0.6		0.458		37-56 [1]
37-56 [10]	364	4840	2924	547			2168	2129	1680		5.3	*	4.4	*	0.356		37-56 [10]
37-56 [20]	456	1375	4460	1862			2038	1716	1549		5.0	*	4.2	*	0.300		37-56 [20]
54-73 [1]	76	161	155	99			128	48	-361		0.1		0.3		0.158		54-73 [1]
54-73 [10]	292	854	412	153			428	303	-61		0.8		0.9		0.852		54-73 [10]
54-73 [20]	248	146	1380	942			679	586	190		1.5		1.4		0.715		54-73 [20]
71-90 [1]	131	89	45	34			75	44	-414		-0.1		0.2		0.117		71-90 [1]
71-90 [10]	142	80	74	73			92	33	-397		0.0		0.2		0.127		71-90 [10]
71-90 [20]	93	98	93	87			92	5	-396		0.0		0.2		0.125		71-90 [20]
87-106 [1]	84	88	65	52			72	17	-416		-0.1		0.1		0.112		87-106 [1]
87-106 [10]	101	113	97	121			108	11	-381		0.0		0.2		0.137		87-106 [10]
87-106 [20]	69	123	111	110			103	24	-386		0.0		0.2		0.134		87-106 [20]
101-120 [1]	247	251	195	260			238	29	-251		0.4		0.5		0.290		101-120 [1]
101-120 [10]	236	158	500	248			285	148	-203		0.5		0.6		0.432		101-120 [10]
101-120 [20]	89	127	175	260			163	74	-326		0.2		0.3		0.199		101-120 [20]
116-130 [1]	165	93	68	252			144	83	-344		0.1		0.3		0.183		116-130 [1]
116-130 [10]	154	451	270	306			295	123	-193		0.5		0.6		0.438		116-130 [10]
116-130 [20]	349	2624	1654	56			1171	1192	682		2.8	*	2.4	*	0.498		116-130 [20]
126-140 [1]	362	1107	757	463			672	335	184		1.5		1.4		0.601		126-140 [1]
126-140 [10]	138	142	994	122			349	430	-140		0.6		0.7		0.733		126-140 [10]
126-140 [20]	131	153	172	122			145	22	-344		0.1		0.3		0.170		126-140 [20]
136-150 [1]	102	96	125	212			133	54	-355		0.1		0.3		0.164		136-150 [1]
136-150 [10]	124	188	125	92			132	40	-356		0.1		0.3		0.160		136-150 [10]
136-150 [20]	208	130	65	44			112	74	-377		0.0		0.2		0.150		136-150 [20]
146-160 [1]	209	143	76	45			118	73	-371		0.0		0.2		0.155		146-160 [1]
146-160 [10]	259	252	116	65			173	98	-316		0.2		0.4		0.220		146-160 [10]
146-160 [20]	288	179	64	81			153	103	-336		0.1		0.3		0.200		146-160 [20]
156-170 [1]	187	2112	52	167			629	990	141		1.4		1.3		0.864		156-170 [1]
156-170 [10]	36	530	57	102			181	234	-308		0.2		0.4		0.318		156-170 [10]
156-170 [20]	60	64	36	119			69	35	-419		-0.1		0.1		0.112		156-170 [20]
166-180 [1]	219	115	249	150			183	62	-305		0.2		0.4		0.220		166-180 [1]
166-180 [10]	210	255	472	110			262	153	-227		0.4		0.5		0.388		166-180 [10]
166-180 [20]	175	985	3300	266			1181	1458	693		2.8	*	2.4	*	0.567		166-180 [20]
176-195 [1]	940	2517	2862	325			1661	1222	1172		4.0	*	3.4	*	0.280		176-195 [1]
176-195 [10]	904	1827	2769	499			1500	1012	1011		3.6	*	3.1	*	0.268		176-195 [10]
176-195 [20]	2180	5668	3574	1343			3191	1890	2703		8.0	*	6.5	*	0.132		176-195 [20]
191-210 [1]	58	52	53	42			51	7	-438		-0.1		0.1		0.100		191-210 [1]
191-210 [10]	147	128	63	53			98	47	-391		0.0		0.2		0.133		191-210 [10]
191-210 [20]	86	132	13	15			61	58	-427		-0.1		0.1		0.111		191-210 [20]
210-229 [1]	842	5074	3482	17			2354	2340	1865		5.8	*	4.8	*	0.351		210-229 [1]
210-229 [10]	644	8250	4076	17			3247	3782	2758		8.1	*	6.6	*	0.387		210-229 [10]
210-229 [20]	480	2469	580	758			1071	939	583		2.5	*	2.2	*	0.471		210-229 [20]
229-248 [1]	857	3481	5992	662			2748	2516	2259		6.8	*	5.6	*	0.300		229-248 [1]
229-248 [10]	707	4814	1011	322			1713	2086	1225		4.2	*	3.5	*	0.481		229-248 [10]
229-248 [20]	218	5098	4859	602			2694	2644	2206		6.7	*	5.5	*	0.331		229-248 [20]
248-267 [1]	75	80	271	185			153	94	-336		0.1		0.3		0.196		248-267 [1]
248-267 [10]	315	542	203	120			295	183	-194		0.5		0.6		0.476		248-267 [10]
248-267 [20]	322	2655	9707	4039			4181	3991	3692		10.5	*	8.6	*	0.286		248-267 [20]
267-286 [1]	1002	6782	4404	9194			5345	3494	4857		13.6	*	10.9	*	0.138		267-286 [1]
267-286 [10]	194	14246	6771	3009			6055	6089	5566	*	15.4	*	12.4	*	0.290		267-286 [10]
267-286 [20]	85	5720	12139	1466			4853	5417	4364		12.3	*	9.9	*	0.344		267-286 [20]
287-306 [1]	30	36	26	23			28	6	-460		-0.2		0.1		0.088		287-306 [1]
287-306 [10]	68	86	25	28			52	30	-437		-0.1		0.1		0.101		287-306 [10]
287-306 [20]	153	136	118	91			124	27	-364		0.1		0.3		0.151		287-306 [20]
307-326 [1]	86	138	107	117			112	22	-377		0.0		0.2		0.141		307-326 [1]
307-326 [10]	57	171	119	87			108	49	-380		0.0		0.2		0.142		307-326 [10]
307-326 [20]	35	126	51	21			58	47	-431		-0.1		0.1		0.107		307-326 [20]
sAg 10	101	74	97	161	171		121	43	-368		0.0		0.2		0.096		sAg 10
sAg 100	87	93	58	59	243		108	77	-381		0.0		0.2		0.096		sAg 100
N	118	18	232	192	42		120	92	-368		0.0		0.2		0.110		N
N	87	89	77	64			79	12	-410		-0.1		0.2		0.116		N
3H																	3H
3H																	3H
3H																	3H
3H	154		823				489	473	0		1.0		1.0		1.000		3H
3H																	3H
	SMC																
N	314	327	17				219	175	-5480		0.0		0.0		0.00	*	N
3H	5677	6224	5197				5699	514	0		1.0		1.0		1.00		3H
PHA - 1	25307	34306	21047				26886	6769	21187	*	4.9	*	4.7	*	0.01	*	PHA - 1
PHA - 5	50902	57676	46035				51538	5846	45839	*	9.4	*	9.0	*	0.00	*	PHA - 5
PHA - 10	42286	45471	29218				38991	8613	33292	*	7.1	*	6.8	*	0.00	*	PHA - 10
LPS - 1	8440	10228	7698				8789	1301	3090		1.6		1.5		0.02	*	LPS - 1
LPS - 5	14612	10521	11062				12065	2222	6366	*	2.2	*	2.1	*	0.01	*	LPS - 5
LPS - 10	10555	12349	11738				11547	912	5848	*	2.1		2.0		0.00	*	LPS - 10
LPS - 20	6244	9361	9337				8314	1793	2615		1.5		1.5		0.07		LPS - 20
LPS - 40	128	3060	3719				2302	1912	-3397		0.4		0.4		0.04	*	LPS - 40
	PBMC																
N	229	148	43	33	214	174	140	84	-316		0.0		0.3		0.00	*	N
3H	348	511	424	463	476	515	456	63	0		1.0		1.0		1.00		3H
PHA - 1	1534	3903	5361	4735	3974	4880	4065	1359	3608		12.4	*	8.9	*	0.00	*	PHA - 1
PHA - 5	8153	8303	7972	11054	7223	9132	8639	1332	8183	*	26.9	*	18.9	*	0.00	*	PHA - 5
PHA - 10	2969	3112	3197	2458	4724	4116	3429	832	2973		10.4	*	7.5	*	0.00	*	PHA - 10
LPS - 1	131	495	698	531	551	94	417	246	-40		0.9		0.9		0.71		LPS - 1
LPS - 5	171	484	739	628	371	60	409	262	-48		0.8		0.9		0.67		LPS - 5
LPS - 10	84	455	512	588	341	40	337	228	-120		0.6		0.7		0.24		LPS - 10
LPS - 20	130	243	183	159	106	168	165	47	-292		0.1		0.4		0.00	*	LPS - 20
LPS - 40	51	103	36	23	109	99	70	38	-386		-0.2		0.2		0.00	*	LPS - 40

Raw data for Negative control duck 1D

1D	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	98	58															
Total 3H	901	519															
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1		<0.05	
1-15 [1]	796	2751	1715	1485	987	276	1335	861	433		1.5		1.5		0.287		1-15 [1]
1-15 [10]	1415	2038	1843	1827	1719	457	1550	573	648		1.8		1.7		0.056		1-15 [10]
1-15 [20]	470	1596	1292	2065	1129	4043	1766	1234	864		2.1		2.0		0.118		1-15 [20]
7-14W-27 [1]	1680	1313	779	3020	2121	500	1569	922	667		1.8		1.7		0.129		7-14W-27 [1]
7-14W-27 [10]	123	813	1832	1692	628	245	889	722	-13		1.0		1.0		0.971		7-14W-27 [10]
7-14W-27 [20]	42	39	242	84	45	126	96	79	-805		0.0		0.1		0.003	*	7-14W-27 [20]
7-14R-27 [1]	91	112	107	124	151	264	141	63	-760		0.1		0.2		0.005	*	7-14R-27 [1]
7-14R-27 [10]	683	633	2827	682	522	46	899	975	-3		1.0		1.0		0.995		7-14R-27 [10]
7-14R-27 [20]	2728	4560	2278	2757	3958	536	2802	1403	1901		3.4	*	3.1	*	0.007	*	7-14R-27 [20]
22-41 [1]	1628	1624	2429	2265	3565	707	2036	964	1135		2.4	*	2.3	*	0.021	*	22-41 [1]
22-41 [10]	1525	959	1551	1188	2455	2126	1634	564	732		1.9		1.8		0.033	*	22-41 [10]
22-41 [20]	1000	1719	729	2216	2378	154	1366	881	464		1.6		1.5		0.263		22-41 [20]
37-56 [1]	83	200	96	104	275	223	163	80	-738		0.1		0.2		0.006	*	37-56 [1]
37-56 [10]	24	256	792	1684	567	84	568	620	-334		0.6		0.6		0.313		37-56 [10]
37-56 [20]	72	3160	2415	2696	3553	781	2113	1381	1211		2.5	*	2.3	*	0.054		37-56 [20]
54-73 [1]	2362	3448	2372	2666	2494	1086	2404	763	1503		2.9	*	2.7	*	0.001	*	54-73 [1]
54-73 [10]	1491	1800	3019	3526	2224	824	2147	997	1246		2.6	*	2.4	*	0.015	*	54-73 [10]
54-73 [20]	182	3984	2407	2307	3491	124	2082	1625	1181		2.5	*	2.3	*	0.095		54-73 [20]
71-90 [1]	55	444	2182	938	106	75	633	831	-268		0.7		0.7		0.492		71-90 [1]
71-90 [10]	44	203	368	367	108	147	206	135	-695		0.1		0.2		0.009	*	71-90 [10]
71-90 [20]	254	237	149	76	72	51	140	88	-762		0.1		0.2		0.005	*	71-90 [20]
87-106 [1]	561	1920	2078	3564	2725	55	1817	1314	916		2.1	*	2.0		0.116		87-106 [1]
87-106 [10]	2931	1491	2372	3153	3705	490	2357	1185	1455		2.8	*	2.6	*	0.013	*	87-106 [10]
87-106 [20]	4175	3096	3087	2755	1949	539	2600	1238	1699		3.1	*	2.9	*	0.007	*	87-106 [20]
101-120 [1]	1810	2388	1647	2938	2066	395	1874	857	972		2.2	*	2.1		0.028	*	101-120 [1]
101-120 [10]	747	1293	1903	2693	2647	83	1561	1049	659		1.8		1.7		0.169		101-120 [10]
101-120 [20]	50	260	405	789	281	67	309	271	-593		0.3		0.3		0.029	*	101-120 [20]
116-130 [1]	160	340	196	229	213	186	220	63	-681		0.2		0.2		0.009	*	116-130 [1]
116-130 [10]	111	1768	3572	1524	696	133	1300	1309	399		1.5		1.4		0.472		116-130 [10]
116-130 [20]	2474	3271	3305	3000	2820	204	2512	1172	1611		3.0	*	2.8	*	0.007	*	116-130 [20]
126-140 [1]	3132	2595	3134	3680	2732	1825	2849	629	1948		3.4	*	3.2	*	0.000	*	126-140 [1]
126-140 [10]	2356	4084	2632	3992	2732	1335	2855	1042	1954		3.4	*	3.2	*	0.001	*	126-140 [10]
126-140 [20]	3767	3643	3789	1974	2046	1299	2753	1106	1851		3.3	*	3.1	*	0.002	*	126-140 [20]
136-150 [1]	55	114	1638	390	463	43	450	608	-451		0.4		0.5		0.176		136-150 [1]
136-150 [10]	78	58	42	147	108	138	95	43	-807		0.0		0.1		0.003	*	136-150 [10]
136-150 [20]	52	52	101	158	122	173	110	51	-792		0.0		0.1		0.004	*	136-150 [20]
146-160 [1]	966	1554	1631	2025	814	304	1215	632	314		1.4		1.3		0.346		146-160 [1]
146-160 [10]	1024	1539	1713	2436	2550	405	1611	822	710		1.9		1.8		0.085		146-160 [10]
146-160 [20]	3080	1493	2309	2090	2226	726	1987	800	1086		2.4	*	2.2	*	0.013	*	146-160 [20]
156-170 [1]	701	1305	2318	3221	2367	3443	2226	1065	1324		2.6	*	2.5	*	0.014	*	156-170 [1]
156-170 [10]	75	1357	2947	1299	2049	90	1303	1117	401		1.5		1.4		0.412		156-170 [10]
156-170 [20]	49	65	143	288	217	158	153	91	-748		0.1		0.2		0.005	*	156-170 [20]
166-180 [1]	93	56	27	36	130	75	69	38	-832		0.0		0.1		0.003	*	166-180 [1]
166-180 [10]	206	3410	3066	1908	228	38	1476	1529	574		1.7		1.6		0.368		166-180 [10]
166-180 [20]	871	3777	3631	3146	3229	249	2484	1522	1582		3.0	*	2.8	*	0.025	*	166-180 [20]
176-195 [1]	1221	2715	2908	1259	2178	674	1826	906	924		2.2	*	2.0		0.042	*	176-195 [1]
176-195 [10]	507	2963	2355	1483	2901	5104	2552	1560	1651		3.1	*	2.8	*	0.023	*	176-195 [10]
176-195 [20]	1651	3025	2310	3507	3859	1740	2682	924	1780		3.2	*	3.0	*	0.001	*	176-195 [20]
191-210 [1]	96	190	444	824	233	259	341	263	-561		0.3		0.4		0.036	*	191-210 [1]
191-210 [10]	86	124	195	453	136	282	212	136	-689		0.1		0.2		0.009	*	191-210 [10]
191-210 [20]	75	66	47	80	82	70	70	13	-832		0.0		0.1		0.002	*	191-210 [20]
210-229 [1]	732	4046	4888	2233	3685	831	2736	1741	1834		3.3	*	3.0	*	0.022	*	210-229 [1]
210-229 [10]	1395	2421	2564	1483	1535	1419	1803	538	901		2.1	*	2.0		0.011	*	210-229 [10]
210-229 [20]	2806	3631	2249	622	2030	600	1989	1202	1088		2.4	*	2.2	*	0.052		210-229 [20]
229-248 [1]	3044	6593	3333	3362	4590	1383	3717	1744	2816		4.5	*	4.1	*	0.002	*	229-248 [1]
229-248 [10]	1204	3667	5552	3741	3695	185	3007	1956	2106		3.6	*	3.3	*	0.019	*	229-248 [10]
229-248 [20]	97	1270	1785	273	550	41	669	707	-232		0.7		0.7		0.509		229-248 [20]
248-267 [1]	222	206	176	237	163	173	196	30	-705		0.1		0.2		0.007	*	248-267 [1]
248-267 [10]	453	2885	2446	3089	837	187	1649	1301	748		1.9		1.8		0.188		248-267 [10]
248-267 [20]	1110	3849	3853	3719	2979	883	2732	1385	1830		3.3	*	3.0	*	0.008	*	248-267 [20]
267-286 [1]	2486	2456	4463	3769	3470	2397	3173	860	2272		3.8	*	3.5	*	0.000	*	267-286 [1]
267-286 [10]	3408	1738	1691	3464	3374	1139	2469	1058	1567		3.0	*	2.7	*	0.005	*	267-286 [10]
267-286 [20]	558	1717	2908	3073	2466	2297	2170	924	1268		2.6	*	2.4	*	0.010	*	267-286 [20]
287-306 [1]	131	42	180	86	121	173	122	52	-780		0.0		0.1		0.004	*	287-306 [1]
287-306 [10]	61	1343	2799	1935	388	233	1126	1093	225		1.3		1.2		0.636		287-306 [10]
287-306 [20]	1248	3667	3115	4952	1716	315	2502	1715	1601		3.0	*	2.8	*	0.038	*	287-306 [20]
307-326 [1]	3674	3647	4589	4151	3958	2191	3701	818	2800		4.5	*	4.1	*	0.000	*	307-326 [1]
307-326 [10]	2303	5192	3586	3661	7566	2471	4130	1977	3228		5.0	*	4.6	*	0.002	*	307-326 [10]
307-326 [20]	3403	7857	3730	5375	4127	2931	4570	1811	3669		5.6	*	5.1	*	0.000	*	307-326 [20]
sAg 10	68	112	905	367	135	54	273	330	-628		0.2		0.3		0.027	*	sAg 10
sAg 100	87	189	68	100	76	140	110	46	-792		0.0		0.1		0.004	*	sAg 100
N	185	216	37	86	68	171	127	73	-775		0.0		0.1		0.004	*	N
N	93	71	72	48	58	76	69	15	-832		0.0		0.1		0.002	*	N
3H		1141	1627	1409			1392	244	491		1.6		1.5		0.165		3H
3H																	3H
3H	279	776	775	305			534	279	-368		0.5		0.6		0.228		3H
3H																	3H
	SMC																
N	155	233	175	2246	1281	77	694	884	-786		0.0		0.5		0.44		N
3H	4940	3624	213	74	16	17	1481	2211	0		1.0		1.0		1.00		3H
PHA - 1	11639	11440	4266	11277	8685	10831	9690	2867	8209	*	11.4	*	6.5	*	0.00	*	PHA - 1
PHA - 5	13545	9067	6505	10222	8758	7787	9314	2421	7833	*	11.0	*	6.3	*	0.00	*	PHA - 5
PHA - 10	12190	11106	9031	10392	10209	8831	10293	1265	8812	*	12.2	*	7.0	*	0.00	*	PHA - 10
LPS - 1	10984	8393	3223	7079	6785	6358	7137	2547	5656	*	8.2	*	4.8	*	0.00	*	LPS - 1
LPS - 5	9707	7011	2213	7768	5935	6904	6590	2489	5109	*	7.5	*	4.5	*	0.00	*	LPS - 5
LPS - 10	9955	5119	942	6565	6881	4411	5645	2996	4165		6.3	*	3.8	*	0.02	*	LPS - 10
LPS - 20	4105	2196	149	5361	3827	1513	2858	1915	1378		2.8	*	1.9		0.28		LPS - 20
LPS - 40	187	198	93	173	80	97	138	54	-1343		-0.7		0.1		0.17		LPS - 40
	PBMC																
N	164	168	198	46	24	275	146	95	-77		0.0		0.7		0.15		N
3H	158	348	242	147	202	240	223	73	0		1.0		1.0		1.00		3H
PHA - 1	747	1997	1907	1233	1936	925	1457	559	1235		17.1	*	6.5	*	0.00	*	PHA - 1
PHA - 5	1669	1850	1768	1304	1806	2661	1843	447	1620		22.1	*	8.3	*	0.00	*	PHA - 5
PHA - 10	667	815	633	489	560	396	593	146	371		5.8	*	2.7	*	0.00	*	PHA - 10
LPS - 1	71	199	657	804	549	131	402	308	179		3.3	*	1.8		0.20		LPS - 1
LPS - 5	67	273	420	541	352	112	294	182	71		1.9		1.3		0.39		LPS - 5
LPS - 10	67	98	517	433	332	105	259	194	36		1.5		1.2		0.68		LPS - 10
LPS - 20	126	93	189	122	105	120	126	33	-97		-0.3		0.6		0.01	*	LPS - 20
LPS - 40	77	69	45	41	174	67	79	49	-144		-0.9		0.4		0.00	*	LPS - 40

Raw data for Negative control duck 1E

1E	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	106	82															
Total 3H	825	2062								>5000		>2.1		>2.1		<0.05	
	R1	R2	R3	R4	R5	R6	Mean	SD									
1-15 [1]	2094	4052	1572	589	515	159	1496	1447	671		1.9		1.8		0.46		1-15 [1]
1-15 [10]	4749	1663	570	624	617	112	1389	1723	564		1.8		1.7		0.54		1-15 [10]
1-15 [20]	605	310	754	1569	635	1311	864	477	38		1.1		1.0		0.96		1-15 [20]
7-14W-27 [1]	2650	73	52	553	1932	145	901	1115	75		1.1		1.1		0.93		7-14W-27 [1]
7-14W-27 [10]	51	1926	1298	131	1717	95	869	876	44		1.1		1.1		0.96		7-14W-27 [10]
7-14W-27 [20]	71	253	152	2629	107	98	552	1020	-274		0.6		0.7		0.76		7-14W-27 [20]
7-14R-27 [1]	115	105	141	173	36	52	103	52	-722		0.0		0.1		0.40		7-14R-27 [1]
7-14R-27 [10]	83	330	9045	378	444	76	1726	3589	900		2.3	*	2.1		0.42		7-14R-27 [10]
7-14R-27 [20]	10298	460	442	3248	3893	132	3079	3881	2253		4.1	*	3.7	*	0.06		7-14R-27 [20]
22-41 [1]	3022	598	297	1984	562	7275	2289	2657	1464		3.0	*	2.8	*	0.15		22-41 [1]
22-41 [10]	4689	259	238	1466	812	4855	2053	2154	1228		2.7	*	2.5	*	0.21		22-41 [10]
22-41 [20]																	22-41 [20]
37-56 [1]	79	82	66	48	96	116	81	24	-744		0.0		0.1		0.39		37-56 [1]
37-56 [10]	68	87	456	202	356	96	211	161	-615		0.1		0.3		0.48		37-56 [10]
37-56 [20]	103	6672	783	375	349	114	1399	2595	574		1.8		1.7		0.57		37-56 [20]
54-73 [1]	180	330	249	593	325	263	323	143	-502		0.3		0.4		0.56		54-73 [1]
54-73 [10]	571	216	618	172	4453	565	1099	1654	274		1.4		1.3		0.77		54-73 [10]
54-73 [20]	774	214	310	173	264	144	313	234	-512		0.3		0.4		0.55		54-73 [20]
71-90 [1]	154	113	94	859	2032	124	562	778	-263		0.6		0.7		0.76		71-90 [1]
71-90 [10]	173	402	61	38	116	151	157	131	-669		0.1		0.2		0.44		71-90 [10]
71-90 [20]	117	174	100	77	126	64	110	39	-716		0.0		0.1		0.41		71-90 [20]
87-106 [1]	464	1573	202	127	83	945	566	588	-260		0.6		0.7		0.76		87-106 [1]
87-106 [10]	1353	1615	961	1108	411	3818	1544	1185	719		2.0		1.9		0.42		87-106 [10]
87-106 [20]	863	651	451	529	461	5828	1463	2144	638		1.9		1.8		0.51		87-106 [20]
101-120 [1]	922	289	260	583	1119	145	553	395	-272		0.6		0.7		0.75		101-120 [1]
101-120 [10]	1146	214	339	1403	70	190	560	566	-265		0.6		0.7		0.76		101-120 [10]
101-120 [20]	185	6018	6404	145	61	161	2162	3139	1337		2.9	*	2.6	*	0.21		101-120 [20]
116-130 [1]	122	241	79	55	46	39	97	77	-729		0.0		0.1		0.40		116-130 [1]
116-130 [10]	138	92	1066	872	39	35	373	467	-452		0.4		0.5		0.60		116-130 [10]
116-130 [20]	143	363	1693	1060	557	35	642	630	-184		0.7		0.8		0.83		116-130 [20]
126-140 [1]	5916	105	130	1468	794	337	1458	2244	633		1.9		1.8		0.51		126-140 [1]
126-140 [10]	8004	674	3742	549	396	171	2256	3113	1430		3.0	*	2.7	*	0.18		126-140 [10]
126-140 [20]	1547	579	783	437	1461	6115	1820	2153	995		2.4	*	2.2	*	0.30		126-140 [20]
136-150 [1]	181	785	262	1374	42	41	447	531	-378		0.5		0.5		0.66		136-150 [1]
136-150 [10]	146	181	90	142	44	48	108	56	-717		0.0		0.1		0.41		136-150 [10]
136-150 [20]	42	42	80	89	297	37	98	100	-728		0.0		0.1		0.40		136-150 [20]
146-160 [1]	494	904	177	372	617	379	490	250	-335		0.5		0.6		0.70		146-160 [1]
146-160 [10]	9319	5349	1566	388	3936	3238	3966	3151	3140		5.4	*	4.8	*	0.01	*	146-160 [10]
146-160 [20]	1225	554	174	199	1161	7500	1802	2828	976		2.4	*	2.2	*	0.34		146-160 [20]
156-170 [1]	1048	486	388	464	1443	1756	931	577	105		1.1		1.1		0.90		156-170 [1]
156-170 [10]	32	159	207	658	1006	98	360	386	-466		0.4		0.4		0.59		156-170 [10]
156-170 [20]	39	33	4243	1723	138	65	1040	1703	215		1.3		1.3		0.82		156-170 [20]
166-180 [1]	42	72	40	49	86	59	58	18	-768		-0.1		0.1		0.38		166-180 [1]
166-180 [10]	47	696	641	156	57	86	280	304	-545		0.2		0.3		0.53		166-180 [10]
166-180 [20]	798	855	280	190	6197	98	1403	2370	577		1.8		1.7		0.56		166-180 [20]
176-195 [1]	2454	128	341	165	2452	152	948	1168	123		1.2		1.1		0.89		176-195 [1]
176-195 [10]	2464	783	391	128	661	267	782	859	-43		0.9		0.9		0.96		176-195 [10]
176-195 [20]	273	815	258	375	447	103	378	243	-447		0.4		0.5		0.60		176-195 [20]
191-210 [1]	52	122	292	52	58	94	112	92	-714		0.0		0.1		0.41		191-210 [1]
191-210 [10]	53	79	66	65	81	69	69	10	-757		-0.1		0.1		0.38		191-210 [10]
191-210 [20]	126	64	52	30	40	143	76	47	-750		0.0		0.1		0.39		191-210 [20]
210-229 [1]	563	441	1460	564	475	70	595	461	-230		0.7		0.7		0.79		210-229 [1]
210-229 [10]	299	984	200	97	1185	61	471	487	-355		0.5		0.6		0.68		210-229 [10]
210-229 [20]	2129	653	353	767	294	87	714	736	-112		0.8		0.9		0.90		210-229 [20]
229-248 [1]	2572	255	420	291	122	219	646	948	-179		0.8		0.8		0.84		229-248 [1]
229-248 [10]	295	152	90	282	610	59	248	202	-578		0.2		0.3		0.50		229-248 [10]
229-248 [20]	111	365	127	59	48	50	126	121	-699		0.0		0.2		0.42		229-248 [20]
248-267 [1]	58	62	135	86	66	53	76	31	-749		0.0		0.1		0.39		248-267 [1]
248-267 [10]	49	586	2244	265	7001	185	1721	2710	896		2.2	*	2.1		0.38		248-267 [10]
248-267 [20]	12489	484	660	141	4832	99	3117	4934	2292		4.2	*	3.8	*	0.08		248-267 [20]
267-286 [1]	1488	1248	624	274	2119	44	966	791	141		1.2		1.2		0.87		267-286 [1]
267-286 [10]	1048	256	375	1130	395	197	567	412	-259		0.6		0.7		0.76		267-286 [10]
267-286 [20]	98	493	250	260	187	73	227	151	-599		0.2		0.3		0.49		267-286 [20]
287-306 [1]	52	64	59	91	149	69	80	36	-745		0.0		0.1		0.39		287-306 [1]
287-306 [10]	56	59	355	1246	228	60	334	463	-491		0.3		0.4		0.57		287-306 [10]
287-306 [20]	46	56	1071	720	1631	58	597	663	-229		0.7		0.7		0.79		287-306 [20]
307-326 [1]	2437	210	268	490	1123	2051	1096	954	271		1.4		1.3		0.76		307-326 [1]
307-326 [10]	5455	2341	411	204	645	324	1563	2064	738		2.0		1.9		0.44		307-326 [10]
307-326 [20]	39	381	138	132	171	122	164	115	-662		0.1		0.2		0.44		307-326 [20]
sAg 10	90	1917	4954	2124	230	319	1605	1867	780		2.1		1.9		0.41		sAg 10
sAg 100	121	163	90	248	329	435	231	133	-595		0.2		0.3		0.49		sAg 100
N	178	33	68	74	63	146	94	56	-732		0.0		0.1		0.40		N
N	56	331	94	114	76	45	119	107	-706		0.0		0.1		0.41		N
3H	43	43	419	3295	79	68	658	1300	-168		0.8		0.8		0.85		3H
3H	40	35	43	63	75	78	56	19	-770		-0.1		0.1		0.37		3H
3H	111	130	7284	7058	118	117	2469	3642	1644		3.3	*	3.0	*	0.15		3H
3H	67	400	123	60	29	36	119	141	-706		0.0		0.1		0.41		3H
	SMC																
N	181	55	166	127	87	54	111	55	-1052		0.0		0.1		0.02	*	N
3H	228	1313	2306	1841	1229	63	1163	881	0		1.0		1.0		1.00		3H
PHA - 1	2983	9492	11950	10355	13089	4604	8745	4065	7582	*	8.2	*	7.5	*	0.00	*	PHA - 1
PHA - 5	5247	10054	11333	10450	12272	9311	9777	2446	8614	*	9.2	*	8.4	*	0.00	*	PHA - 5
PHA - 10	4391	7851	8231	7340	7868	5743	6904	1512	5741	*	6.5	*	5.9	*	0.00	*	PHA - 10
LPS - 1	554	1153	1273	2113	1633	1215	1323	520	160		1.2		1.1		0.71		LPS - 1
LPS - 5	363	1225	1143	1183	1713	666	1049	472	-114		0.9		0.9		0.79		LPS - 5
LPS - 10	276	1165	1174	1182	1141	142	846	496	-317		0.7		0.7		0.46		LPS - 10
LPS - 20	172	367	565	1040	845	130	520	367	-644		0.4		0.4		0.13		LPS - 20
LPS - 40	81	206	117	116	119	138	129	42	-1034		0.0		0.1		0.02	*	LPS - 40
	PBMC																
N	271	309	161	212	192	109	209	73	-716		0.0		0.2		0.00	*	N
3H	776	1038	1325	1419	885	105	925	472	0		1.0		1.0		1.00		3H
PHA - 1	4839	6306	6127	6141	6078	5087	5763	629	4838		7.8	*	6.2	*	0.00	*	PHA - 1
PHA - 5	12509	12992	11598	10332	13655	12511	12266	1163	11342	*	16.8	*	13.3	*	0.00	*	PHA - 5
PHA - 10	11841	6487	6931	7920	15894	11961	10172	3685	9248	*	13.9	*	11.0	*	0.00	*	PHA - 10
LPS - 1	167	1376	1455	1618	1191	180	998	653	73		1.1		1.1		0.83		LPS - 1
LPS - 5	113	836	1441	1294	1014	103	800	576	-125		0.8		0.9		0.69		LPS - 5
LPS - 10	255	458	808	902	294	50	461	333	-464		0.4		0.5		0.08		LPS - 10
LPS - 20	97	60	110	118	103	76	94	22	-831		-0.2		0.1		0.00	*	LPS - 20
LPS - 40	92	94	72	92	85	86	87	8	-838		-0.2		0.1		0.00	*	LPS - 40

Raw data for Negative control duck 1F

1F	Mean	SD							CPM-3H		S.I.		P/N		t-Test	
Total N	76	34														
Total 3H	119	217								>5000		>2.1		>2.1	<0.05	
	R1	R2	R3	R4	R5	R6	Mean	SD								
1-15 [1]	60	92	75	134	92	107	93	26	-26		0.4		0.8		0.773	1-15 [1]
1-15 [10]	91	286	252	170	966	164	321	323	202		5.6	*	2.7	*	0.075	1-15 [10]
1-15 [20]	130	248	267	284	294	367	265	78	145		4.3	*	2.2	*	0.121	1-15 [20]
7-14W-27 [1]	96	117	176	283	171	105	158	70	38		1.9		1.3		0.676	7-14W-27 [1]
7-14W-27 [10]	112	198	253	294	387	83	221	115	102		3.3	*	1.8		0.282	7-14W-27 [10]
7-14W-27 [20]	35	125	194	139	91	72	109	56	-10		0.8		0.9		0.910	7-14W-27 [20]
7-14R-27 [1]	138	261	66	39	45	325	146	121	26		1.6		1.2		0.781	7-14R-27 [1]
7-14R-27 [10]	118	83	112	58	77	65	85	25	-34		0.2		0.7		0.706	7-14R-27 [10]
7-14R-27 [20]	112	124	221	203	158	64	147	59	27		1.6		1.2		0.766	7-14R-27 [20]
22-41 [1]	446	177	295	107	169	160	226	124	106		3.4	*	1.9		0.263	22-41 [1]
22-41 [10]	292	489	377	154	168	193	279	133	159		4.6	*	2.3	*	0.099	22-41 [10]
22-41 [20]	387	191	115	100	108	149	175	109	55		2.3	*	1.5		0.553	22-41 [20]
37-56 [1]	70	260	85	106	217	133	145	77	26		1.6		1.2		0.781	37-56 [1]
37-56 [10]	64	96	149	204	167	151	138	51	19		1.4		1.2		0.837	37-56 [10]
37-56 [20]	63	92	212	140	92	95	115	53	-4		0.9		1.0		0.965	37-56 [20]
54-73 [1]	152	252	160	117	216	179	179	48	59		2.4	*	1.5		0.516	54-73 [1]
54-73 [10]	188	276	210	312	211	97	215	75	96		3.2	*	1.8		0.300	54-73 [10]
54-73 [20]	98	186	353	186	212	168	200	84	81		2.8	*	1.7		0.384	54-73 [20]
71-90 [1]	73	147	147	185	97	52	117	51	-3		0.9		1.0		0.977	71-90 [1]
71-90 [10]	116	135	254	166	193	203	178	50	58		2.3	*	1.5		0.525	71-90 [10]
71-90 [20]	92	107	90	44	61	201	99	55	-21		0.5		0.8		0.822	71-90 [20]
87-106 [1]	169	100	83	145	93	71	110	38	-9		0.8		0.9		0.918	87-106 [1]
87-106 [10]	98	113	131	171	184	183	147	38	27		1.6		1.2		0.766	87-106 [10]
87-106 [20]	73	98	111	121	126	115	107	19	-13		0.7		0.9		0.890	87-106 [20]
101-120 [1]	215	178	168	158	138	249	184	41	65		2.5	*	1.5		0.479	101-120 [1]
101-120 [10]	95	186	96	93	46	122	106	46	-13		0.7		0.9		0.885	101-120 [10]
101-120 [20]	97	42	46	40	48	72	57	23	-62		-0.4		0.5		0.496	101-120 [20]
116-130 [1]	250	217	132	307	266	120	215	75	95		3.2	*	1.8		0.303	116-130 [1]
116-130 [10]	91	71	109	150	123	130	112	28	-7		0.8		0.9		0.936	116-130 [10]
116-130 [20]	110	87	128	129	120	52	104	30	-16		0.6		0.9		0.864	116-130 [20]
126-140 [1]	243	150	208	172	217	38	171	73	52		2.2	*	1.4		0.574	126-140 [1]
126-140 [10]	146	161	199	247	134	70	159	60	40		1.9		1.3		0.663	126-140 [10]
126-140 [20]	175	270	524	223	321	135	274	139	155		4.5	*	2.3	*	0.109	126-140 [20]
136-150 [1]	141	207	87	125	108	102	128	43	9		1.2		1.1		0.924	136-150 [1]
136-150 [10]	137	209	115	194	127	145	154	38	35		1.8		1.3		0.701	136-150 [10]
136-150 [20]	67	262	304	290	309	151	230	99	111		3.5	*	1.9		0.238	136-150 [20]
146-160 [1]	61	163	263	118	90	46	123	80	4		1.1		1.0		0.967	146-160 [1]
146-160 [10]	78	335	236	193	320	251	235	94	116		3.6	*	2.0		0.216	146-160 [10]
146-160 [20]	145	168	120	191	222	107	159	44	39		1.9		1.3		0.667	146-160 [20]
156-170 [1]	145	205	231	294	126	147	191	64	72		2.6	*	1.6		0.436	156-170 [1]
156-170 [10]	48	166	135	107	63	140	110	46	-10		0.8		0.9		0.915	156-170 [10]
156-170 [20]	45	111	103	119	109	69	92	29	-27		0.4		0.8		0.766	156-170 [20]
166-180 [1]	144	70	46	136	206	207	135	67	15		1.3		1.1		0.868	166-180 [1]
166-180 [10]	111	83	55	147	103	189	114	47	-5		0.9		1.0		0.956	166-180 [10]
166-180 [20]	39	125	57	123	151	78	95	44	-24		0.4		0.8		0.791	166-180 [20]
176-195 [1]	85	81	82	163	294	217	153	88	34		1.8		1.3		0.713	176-195 [1]
176-195 [10]	107	106	132	131	196	185	143	39	23		1.5		1.2		0.799	176-195 [10]
176-195 [20]	143	206	286	401	188	126	225	103	105		3.4	*	1.9		0.262	176-195 [20]
191-210 [1]	81	39	36	126	131	56	78	42	-41		0.1		0.7		0.650	191-210 [1]
191-210 [10]	141	89	49	155	165	164	127	47	8		1.2		1.1		0.934	191-210 [10]
191-210 [20]	48	76	85	59	58	289	102	92	-17		0.6		0.9		0.853	191-210 [20]
210-229 [1]	75	169	173	237	270	30	159	92	39		1.9		1.3		0.670	210-229 [1]
210-229 [10]	165	399	972	437	425	160	426	296	307		8.0	*	3.6	*	0.008	* 210-229 [10]
210-229 [20]	134	497	514	538	389	209	380	171	260		6.9	*	3.2	*	0.011	* 210-229 [20]
229-248 [1]	83	171	222	239	380	120	202	105	83		2.9	*	1.7		0.376	229-248 [1]
229-248 [10]	66	113	170	366	169	120	167	105	48		2.1		1.4		0.609	229-248 [10]
229-248 [20]	72	56	279	56	90	69	104	87	-16		0.6		0.9		0.863	229-248 [20]
248-267 [1]	229	192	289	190	102	67	178	82	59		2.3	*	1.5		0.525	248-267 [1]
248-267 [10]	109	191	245	131	96	47	136	71	17		1.4		1.1		0.855	248-267 [10]
248-267 [20]	78	171	168	87	61	42	101	55	-19		0.6		0.8		0.839	248-267 [20]
267-286 [1]	120	197	131	61	110	91	118	46	-1		1.0		1.0		0.989	267-286 [1]
267-286 [10]	143	87	154	474	93	152	184	145	64		2.5	*	1.5		0.501	267-286 [10]
267-286 [20]	198	287	231	218	308	117	226	68	107		3.4	*	1.9		0.249	267-286 [20]
287-306 [1]	35	104	117	58	67	192	95	56	-24		0.5		0.8		0.793	287-306 [1]
287-306 [10]	76	184	91	55	68	148	104	51	-16		0.6		0.9		0.861	287-306 [10]
287-306 [20]	79	90	89	81	81	138	93	23	-27		0.4		0.8		0.768	287-306 [20]
307-326 [1]	85	116	145	129	109	189	129	35	9		1.2		1.1		0.920	307-326 [1]
307-326 [10]	193	270	323	156	204	196	223	61	104		3.4	*	1.9		0.261	307-326 [10]
307-326 [20]	152	246	479	675	315	335	367	186	247		6.6	*	3.1	*	0.016	* 307-326 [20]
sAg 10	99	94	53	86	45	50	71	24	-48		-0.1		0.6		0.595	sAg 10
sAg 100	117	124	69	55	70	100	89	29	-31		0.3		0.7		0.737	sAg 100
N	58	46	49	77	64	158	75	42	-44		0.0		0.6		0.626	N
N	67	96	101	73	97	24	76	29	-43		0.0		0.6		0.635	N
3H	36	67	95	40	23	51	52	26	-68		-0.5		0.4		0.459	3H
3H	29	119	129	50	76	147	91	47	-28		0.4		0.8		0.757	3H
3H	1127	91	54	83	81	93	254	427	135		4.1	*	2.1	*	0.278	3H
3H	107	64	112	84	91	24	80	33	-39		0.1		0.7		0.666	3H
	SMC															
N	17	37	313	213	215	243	173	119	-6751		0.0		0.0		0.16	N
3H	1396	29161	3247	3105	3493	1142	6924	10939	0		1.0		1.0		1.00	3H
PHA - 1	12988	22654	19539	15505	28368	16073	19188	5623	12264	*	2.8	*	2.8	*	0.03	* PHA - 1
PHA - 5	14893	15	19293	22201	22291	12661	15225	8404	8301	*	2.2	*	2.2	*	0.17	PHA - 5
PHA - 10	3569	21672	14342	18961	3846	12894	12547	7538	5623	*	1.8		1.8		0.32	PHA - 10
LPS - 1	3268	7074	5757	6769	8210	5078	6026	1732	-898		0.9		0.9		0.85	LPS - 1
LPS - 5	1791	7103	5217	6442	8715	2874	5357	2623	-1567		0.8		0.8		0.74	LPS - 5
LPS - 10	126	4334	7522	7060	7376	3770	5031	2897	-1893		0.7		0.7		0.69	LPS - 10
LPS - 20	63	2290	3753	4347	4857	822	2688	1956	-4236		0.4		0.4		0.37	LPS - 20
LPS - 40	70	173	50	101	90	73	92	43	-6832		0.0		0.0		0.16	LPS - 40
	PBMC															
N	166	139	15	38	96	228	114	80	-43		0.0		0.7		0.33	N
3H	207	220	87	88	223	117	157	67	0		1.0		1.0		1.00	3H
PHA - 1	6010	5939	5804	4713	5246	7777	5915	1037	5758	*	133.9	*	37.7	*	0.00	* PHA - 1
PHA - 5	8839	9779	7658	11326	7232	12970	9634	2205	9477	*	219.7	*	61.4	*	0.00	* PHA - 5
PHA - 10	6844	4073	1364	6743	11	5661	4116	2869	3959		92.4	*	26.2	*	0.01	* PHA - 10
LPS - 1	205	1051	943	917	7852	120	1848	2968	1691		40.0	*	11.8	*	0.19	LPS - 1
LPS - 5	79	678	632	905	1098	69	577	424	420		10.7	*	3.7	*	0.04	* LPS - 5
LPS - 10	97	563	304	297	127	81	245	184	88		3.0	*	1.6		0.30	LPS - 10
LPS - 20	74	103	65	28	91	44	67	28	-90		-1.1		0.4		0.01	* LPS - 20
LPS - 40	83	112	32	41	64	87	70	30	-87		-1.0		0.4		0.01	* LPS - 40

Raw data for Negative control duck 1G

1G	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	89	73															
Total 3H	67	62															
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000	>2.1		>2.1		<0.05		
1-15 [1]	190	217	248	235	164	94	191	57	124		-4.6		2.9	*	0.000	*	1-15 [1]
1-15 [10]	314	417	966	783	384	408	545	264	478		-20.5		8.1	*	0.000	*	1-15 [10]
1-15 [20]	114	823	847	445	492	201	487	305	420		-17.9		7.3	*	0.000	*	1-15 [20]
7-14W-27 [1]	42	647	553	738	205	34	370	314	303		-12.6		5.5	*	0.000	*	7-14W-27 [1]
7-14W-27 [10]	31	493	2205	666	761	21	696	803	629		-27.3		10.4	*	0.000	*	7-14W-27 [10]
7-14W-27 [20]	71	134	40	104	90	672	185	241	118		-4.3		2.8	*	0.034	*	7-14W-27 [20]
7-14R-27 [1]	32	48	201	164	142	71	110	69	43		-0.9		1.6		0.153		7-14R-27 [1]
7-14R-27 [10]	16	22	162	90	48	92	71	55	4		0.8		1.1		0.881		7-14R-27 [10]
7-14R-27 [20]	42	255	1500	1543	951	44	722	703	655		-28.5		10.8	*	0.000	*	7-14R-27 [20]
22-41 [1]	43	554	458	406	534	385	396	186	329		-13.8		5.9	*	0.000	*	22-41 [1]
22-41 [10]	47	770	1327	445	513	33	522	486	455		-19.5		7.8	*	0.000	*	22-41 [10]
22-41 [20]	20	116	661	592	1278	77	457	487	390		-16.6		6.8	*	0.000	*	22-41 [20]
37-56 [1]	135	163	88	45	92	111	106	41	38		-0.7		1.6		0.165		37-56 [1]
37-56 [10]	50	53	18	27	77	78	50	25	-17		1.8		0.7		0.527		37-56 [10]
37-56 [20]	36	104	174	334	187	129	161	101	93		-3.2		2.4	*	0.007	*	37-56 [20]
54-73 [1]	142	475	420	203	206	260	284	133	217		-8.8		4.2	*	0.000	*	54-73 [1]
54-73 [10]	730	138	351	318	316	1183	506	384	439		-18.8		7.5	*	0.000	*	54-73 [10]
54-73 [20]	279	111	214	259	226	82	195	80	128		-4.8		2.9	*	0.000	*	54-73 [20]
71-90 [1]	61	36	48	30	74	64	52	17	-15		1.7		0.8		0.561		71-90 [1]
71-90 [10]	64	46	20	64	39	57	48	17	-19		1.9		0.7		0.468		71-90 [10]
71-90 [20]	135	160	132	242	299	37	167	92	100		-3.5		2.5	*	0.003	*	71-90 [20]
87-106 [1]	55	528	495	379	590	48	349	241	282		-11.7		5.2	*	0.000	*	87-106 [1]
87-106 [10]	76	450	479	600	607	71	380	246	313		-13.1		5.7	*	0.000	*	87-106 [10]
87-106 [20]	90	273	769	434	348	148	343	243	276		-11.4		5.1	*	0.000	*	87-106 [20]
101-120 [1]	103	96	258	596	255	198	251	183	184		-7.3		3.7	*	0.000	*	101-120 [1]
101-120 [10]	78	52	98	258	352	34	145	129	78		-2.5		2.2	*	0.038	*	101-120 [10]
101-120 [20]	71	30	85	48	54	41	55	20	-12		1.6		0.8		0.636		101-120 [20]
116-130 [1]	42	302	177	74	66	143	134	97	67		-2.0		2.0		0.045	*	116-130 [1]
116-130 [10]	46	69	61	55	49	204	80	61	13		0.4		1.2		0.643		116-130 [10]
116-130 [20]	33	90	127	295	85	114	124	90	57		-1.5		1.8		0.079		116-130 [20]
126-140 [1]	177	187	350	385	273	121	249	105	181		-7.2		3.7	*	0.000	*	126-140 [1]
126-140 [10]	106	512	354	344	169	36	253	179	186		-7.4		3.8	*	0.000	*	126-140 [10]
126-140 [20]	66	155	354	268	77	39	160	126	92		-3.2		2.4	*	0.015	*	126-140 [20]
136-150 [1]	22	81	136	52	75	56	70	38	3		0.9		1.0		0.913		136-150 [1]
136-150 [10]	54	71	83	47	95	62	68	18	1		0.9		1.0		0.963		136-150 [10]
136-150 [20]	51	22	83	297	62	142	109	100	42		-0.9		1.6		0.203		136-150 [20]
146-160 [1]	25	647	278	257	68	32	218	238	151		-5.8		3.2	*	0.008	*	146-160 [1]
146-160 [10]	47	232	582	354	134	37	231	209	164		-6.4		3.4	*	0.002	*	146-160 [10]
146-160 [20]	932	269	362	232	113	55	327	316	260		-10.7		4.9	*	0.001	*	146-160 [20]
156-170 [1]	30	153	347	280	191	123	187	113	120		-4.4		2.8	*	0.001	*	156-170 [1]
156-170 [10]	27	22	86	141	63	64	67	43	0		1.0		1.0		0.994		156-170 [10]
156-170 [20]	56	39	49	43	56	47	48	7	-19		1.9		0.7		0.465		156-170 [20]
166-180 [1]	154	138	75	44	194	83	115	56	48		-1.1		1.7		0.100		166-180 [1]
166-180 [10]	20	98	78	33	96	39	61	34	-7		1.3		0.9		0.805		166-180 [10]
166-180 [20]	35	339	565	442	73	30	247	232	180		-7.1		3.7	*	0.002	*	166-180 [20]
176-195 [1]	42	170	333	255	317	36	192	132	125		-4.6		2.9	*	0.002	*	176-195 [1]
176-195 [10]	1244	320	323	202	323	81	415	417	348		-14.7		6.2	*	0.000	*	176-195 [10]
176-195 [20]	82	313	497	295	394	86	277	166	210		-8.5		4.1	*	0.000	*	176-195 [20]
191-210 [1]	25	50	62	31	84	70	53	23	-14		1.6		0.8		0.604		191-210 [1]
191-210 [10]	36	31	58	25	51	47	41	13	-26		2.2	*	0.6		0.326		191-210 [10]
191-210 [20]	82	54	181	74	31	35	76	55	9		0.6		1.1		0.755		191-210 [20]
210-229 [1]	77	61	310	110	44	31	105	104	38		-0.7		1.6		0.253		210-229 [1]
210-229 [10]	168	736	1973	1382	365	21	774	763	707		-30.8		11.5	*	0.000	*	210-229 [10]
210-229 [20]	1328	1983	1166	1296	520	27	1053	686	986		-43.4		15.7	*	0.000	*	210-229 [20]
229-248 [1]	75	286	426	479	145	46	243	183	175		-6.9		3.6	*	0.000	*	229-248 [1]
229-248 [10]	52	309	962	665	2414	32	739	896	672		-29.2		11.0	*	0.001	*	229-248 [10]
229-248 [20]	65	65	54	110	51	31	63	26	-5		1.2		0.9		0.862		229-248 [20]
248-267 [1]	231	172	238	26	34	110	135	94	68		-2.1		2.0		0.039	*	248-267 [1]
248-267 [10]	87	60	39	30	21	61	50	24	-17		1.8		0.7		0.511		248-267 [10]
248-267 [20]	39	475	281	196	35	40	178	178	111		-4.0		2.6	*	0.016	*	248-267 [20]
267-286 [1]	815	382	288	364	121	37	335	272	267		-11.0		5.0	*	0.000	*	267-286 [1]
267-286 [10]	299	526	471	217	236	32	297	181	230		-9.3		4.4	*	0.000	*	267-286 [10]
267-286 [20]	201	379	645	801	866	46	490	333	423		-18.0		7.3	*	0.000	*	267-286 [20]
287-306 [1]	21	19	177	131	235	122	118	86	50		-1.3		1.8		0.111		287-306 [1]
287-306 [10]	16	17	129	68	118	97	74	49	7		0.7		1.1		0.800		287-306 [10]
287-306 [20]	117	120	293	353	51	38	162	130	95		-3.3		2.4	*	0.014	*	287-306 [20]
307-326 [1]	184	207	494	270	131	49	223	152	155		-6.0		3.3	*	0.000	*	307-326 [1]
307-326 [10]	182	855	871	1073	1254	55	715	486	648		-28.2		10.7	*	0.000	*	307-326 [10]
307-326 [20]	187	2160	1793	2291	920	75	1238	982	1171		-51.7		18.4	*	0.000	*	307-326 [20]
sAg 10	42	43	61	77	45	61	54	14	-13		1.6		0.8		0.625		sAg 10
sAg 100	19	139	140	59	74	30	76	52	9		0.6		1.1		0.740		sAg 100
N	290	117	101	98	70	138	136	79	69		-2.1		2.0		0.030	*	N
N	43	43	80	33	35	24	43	19	-24		2.1		0.6		0.362		N
3H	32	21	54	49	107	88	59	33	-9		1.4		0.9		0.748		3H
3H	23	26	37	61	52	38	40	15	-28		2.2	*	0.6		0.296		3H
3H	44	53	333	83	60	102	113	110	45		-1.0		1.7		0.185		3H
3H	35	28	58	107	75	45	58	29	-9		1.4		0.9		0.732		3H
	SMC																
N	25	66	56	2144	38	36	394	857	-4		0.0		1.0		0.99		N
3H	61	396	607	718	568	36	397	290	0		1.0		1.0		1.00		3H
PHA - 1	8287	9347	5939	3827	13995	5387	7797	3635	7399	*	1974.2	*	19.6	*	0.00	*	PHA - 1
PHA - 5	11042	11098	24347	10769	20619	10952	14804	6064	14407	*	3842.8	*	37.3	*	0.00	*	PHA - 5
PHA - 10	11659	11823	18560	13017	19177	15124	14893	3324	14496	*	3866.5	*	37.5	*	0.00	*	PHA - 10
LPS - 1	2431	6215	5316	4451	4771	2630	4302	1499	3905		1042.3	*	10.8	*	0.00	*	LPS - 1
LPS - 5	1219	5459	6179	4998	4559	1626	4006	2076	3609		963.4	*	10.1	*	0.00	*	LPS - 5
LPS - 10	168	3946	5347	3222	3808	592	2847	2039	2450		654.2	*	7.2	*	0.02	*	LPS - 10
LPS - 20	26	557	2322	1620	696	56	879	914	482		129.5	*	2.2	*	0.25		LPS - 20
LPS - 40	27	47	54	47	39	62	46	12	-352		-92.8		0.1		0.01	*	LPS - 40
	PBMC																
N	38	32	43	59	41	48	43	9	-17		0.0		0.7		0.16		N
3H	60	52	58	112	35	49	61	26	0		1.0		1.0		1.00		3H
PHA - 1	292	163	563	538	688	345	431	197	371		22.4	*	7.1	*	0.00	*	PHA - 1
PHA - 5	6386	1963	10065	4662	14127	15517	8786	5381	8726	*	504.4	*	145.0	*	0.00	*	PHA - 5
PHA - 10	4298	3839	6390	5855	7915	14290	7098	3818	7037	*	407.0	*	117.2	*	0.00	*	PHA - 10
LPS - 1	2790	908	1716	287	3630	1812	1857	1217	1796		104.6	*	30.7	*	0.00	*	LPS - 1
LPS - 5	206	315	312	232	191	520	296	122	235		14.6	*	4.9	*	0.00	*	LPS - 5
LPS - 10	379	93	165	127	274	99	189	114	129		8.4	*	3.1	*	0.02	*	LPS - 10
LPS - 20	255	50	330	230	149	50	177	114	116		7.7	*	2.9	*	0.04	*	LPS - 20
LPS - 40	61	64	69	105	76	56	72	18	11		1.6		1.2		0.42		LPS - 40

Raw data for Negative control duck 1H

1H	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	62	45															
Total 3H	441	663								>5000		>2.1		>2.1		<0.05	
	R1	R2	R3	R4	R5	R6	Mean	SD									
1-15 [1]	1458	55	256	233	101	73	363	543	-79		0.8		0.8		0.810		1-15 [1]
1-15 [10]	137	516	470	528	229	375	376	161	-66		0.8		0.9		0.818		1-15 [10]
1-15 [20]	229	1974	975	1241	939	126	914	682	473		2.2	*	2.1		0.193		1-15 [20]
7-14W-27 [1]	903	372	199	327	241	63	351	291	-91		0.8		0.8		0.758		7-14W-27 [1]
7-14W-27 [10]	997	1289	3986	2215	170	19	1446	1479	1005		3.6	*	3.3	*	0.080		7-14W-27 [10]
7-14W-27 [20]	77	33	118	42	41	26	56	35	-385		0.0		0.1		0.183		7-14W-27 [20]
7-14R-27 [1]	56	106	37	45	40	23	51	29	-390		0.0		0.1		0.177		7-14R-27 [1]
7-14R-27 [10]	40	72	55	140	41	24	62	41	-379		0.0		0.1		0.189		7-14R-27 [10]
7-14R-27 [20]	55	2750	4870	13949	7095	55	4796	5258	4354		12.5	*	10.9	*	0.019	*	7-14R-27 [20]
22-41 [1]	2296	639	602	344	238	627	791	756	350		1.9		1.8		0.348		22-41 [1]
22-41 [10]	1541	675	3444	539	472	1600	1379	1128	937		3.5	*	3.1	*	0.053		22-41 [10]
22-41 [20]	3357	848	1049	874	355	604	1181	1093	740		2.9	*	2.7	*	0.111		22-41 [20]
37-56 [1]	35	33	34	47	96	35	47	25	-395		0.0		0.1		0.172		37-56 [1]
37-56 [10]	32	55	31	44	72	55	48	16	-393		0.0		0.1		0.174		37-56 [10]
37-56 [20]	107	81	219	316	50	24	133	112	-309		0.2		0.3		0.283		37-56 [20]
54-73 [1]	2548	220	203	583	306	906	794	900	353		1.9		1.8		0.381		54-73 [1]
54-73 [10]	444	359	369	450	887	399	485	201	43		1.1		1.1		0.880		54-73 [10]
54-73 [20]	315	274	398	651	469	459	428	134	-14		1.0		1.0		0.961		54-73 [20]
71-90 [1]	31	22	3564	22	33	35	618	1443	176		1.5		1.4		0.741		71-90 [1]
71-90 [10]	209	36	31	30	87	40	72	70	-369		0.0		0.2		0.201		71-90 [10]
71-90 [20]	21	23	18	38	66	70	39	23	-402		-0.1		0.1		0.165		71-90 [20]
87-106 [1]	2636	69	126	168	65	846	652	1017	210		1.6		1.5		0.622		87-106 [1]
87-106 [10]	774	292	791	452	92	379	463	275	22		1.1		1.0		0.940		87-106 [10]
87-106 [20]	942	345	662	577	421	157	517	273	76		1.2		1.2		0.795		87-106 [20]
101-120 [1]	364	1081	190	197	119	1230	530	493	89		1.2		1.2		0.781		101-120 [1]
101-120 [10]	6794	138	69	97	38	52	1198	2742	757		3.0	*	2.7	*	0.409		101-120 [10]
101-120 [20]	12	22	23	27	27	33	24	7	-417		-0.1		0.1		0.151		101-120 [20]
116-130 [1]	31	32	21	89	20	27	37	26	-405		-0.1		0.1		0.163		116-130 [1]
116-130 [10]	35	23	42	21	350	15	81	132	-360		0.1		0.2		0.215		116-130 [10]
116-130 [20]	413	97	505	279	179	28	250	184	-191		0.5		0.6		0.506		116-130 [20]
126-140 [1]	741	458	259	365	200	1262	548	398	106		1.3		1.2		0.729		126-140 [1]
126-140 [10]	885	884	703	514	597	113	616	288	175		1.5		1.4		0.555		126-140 [10]
126-140 [20]	572	939	406	432	318	179	474	262	33		1.1		1.1		0.910		126-140 [20]
136-150 [1]	41	31	25	59	27	33	36	13	-405		-0.1		0.1		0.162		136-150 [1]
136-150 [10]	40	16	25	27	56	24	31	14	-410		-0.1		0.1		0.157		136-150 [10]
136-150 [20]	21	94	33	26	22	24	37	28	-405		-0.1		0.1		0.163		136-150 [20]
146-160 [1]	5999	329	184	260	206	718	1283	2319	841		3.2	*	2.9	*	0.291		146-160 [1]
146-160 [10]	2359	360	178	431	557	12681	2761	4925	2320		7.1	*	6.3	*	0.155		146-160 [10]
146-160 [20]	1508	661	472	603	624	3723	1265	1260	824		3.2	*	2.9	*	0.106		146-160 [20]
156-170 [1]	105	138	258	215	114	612	240	192	-201		0.5		0.5		0.486		156-170 [1]
156-170 [10]	138	135	216	139	30	28	114	73	-327		0.1		0.3		0.255		156-170 [10]
156-170 [20]	28	55	159	30	21	23	53	54	-389		0.0		0.1		0.179		156-170 [20]
166-180 [1]	30	40	50	59	191	402	129	146	-313		0.2		0.3		0.280		166-180 [1]
166-180 [10]	26	38	41	61	124	90	63	37	-378		0.0		0.1		0.190		166-180 [10]
166-180 [20]	45	258	635	391	150	51	255	228	-186		0.5		0.6		0.521		166-180 [20]
176-195 [1]	658	1289	587	481	316	1698	838	536	397		2.0		1.9		0.236		176-195 [1]
176-195 [10]	571	461	446	276	400	18156	3385	7237	2944		8.8	*	7.7	*	0.212		176-195 [10]
176-195 [20]	527	772	845	701	344	1443	772	375	331		1.9		1.7		0.286		176-195 [20]
191-210 [1]	32	56	44	89	114	94	72	32	-370		0.0		0.2		0.199		191-210 [1]
191-210 [10]	119	39	37	32	79	83	65	35	-377		0.0		0.1		0.192		191-210 [10]
191-210 [20]	189	127	154	114	31	70	114	57	-327		0.1		0.3		0.254		191-210 [20]
210-229 [1]	45	180	1449	497	80	81	389	545	-53		0.9		0.9		0.872		210-229 [1]
210-229 [10]	759	4143	4070	8576	1167	73	3131	3175	2690		8.1	*	7.1	*	0.019	*	210-229 [10]
210-229 [20]	4733	11490	14070	17114	8469	149	9338	6227	8896	*	24.4	*	21.2	*	0.000	*	210-229 [20]
229-248 [1]	249	1518	1174	1528	558	255	880	601	439		2.2	*	2.0		0.206		229-248 [1]
229-248 [10]	110	396	759	1273	71	58	445	488	3		1.0		1.0		0.992		229-248 [10]
229-248 [20]	95	98	83	67	84	45	79	20	-363		0.0		0.2		0.208		229-248 [20]
248-267 [1]	320	306	389	242	313	168	290	76	-152		0.6		0.7		0.590		248-267 [1]
248-267 [10]	110	181	318	222	225	42	183	97	-258		0.3		0.4		0.365		248-267 [10]
248-267 [20]	4900	763	336	103	193	1003	1216	1838	775		3.0	*	2.8	*	0.239		248-267 [20]
267-286 [1]	401	836	324	432	309	1721	671	550	229		1.6		1.5		0.489		267-286 [1]
267-286 [10]	408	1301	820	738	543	1379	865	396	423		2.1	*	2.0		0.181		267-286 [10]
267-286 [20]	662	1972	1803	714	1463	708	1220	599	779		3.1	*	2.8	*	0.034	*	267-286 [20]
287-306 [1]	119	138	167	193	166	151	156	26	-286		0.2		0.4		0.316		287-306 [1]
287-306 [10]	33	123	94	77	97	61	81	31	-361		0.1		0.2		0.210		287-306 [10]
287-306 [20]	46	242	812	410	135	59	284	292	-157		0.6		0.6		0.594		287-306 [20]
307-326 [1]	10277	413	1252	874	535	905	2376	3882	1935		6.1	*	5.4	*	0.138		307-326 [1]
307-326 [10]	9911	2151	3408	1875	2283		3926	3397	3484		10.2	*	8.9	*	0.006	*	307-326 [10]
307-326 [20]	1712	3428	3570	5756	910	2226	2934	1715	2492		7.6	*	6.6	*	0.001	*	307-326 [20]
sAg 10	31	60	85	82	86	34	63	25	-378		0.0		0.1		0.190		sAg 10
sAg 100	28	194	96	75	247	66	118	84	-324		0.1		0.3		0.260		sAg 100
N	32	31	46	38	24	29	33	8	-408		-0.1		0.1		0.159		N
N	43	101	149	118	113	16	90	50	-351		0.1		0.2		0.222		N
3H					2318		2318		1877		5.9	*	5.3	*			3H
3H		240		165	167		191	43	-251		0.3		0.4		0.538		3H
3H					393		393		-48		0.9		0.9				3H
3H	257	210	244	258	162		226	41	-215		0.4		0.5		0.489		3H
	SMC																
N	55	51	61	56	72	192	81	55	-641		0.0		0.1		0.00	*	N
3H	114	1098	610	1212	921	377	722	429	0		1.0		1.0		1.00		3H
PHA - 1	13548	6504	5246	10334	9146	10093	9145	2962	8423	*	14.1	*	12.7	*	0.00	*	PHA - 1
PHA - 5	14832	11466	13599	25561	16176	18788	16737	4973	16015	*	26.0	*	23.2	*	0.00	*	PHA - 5
PHA - 10	21793	17686	27799	23471	17565	19546	21310	3932	20588	*	33.1	*	29.5	*	0.00	*	PHA - 10
LPS - 1	3375	4252	4515	4186	5637	2210	4029	1152	3307		6.2	*	5.6	*	0.00	*	LPS - 1
LPS - 5	1850	3916	4439	4923	2372	1627	3188	1414	2466		4.8	*	4.4	*	0.00	*	LPS - 5
LPS - 10	417	2774	4250	4505	2506	572	2504	1744	1782		3.8	*	3.5	*	0.04	*	LPS - 10
LPS - 20	36	339	1382	1100	679	104	607	548	-115		0.8		0.8		0.69		LPS - 20
LPS - 40	46	121	82	96	140	137	104	36	-618		0.0		0.1		0.01	*	LPS - 40
	PBMC																
N	65	35	35	30	46	36	41	13	-27		0.0		0.6		0.01	*	N
3H	52	51	69	62	92	83	68	16	0		1.0		1.0		1.00		3H
PHA - 1	633	478	750	565	697	292	569	166	501		19.6	*	8.4	*	0.00	*	PHA - 1
PHA - 5	5559	2170	3273	2645	2256	1717	2936	1387	2869		107.2	*	43.2	*	0.00	*	PHA - 5
PHA - 10	3241	1940	1585	1812	1624	1505	1951	652	1883		70.7	*	28.7	*	0.00	*	PHA - 10
LPS - 1	1522	1320	1329	1105	961	852	1181	253	1113		42.2	*	17.4	*	0.00	*	LPS - 1
LPS - 5	875	693	818	1091	969	1144	932	170	864		33.0	*	13.7	*	0.00	*	LPS - 5
LPS - 10	328	446	453	380	598	530	456	98	388		15.4	*	6.7	*	0.00	*	LPS - 10
LPS - 20	224	145	275	390	654	401	348	179	280		11.4	*	5.1	*	0.00	*	LPS - 20
LPS - 40	130	56	77	119	65	67	85	31	17		1.6		1.3		0.25		LPS - 40

Raw data for Negative control duck 1I

1I	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	75	76															
Total 3H	227	125															
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1		<0.05	
1-15 [1]	60	127	151	162	71	76	108	44	-119		0.2		0.5		0.063		1-15 [1]
1-15 [10]	88	152	228	399	207	180	209	105	-18		0.9		0.9		0.825		1-15 [10]
1-15 [20]	252	148	101	167	132	59	143	65	-84		0.5		0.6		0.213		1-15 [20]
7-14W-27 [1]	109	94	161	251	130	93	140	60	-87		0.4		0.6		0.184		7-14W-27 [1]
7-14W-27 [10]	460	454	445	448	322	192	387	109	160		2.0		1.7		0.087		7-14W-27 [10]
7-14W-27 [20]	50	114	161	83	267	79	126	79	-101		0.3		0.6		0.172		7-14W-27 [20]
7-14R-27 [1]	148	94	82	49	56	83	85	35	-142		0.1		0.4		0.029	*	7-14R-27 [1]
7-14R-27 [10]	106	56	40	30	38	79	58	29	-169		-0.1		0.3		0.012	*	7-14R-27 [10]
7-14R-27 [20]	1247	177	158	482	149	169	397	436	170		2.1	*	1.7		0.541		7-14R-27 [20]
22-41 [1]	146	180	129	257	124	94	155	57	-72		0.5		0.7		0.257		22-41 [1]
22-41 [10]	248	164	208	206	124	59	168	68	-59		0.6		0.7		0.377		22-41 [10]
22-41 [20]	198	217	155	112	95	96	146	53	-82		0.5		0.6		0.195		22-41 [20]
37-56 [1]	61	94	143	55	111	102	94	33	-133		0.1		0.4		0.035	*	37-56 [1]
37-56 [10]	156	253	78	114	135	105	140	61	-87		0.4		0.6		0.189		37-56 [10]
37-56 [20]	446	122	121	116	109	115	172	135	-56		0.6		0.8		0.570		37-56 [20]
54-73 [1]	181	168	255	141	115	122	164	52	-63		0.6		0.7		0.298		54-73 [1]
54-73 [10]	130	130	179	285	77	66	145	80	-83		0.5		0.6		0.259		54-73 [10]
54-73 [20]	71	114	157	285	110	108	141	76	-86		0.4		0.6		0.229		54-73 [20]
71-90 [1]	137	161	121	195	181	161	159	27	-68		0.6		0.7		0.217		71-90 [1]
71-90 [10]	94	81	99	188	92	127	114	40	-114		0.3		0.5		0.068		71-90 [10]
71-90 [20]	25	156	291	82	143	113	135	90	-92		0.4		0.6		0.238		71-90 [20]
87-106 [1]	167	221	98	166	95	234	164	59	-64		0.6		0.7		0.316		87-106 [1]
87-106 [10]	231	175	234	173	120	164	183	43	-44		0.7		0.8		0.439		87-106 [10]
87-106 [20]	115	130	151	157	56	60	112	44	-116		0.2		0.5		0.070		87-106 [20]
101-120 [1]	135	197	223	220	173	103	175	48	-52		0.7		0.8		0.379		101-120 [1]
101-120 [10]	136	53	115	111	225	27	111	69	-116		0.2		0.5		0.108		101-120 [10]
101-120 [20]	64	166	123	174	53	113	116	50	-112		0.3		0.5		0.086		101-120 [20]
116-130 [1]	51	16	32	226	391	152	145	145	-82		0.5		0.6		0.432		116-130 [1]
116-130 [10]	108	125	60	72	109	38	85	34	-142		0.1		0.4		0.028	*	116-130 [10]
116-130 [20]	173	103	196	122	133	17	124	63	-103		0.3		0.5		0.131		116-130 [20]
126-140 [1]	199	205	160	224	306	154	208	55	-19		0.9		0.9		0.751		126-140 [1]
126-140 [10]	149	202	226	130	275	195	196	52	-31		0.8		0.9		0.603		126-140 [10]
126-140 [20]	79	110	218	113	227	139	148	61	-79		0.5		0.7		0.225		126-140 [20]
136-150 [1]	196	85	91	40	64	102	96	54	-131		0.1		0.4		0.056		136-150 [1]
136-150 [10]	74	44	124	121	112	101	96	31	-131		0.1		0.4		0.036	*	136-150 [10]
136-150 [20]	68	470	76	190	42	36	147	168	-80		0.5		0.6		0.494		136-150 [20]
146-160 [1]	114	139	167	187	100	69	129	44	-98		0.4		0.6		0.113		146-160 [1]
146-160 [10]	192	144	105	180	128	119	145	35	-82		0.5		0.6		0.154		146-160 [10]
146-160 [20]	310	106	177	149	303	542	265	159	38		1.2		1.2		0.734		146-160 [20]
156-170 [1]	75	118	122	127	148	152	124	28	-103		0.3		0.5		0.077		156-170 [1]
156-170 [10]	83	75	42	106	40	194	90	57	-137		0.1		0.4		0.050		156-170 [10]
156-170 [20]	129	53	99	144	82	52	93	38	-134		0.1		0.4		0.038	*	156-170 [20]
166-180 [1]	26	15	39	54	52	62	41	18	-186		-0.2		0.2		0.006	*	166-180 [1]
166-180 [10]	74	129	72	109	67	33	81	34	-146		0.0		0.4		0.025	*	166-180 [10]
166-180 [20]	34	41	74	129	103	75	76	36	-151		0.0		0.3		0.023	*	166-180 [20]
176-195 [1]	143	103	61	98	81	80	94	28	-133		0.1		0.4		0.033	*	176-195 [1]
176-195 [10]	160	106	82	69	82	108	101	33	-126		0.2		0.4		0.043	*	176-195 [10]
176-195 [20]	52	127	165	85	123	79	105	41	-122		0.2		0.5		0.055		176-195 [20]
191-210 [1]	24	31	38	36	31	42	34	6	-193		-0.3		0.1		0.005	*	191-210 [1]
191-210 [10]	35	37	18	43	72	46	42	18	-185		-0.2		0.2		0.006	*	191-210 [10]
191-210 [20]	35	163	81	290	75	204	141	96	-86		0.4		0.6		0.286		191-210 [20]
210-229 [1]	95	103	168	140	246	41	132	70	-95		0.4		0.6		0.177		210-229 [1]
210-229 [10]	40	381	1012	738	292	511	496	343	269		2.8	*	2.2	*	0.242		210-229 [10]
210-229 [20]	130	425	1084	1443	595	594	712	474	485		4.2	*	3.1	*	0.135		210-229 [20]
229-248 [1]	81	197	318	331	292	229	241	94	14		1.1		1.1		0.851		229-248 [1]
229-248 [10]	61	136	137	99	148	92	112	34	-115		0.2		0.5		0.060		229-248 [10]
229-248 [20]	48	113	127	178	139	65	112	48	-115		0.2		0.5		0.075		229-248 [20]
248-267 [1]	33	71	355	159	82	133	139	115	-88		0.4		0.6		0.325		248-267 [1]
248-267 [10]	18	26	41	142	97	89	69	48	-158		0.0		0.3		0.024	*	248-267 [10]
248-267 [20]	28	143	187	85	107	70	103	56	-124		0.2		0.5		0.070		248-267 [20]
267-286 [1]	43	206	153	160	88	105	126	58	-101		0.3		0.6		0.128		267-286 [1]
267-286 [10]	37	143	158	126	136	96	116	44	-111		0.3		0.5		0.079		267-286 [10]
267-286 [20]	120	327	219	150	110	133	177	83	-51		0.7		0.8		0.485		267-286 [20]
287-306 [1]	84	90	40	85	71	68	73	18	-154		0.0		0.3		0.015	*	287-306 [1]
287-306 [10]	57	72	47	81	52	109	70	23	-157		0.0		0.3		0.015	*	287-306 [10]
287-306 [20]	58	115	66	163	89	60	92	41	-135		0.1		0.4		0.038	*	287-306 [20]
307-326 [1]	65	131	199	125	199	271	165	73	-62		0.6		0.7		0.365		307-326 [1]
307-326 [10]	129	194	355	205	147	108	190	89	-37		0.8		0.8		0.616		307-326 [10]
307-326 [20]	160	387	496	411	452	50	326	179	99		1.6		1.4		0.424		307-326 [20]
sAg 10	167	53	73	53	36	65	75	47	-153		0.0		0.3		0.027	*	sAg 10
sAg 100	111	70	74	57	57	100	78	23	-149		0.0		0.3		0.019	*	sAg 100
N	42	48	89	99	158	276	119	88	-108		0.3		0.5		0.168		N
N	31	12	20	17	41	61	30	18	-197		-0.3		0.1		0.005	*	N
3H					371		371		144		1.9		1.6				3H
3H																	3H
3H					153		153		-74		0.5		0.7				3H
3H				157			157		-70		0.5		0.7				3H
	SMC																
N	33	38	42	35	65	41	42	12	-141		0.0		0.2		0.05	*	N
3H	64	84	86	388	375	103	183	154	0		1.0		1.0		1.00		3H
PHA - 1	542	743	313	34889	9976	4466	8,488	13,456	8,305	*	59.9	*	46.3	*	0.16		PHA - 1
PHA - 5	2667	2294	1640	23187	30758	19372	13,319	12,725	13,136	*	94.1	*	72.7	*	0.03	*	PHA - 5
PHA - 10	1824	1652	1552	16199	21382	5322	7,988	8,643	7,805	*	56.3	*	43.6	*	0.05		PHA - 10
LPS - 1	1133	928	789	2508	3035	3059	1,908	1,074	1,725		13.2	*	10.4	*	0.00	*	LPS - 1
LPS - 5	1076	991	1161	1923	2217	1600	1,495	501	1,311		10.3	*	8.2	*	0.00	*	LPS - 5
LPS - 10	408	603	510	1412	2814	2899	1,441	1,153	1,258		9.9	*	7.9	*	0.02	*	LPS - 10
LPS - 20	375	627	392	101	823	1599	653	524	470		4.3	*	3.6	*	0.06		LPS - 20
LPS - 40	120	66	61	52	68	86	75	24	-108		0.2		0.4		0.12		LPS - 40
	PBMC																
N	17	44	44	236	304	107	125	118	-12		0.0		0.9		0.87		N
3H	49	52	53	307	330	35	137	140	0		1.0		1.0		1.00		3H
PHA - 1	8975	12022	9788	11602	15165	16822	12,395	3,051	12,258	*	1008.5	*	90.2	*	0.00	*	PHA - 1
PHA - 5	13535	12916	10080	11299	12062	11926	11,969	1,215	11,832	*	973.5	*	87.1	*	0.00	*	PHA - 5
PHA - 10	3966	5858	3441	2590	5635	5102	4,432	1,306	4,294		354.0	*	32.3	*	0.00	*	PHA - 10
LPS - 1	890	594	855	579	1099	1197	869	253	731		61.1	*	6.3	*	0.00	*	LPS - 1
LPS - 5	124	392	507	347	542	172	347	171	210		18.2	*	2.5	*	0.04	*	LPS - 5
LPS - 10	57	287	438	336	533	82	289	190	151		13.4	*	2.1		0.15		LPS - 10
LPS - 20	137	132	245	328	226	56	187	97	50		5.1	*	1.4		0.49		LPS - 20
LPS - 40	149	85	117	181	95	64	115	44	-22		-0.8		0.8		0.72		LPS - 40

Raw data for Negative control duck 1J

1J	Mean	SD													
Total N	59	45													
Total 3H	275	195							CPM-3H		S.I.		P/N	t-Test	
	R1	R2	R3	R4	R5	R6	Mean	SD	>5000		>2.1		>2.1	<0.05	
1-15 [1]	973	413	2597	7918	2532	1289	2,620	2,736	2,345		11.8	*	9.5	* 0.091	1-15 [1]
1-15 [10]	1306	1002	7217	594	1262	1410	2,132	2,508	1,857		9.6	*	7.8	* 0.136	1-15 [10]
1-15 [20]	658	2366	3855	2424	596	3214	2,186	1,326	1,911		9.8	*	7.9	* 0.011 *	1-15 [20]
7-14W-27 [1]	562	1776	3832	1129	1130	2531	1,827	1,191	1,552		8.2	*	6.6	* 0.019 *	7-14W-27 [1]
7-14W-27 [10]	1261	3888	4322	7053	5128	1446	3,850	2,218	3,575		17.5	*	14.0	* 0.006 *	7-14W-27 [10]
7-14W-27 [20]	30	83	1327	3475	4100	33	1,508	1,845	1,233		6.7	*	5.5	* 0.174	7-14W-27 [20]
7-14R-27 [1]	214	66	62	75	30	40	81	67	-194		0.1		0.3	0.048 *	7-14R-27 [1]
7-14R-27 [10]	278	1025	3098	1043	3261	13	1,453	1,399	1,178		6.4	*	5.3	* 0.097	7-14R-27 [10]
7-14R-27 [20]	2229	1477	1244	11674	2352	2689	3,611	3,988	3,336		16.4	*	13.1	* 0.097	7-14R-27 [20]
22-41 [1]	2732	346	1760	1026	2573	1413	1,642	914	1,367		7.3	*	6.0	* 0.010 *	22-41 [1]
22-41 [10]	495	1557	1061	6927	292	1418	1,958	2,485	1,683		8.8	*	7.1	* 0.168	22-41 [10]
22-41 [20]	3283	2685	1345	1561	372	480	1,621	1,169	1,346		7.2	*	5.9	* 0.033 *	22-41 [20]
37-56 [1]	23	46	25	33	43	49	37	11	-239		-0.1		0.1	0.015 *	37-56 [1]
37-56 [10]	12	9055	1164	2947	1760	98	2,506	3,390	2,231		11.3	*	9.1	* 0.179	37-56 [10]
37-56 [20]	228	864	651	417	820	7996	1,829	3,031	1,554		8.2	*	6.7	* 0.286	37-56 [20]
54-73 [1]	100	186	581	247	1303	203	437	456	162		1.7		1.6	0.482	54-73 [1]
54-73 [10]	654	1704	1284	1010	624	5948	1,871	2,038	1,596		8.4	*	6.8	* 0.118	54-73 [10]
54-73 [20]	2472	513	596	571	1067	562	964	767	689		4.2	*	3.5	* 0.084	54-73 [20]
71-90 [1]	42	1193	1842	438	3061	43	1,103	1,190	828		4.8	*	4.0	* 0.161	71-90 [1]
71-90 [10]	111	70	47	95	69	69	77	23	-198		0.1		0.3	0.034 *	71-90 [10]
71-90 [20]	52	62	88	77	50	61	65	15	-210		0.0		0.2	0.026 *	71-90 [20]
87-106 [1]	1412	545	547	525	451	221	617	409	342		2.6	*	2.2	* 0.123	87-106 [1]
87-106 [10]	72	320	1386	791	3266	475	1,052	1,176	777		4.6	*	3.8	* 0.182	87-106 [10]
87-106 [20]	1267	522	738	300	535	373	623	350	348		2.6	*	2.3	* 0.081	87-106 [20]
101-120 [1]	185	671	579	418	381	4241	1,079	1,558	804		4.7	*	3.9	* 0.285	101-120 [1]
101-120 [10]	32	1783	2475	571	1054	56	995	980	720		4.3	*	3.6	* 0.143	101-120 [10]
101-120 [20]	33	502	113	331	968	44	332	362	57		1.3		1.2	0.761	101-120 [20]
116-130 [1]	68	114	50	27	28	63	58	32	-217		0.0		0.2	0.024 *	116-130 [1]
116-130 [10]	34	268	840	568	131	41	314	325	39		1.2		1.1	0.822	116-130 [10]
116-130 [20]	222	944	616	108	220	70	363	344	88		1.4		1.3	0.624	116-130 [20]
126-140 [1]	1321	203	872	725	286	486	649	416	374		2.7	*	2.4	* 0.099	126-140 [1]
126-140 [10]	1005	619	1493	307	378	1670	912	576	637		3.9	*	3.3	* 0.044 *	126-140 [10]
126-140 [20]	247	1879	1010	1714	323	7196	2,062	2,605	1,787		9.3	*	7.5	* 0.164	126-140 [20]
136-150 [1]	53	112	65	8921	98	21	1,545	3,614	1,270		6.9	*	5.6	* 0.457	136-150 [1]
136-150 [10]	102	135	52	55	69	69	80	32	-195		0.1		0.3	0.038 *	136-150 [10]
136-150 [20]	145	149	74	86	35	51	90	48	-185		0.1		0.3	0.050	136-150 [20]
146-160 [1]	110	636	413	258	250	57	287	212	12		1.1		1.0	0.923	146-160 [1]
146-160 [10]	180	2065	827	1126	1008	53	877	729	602		3.8	*	3.2	* 0.109	146-160 [10]
146-160 [20]	365	524	1122	4059	712	55	1,140	1,474	865		5.0	*	4.1	* 0.229	146-160 [20]
156-170 [1]	63	414	1211	728	356	215	498	414	223		2.0		1.8	0.301	156-170 [1]
156-170 [10]	62	2047	347	378	90	32	493	776	218		2.0		1.8	0.559	156-170 [10]
156-170 [20]	108	103	87	683	25	26	172	253	-103		0.5		0.6	0.477	156-170 [20]
166-180 [1]	78	279	116	283	69	77	150	103	-125		0.4		0.5	0.206	166-180 [1]
166-180 [10]	89	215	312	415	111	70	202	139	-73		0.7		0.7	0.487	166-180 [10]
166-180 [20]	75	229	2903	1316	571	43	856	1,109	581		3.7	*	3.1	* 0.281	166-180 [20]
176-195 [1]	3921	482	3357	778	1407	415	1,727	1,533	1,452		7.7	*	6.3	* 0.067	176-195 [1]
176-195 [10]	684	12922	1199	3439	388	1144	3,296	4,837	3,021		15.0	*	12.0	* 0.200	176-195 [10]
176-195 [20]	261	876	710	2334	1170	9383	2,456	3,465	2,181		11.1	*	8.9	* 0.197	176-195 [20]
191-210 [1]	42	89	319	2243	1972	47	785	1,033	510		3.4	*	2.9	* 0.308	191-210 [1]
191-210 [10]	52	90	64	75	140	93	86	31	-189		0.1		0.3	0.042 *	191-210 [10]
191-210 [20]	62	130	155	219	253	20129	3,491	8,151	3,216		15.9	*	12.7	* 0.405	191-210 [20]
210-229 [1]	1272	406	303	347	506	232	511	384	236		2.1		1.9	0.247	210-229 [1]
210-229 [10]	692	3202	2896	3138	6693	59	2,780	2,341	2,505		12.6	*	10.1	* 0.042 *	210-229 [10]
210-229 [20]	1082	7263	3094	1356	12401	82	4,213	4,746	3,938		19.2	*	15.3	* 0.099	210-229 [20]
229-248 [1]	3293	1080	866	1765	4100	80	1,864	1,539	1,589		8.3	*	6.8	* 0.049 *	229-248 [1]
229-248 [10]	125	1355	767	1543	725	3857	1,395	1,307	1,120		6.2	*	5.1	* 0.092	229-248 [10]
229-248 [20]	23	48	75	136	82	185	92	59	-184		0.2		0.3	0.055	229-248 [20]
248-267 [1]	194	170	90	57	139	158	135	52	-140		0.4		0.5	0.122	248-267 [1]
248-267 [10]	167	130	167	875	2063	86	581	784	306		2.4	*	2.1	* 0.420	248-267 [10]
248-267 [20]	2032	433	481	1038	1018	12668	2,945	4,798	2,670		13.3	*	10.7	* 0.249	248-267 [20]
267-286 [1]	1875	1417	606	2622	486	10153	2,860	3,661	2,585		13.0	*	10.4	* 0.153	267-286 [1]
267-286 [10]	580	4609	348	1752	251	19667	4,535	7,593	4,260		20.7	*	16.5	* 0.245	267-286 [10]
267-286 [20]	204	1061	613	2043	470	124	753	715	478		3.2	*	2.7	* 0.184	267-286 [20]
287-306 [1]	182	218	264	152	174	130	187	48	-88		0.6		0.7	0.309	287-306 [1]
287-306 [10]	54	2296	923	188	135	35	605	893	330		2.5	*	2.2	* 0.442	287-306 [10]
287-306 [20]	50	418	1290	262	989	70	513	512	238		2.1	*	1.9	0.355	287-306 [20]
307-326 [1]	3256	1138	500	2798	1047	60	1,467	1,278	1,192		6.5	*	5.3	* 0.071	307-326 [1]
307-326 [10]	481	532	4579	3944	1074	1574	2,031	1,785	1,756		9.1	*	7.4	* 0.058	307-326 [10]
307-326 [20]	443	983	4830	1566	1644	188	1,609	1,682	1,334		7.2	*	5.9	* 0.114	307-326 [20]
sAg 10	29	80	60	155	35	32	65	48	-210		0.0		0.2	0.031 *	sAg 10
sAg 100	79	41	66	30	42	74	55	20	-220		0.0		0.2	0.022 *	sAg 100
N	59	125	34	19	36	155	71	56	-204		0.1		0.3	0.036 *	N
N	86	34	34	17	20	86	46	32	-229		-0.1		0.2	0.019 *	N
3H															3H
3H	244	169					207	53	-69		0.7		0.8	0.662	3H
3H		174	620	168			321	259	46		1.2		1.2	0.785	3H
3H															3H
	SMC														
N	119	103	183	126	22	39	98	60	-173		0.0		0.4	0.02 *	N
3H	169	240	502	246	363	112	272	141	0		1.0		1.0	1.00	3H
PHA - 1	14980	10955	18325	14634	11615	16412	14,487	2,808	14,215	*	83.1	*	53.4	* 0.00 *	PHA - 1
PHA - 5	52130	55856	64049	55314	42551	49637	53,256	7,162	52,984	*	306.8	*	196.2	* 0.00 *	PHA - 5
PHA - 10	119765	83707	87135	86836	74678	83670	89,298	15,593	89,027	*	514.9	*	328.9	* 0.00 *	PHA - 10
LPS - 1	1359	978	1780	2190	1384	1228	1,486	432	1,215		8.0	*	5.5	* 0.00 *	LPS - 1
LPS - 5	1896	2382	1231	1538	1196	2132	1,729	487	1,457		9.4	*	6.4	* 0.00 *	LPS - 5
LPS - 10	1102	1447	62	1948	1309	1243	1,185	622	913		6.3	*	4.4	* 0.01 *	LPS - 10
LPS - 20	212	1341	1428	1801	1722	348	1,142	691	870		6.0	*	4.2	* 0.01 *	LPS - 20
LPS - 40	265	199	101	57	24	140	131	90	-141		0.2		0.5	0.07	LPS - 40
	PBMC														
N	150	165	123	76	34	71	103	51	-536		0.0		0.2	0.04 *	N
3H	369	488	325	316	606	1732	639	546	0		1.0		1.0	1.00	3H
PHA - 1	54539	40140	54420	73667	90819	106889	70,079	25,223	69,440	*	130.5	*	109.6	* 0.00 *	PHA - 1
PHA - 5	46902	22293	44067	50650	68697	99582	55,365	26,261	54,726	*	103.1	*	86.6	* 0.00 *	PHA - 5
PHA - 10	21891	14858	21174	30844	42366	88821	36,659	27,278	36,019	*	68.2	*	57.4	* 0.01 *	PHA - 10
LPS - 1	1284	2056	2059	3358	4625	4481	2,977	1,392	2,338		5.4	*	4.7	* 0.00 *	LPS - 1
LPS - 5	604	1621	1694	2292	2187	1287	1,614	620	975		2.8	*	2.5	* 0.02 *	LPS - 5
LPS - 10	339	1306	952	1371	1812	330	1,018	596	379		1.7		1.6	0.28	LPS - 10
LPS - 20	58	557	453	615	478	54	369	249	-270		0.5		0.6	0.30	LPS - 20
LPS - 40	35	49	41	36	73	101	56	26	-583		-0.1		0.1	0.03 *	LPS - 40

Raw data for Negative control duck 1K

1K	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	115	100															
Total 3H	374	395															
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1		<0.05	
1-15 [1]	142	119	96	196	63	39	109	56	-265		0.0		0.3		0.135		1-15 [1]
1-15 [10]	157	725	688	576	447	377	495	213	121		1.5		1.3		0.533		1-15 [10]
1-15 [20]	158	668	474	455	1013	130	483	331	109		1.4		1.3		0.630		1-15 [20]
7-14W-27 [1]	78	288	438	596	435	178	336	191	-39		0.9		0.9		0.836		7-14W-27 [1]
7-14W-27 [10]	89	250	603	240	312	164	276	178	-98		0.6		0.7		0.597		7-14W-27 [10]
7-14W-27 [20]	161	117	79	158	83	53	109	44	-266		0.0		0.3		0.133		7-14W-27 [20]
7-14R-27 [1]	197	139	139	88	98	86	125	43	-250		0.0		0.3		0.155		7-14R-27 [1]
7-14R-27 [10]	135	63	92	96	30	38	76	40	-299		-0.2		0.2		0.096		7-14R-27 [10]
7-14R-27 [20]	38	829	142	503	451	115	346	303	-28		0.9		0.9		0.897		7-14R-27 [20]
22-41 [1]	68	198	450	402	216	415	292	153	-83		0.7		0.8		0.646		22-41 [1]
22-41 [10]	166	355	570	487	219	89	314	189	-60		0.8		0.8		0.748		22-41 [10]
22-41 [20]	151	549	946	431	140	75	382	333	8		1.0		1.0		0.972		22-41 [20]
37-56 [1]	84	70	104	67	92	388	134	125	-240		0.1		0.4		0.190		37-56 [1]
37-56 [10]	22	49	46	117	78	101	69	36	-305		-0.2		0.2		0.089		37-56 [10]
37-56 [20]	604	131	2104	240	772	242	682	739	308		2.2	*	1.8		0.426		37-56 [20]
54-73 [1]		738	4441	2334	2158	200	1,974	1,653	1,600		7.2	*	5.3	*	0.068		54-73 [1]
54-73 [10]		2286	6664	1522	2771	203	2,689	2,424	2,315		9.9	*	7.2	*	0.068		54-73 [10]
54-73 [20]		528	1419	5946	1555	240	1,938	2,310	1,563		7.0	*	5.2	*	0.174		54-73 [20]
71-90 [1]	161	86	67	81	82	238	119	67	-255		0.0		0.3		0.151		71-90 [1]
71-90 [10]	115	121	130	153	143	171	139	21	-235		0.1		0.4		0.175		71-90 [10]
71-90 [20]	175	181	158	107	76	127	137	41	-237		0.1		0.4		0.174		71-90 [20]
87-106 [1]	155	180	2986	1915	1771	158	1,194	1,204	820		4.2	*	3.2	*	0.182		87-106 [1]
87-106 [10]		2067	2993	4410	5571	184	3,045	2,085	2,671		11.3	*	8.1	*	0.023	*	87-106 [10]
87-106 [20]	158	1339	10586	4796	8182	213	4,212	4,414	3,838		15.8	*	11.3	*	0.087		87-106 [20]
101-120 [1]	216	3405	11771	4387	4244	137	4,027	4,247	3,652		15.1	*	10.8	*	0.090		101-120 [1]
101-120 [10]	65	46	901	233	50	154	242	331	-133		0.5		0.6		0.559		101-120 [10]
101-120 [20]	156	202	140	116	107	121	140	35	-234		0.1		0.4		0.179		101-120 [20]
116-130 [1]	61	161	160	106	151	363	167	104	-207		0.2		0.4		0.244		116-130 [1]
116-130 [10]	74	143	155	94	88	133	115	33	-260		0.0		0.3		0.140		116-130 [10]
116-130 [20]	195	983	1888	1238	110	122	756	735	382		2.5	*	2.0		0.327		116-130 [20]
126-140 [1]	260	1188	2256	1523	495	97	970	837	596		3.3	*	2.6	*	0.180		126-140 [1]
126-140 [10]	155	2864	3439	2015	1223	526	1,704	1,302	1,329		6.1	*	4.6	*	0.057		126-140 [10]
126-140 [20]	156	3939	2472	1804	231	282	1,481	1,542	1,106		5.3	*	4.0	*	0.156		126-140 [20]
136-150 [1]	161	101	79	122	88	155	118	34	-257		0.0		0.3		0.144		136-150 [1]
136-150 [10]	98	125	160	178	67	96	121	42	-254		0.0		0.3		0.149		136-150 [10]
136-150 [20]	169	476	100	52	54	74	154	163	-220		0.2		0.4		0.242		136-150 [20]
146-160 [1]	101	469	942	510	728	94	474	337	100		1.4		1.3		0.662		146-160 [1]
146-160 [10]	117	2006	3605	967	4151	136	1,830	1,739	1,456		6.6	*	4.9	*	0.102		146-160 [10]
146-160 [20]	630	1883	1070	539	733	80	823	610	448		2.7	*	2.2	*	0.193		146-160 [20]
156-170 [1]	111	890	930	1706	756	188	764	581	389		2.5	*	2.0		0.237		156-170 [1]
156-170 [10]	58	187	895	284	104	83	269	318	-106		0.6		0.7		0.634		156-170 [10]
156-170 [20]	138	189	430	101	71	76	168	136	-207		0.2		0.4		0.257		156-170 [20]
166-180 [1]	43	42	44	72	108	101	68	30	-306		-0.2		0.2		0.088		166-180 [1]
166-180 [10]	30	38	77	119	174	57	83	55	-292		-0.1		0.2		0.104		166-180 [10]
166-180 [20]	81	106	157	413	120	59	156	130	-218		0.2		0.4		0.231		166-180 [20]
176-195 [1]	78	229	374	478	238	161	260	145	-115		0.6		0.7		0.523		176-195 [1]
176-195 [10]	165	193	340	896	443	72	352	298	-23		0.9		0.9		0.916		176-195 [10]
176-195 [20]	48	166	341	399	246	53	209	146	-165		0.4		0.6		0.363		176-195 [20]
191-210 [1]	54	108	40	50	66	56	62	24	-312		-0.2		0.2		0.083		191-210 [1]
191-210 [10]	47	46	56	53	70	40	52	10	-322		-0.2		0.1		0.074		191-210 [10]
191-210 [20]	95	60	46	92	85	38	69	25	-305		-0.2		0.2		0.089		191-210 [20]
210-229 [1]	61	100	72	138	100	80	92	27	-282		-0.1		0.2		0.111		210-229 [1]
210-229 [10]	55	63	597	853	75	40	281	354	-94		0.6		0.7		0.688		210-229 [10]
210-229 [20]	45	411	1005	1644	235	103	574	628	200		1.8		1.5		0.555		210-229 [20]
229-248 [1]	28	197	753	1013	196	68	376	407	2		1.0		1.0		0.995		229-248 [1]
229-248 [10]	22	19	160	96	21	28	58	58	-317		-0.2		0.2		0.082		229-248 [10]
229-248 [20]	26	31	69	61	66	70	54	20	-320		-0.2		0.1		0.076		229-248 [20]
248-267 [1]	61	58	42	47	29	45	47	12	-327		-0.3		0.1		0.071		248-267 [1]
248-267 [10]	58	75	121	180	51	65	92	50	-283		-0.1		0.2		0.113		248-267 [10]
248-267 [20]	73	169	435	340	185	99	217	142	-157		0.4		0.6		0.384		248-267 [20]
267-286 [1]	57	318	568	1183	792	236	526	413	151		1.6		1.4		0.552		267-286 [1]
267-286 [10]	41	193	305	647	1387	2064	773	794	399		2.5	*	2.1		0.336		267-286 [10]
267-286 [20]	57	66	483	240	246	24	186	174	-188		0.3		0.5		0.318		267-286 [20]
287-306 [1]	23	55	34	83	88	98	64	31	-311		-0.2		0.2		0.084		287-306 [1]
287-306 [10]	60	109	162	87	36	85	90	43	-284		-0.1		0.2		0.111		287-306 [10]
287-306 [20]	39	169	311	109	1165	2286	680	889	306		2.2	*	1.8		0.497		287-306 [20]
307-326 [1]	56	178	402	49	2200	399	547	825	173		1.7		1.5		0.679		307-326 [1]
307-326 [10]	45	623	453	63	2284	2003	912	984	538		3.1	*	2.4	*	0.284		307-326 [10]
307-326 [20]	50	574	1014	39	1254	611	590	493	216		1.8		1.6		0.450		307-326 [20]
sAg 10	64	64	26	138	128	79	83	43	-291		-0.1		0.2		0.104		sAg 10
sAg 100	120	83	48	82	55	76	77	25	-297		-0.1		0.2		0.096		sAg 100
N	56	161	261	330	199	101	185	101	-190		0.3		0.5		0.283		N
N	38	76	45	32	41	42	46	16	-329		-0.3		0.1		0.070		N
3H					1080		1,080		706		3.7	*	2.9	*			3H
3H																	3H
3H		191	193	234	173		198	26	-176		0.3		0.5		0.409		3H
3H																	3H
	SMC																
N	57	36	259	149	118	41	110	86	-656		0.0		0.1		0.01	*	N
3H	107	1040	951	1230	1171	96	766	524	0		1.0		1.0		1.00		3H
PHA - 1	4397	13077	12891	13440	7961	1963	8,954	4,965	8,189	*	13.5	*	11.7	*	0.00	*	PHA - 1
PHA - 5	11788	18620	22115	19043	10581	9246	15,232	5,342	14,467	*	23.1	*	19.9	*	0.00	*	PHA - 5
PHA - 10	6174	17844	15480	19352	18453	12060	14,893	5,013	14,128	*	22.5	*	19.5	*	0.00	*	PHA - 10
LPS - 1	252	3051	4303	2146	2605	904	2,210	1,469	1,445		3.2	*	2.9	*	0.05	*	LPS - 1
LPS - 5	290	3525	3204	4006	2987	463	2,412	1,615	1,647		3.5	*	3.2	*	0.04	*	LPS - 5
LPS - 10	204	1936	2732	3050	2032	141	1,682	1,242	917		2.4	*	2.2	*	0.13		LPS - 10
LPS - 20	85	265	480	425	272	337	310	139	-455		0.3		0.4		0.07		LPS - 20
LPS - 40	237	198	118	137	107	249	174	62	-591		0.1		0.2		0.02	*	LPS - 40
	PBMC																
N	106	128	80	43	105	528	165	180	-250		0.0		0.4		0.03	*	N
3H	230	501	578	503	216	460	415	153	0		1.0		1.0		1.00		3H
PHA - 1	9899	10565	16312	10425	4155	19164	11,753	5,292	11,339	*	46.4	*	28.4	*	0.00	*	PHA - 1
PHA - 5	11292	15750	12789	19284	14844	13818	14,629	2,762	14,215	*	57.9	*	35.3	*	0.00	*	PHA - 5
PHA - 10	3936	6159	3219	4945	2710	3776	4,124	1,248	3,709		15.9	*	9.9	*	0.00	*	PHA - 10
LPS - 1	203	819	780	841	978	199	637	344	222		1.9		1.5		0.18		LPS - 1
LPS - 5	144	631	1063	794	328	112	512	381	97		1.4		1.2		0.58		LPS - 5
LPS - 10	81	496	549	954	465	56	433	333	19		1.1		1.0		0.90		LPS - 10
LPS - 20	121	147	216	239	112	61	149	67	-265		-0.1		0.4		0.00	*	LPS - 20
LPS - 40	101	80	39	56	49	108	72	29	-343		-0.4		0.2		0.00	*	LPS - 40

Raw data for Negative control duck 1L

1L	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	61	34															
Total 3H	183	202								>5000		>2.1		>2.1		<0.05	
	R1	R2	R3	R4	R5	R6	Mean	SD									
1-15 [1]																	1-15 [1]
1-15 [10]																	1-15 [10]
1-15 [20]	402	574	48	19	1462	322	471	530	289		3.4	*	2.6	*	0.037	*	1-15 [20]
7-14W-27 [1]																	7-14W-27 [1]
7-14W-27 [10]	1031	139	31	15		124	268	430	85		1.7		1.5		0.492		7-14W-27 [10]
7-14W-27 [20]	311	56	76	15	17	33	85	113	-98		0.2		0.5		0.265		7-14W-27 [20]
7-14R-27 [1]	25	49	70	32	55	30	44	17	-139		-0.1		0.2		0.106		7-14R-27 [1]
7-14R-27 [10]																	7-14R-27 [10]
7-14R-27 [20]	23	36	131	630	205	1062	348	415	165		2.4	*	1.9		0.164		7-14R-27 [20]
22-41 [1]	185	45	59		841	666	359	369	177		2.5	*	2.0		0.137		22-41 [1]
22-41 [10]																	22-41 [10]
22-41 [20]																	22-41 [20]
37-56 [1]	40	203	197	312	45	77	146	109	-37		0.7		0.8		0.670		37-56 [1]
37-56 [10]	66	83	163	476	96	71	159	159	-24		0.8		0.9		0.793		37-56 [10]
37-56 [20]	156	832	949	657	421	91	518	354	335		3.8	*	2.8	*	0.004	*	37-56 [20]
54-73 [1]		247	739	216	603	122	385	269	203		2.7	*	2.1	*	0.063		54-73 [1]
54-73 [10]		465	498	279	384	985	522	272	340		3.8	*	2.9	*	0.003	*	54-73 [10]
54-73 [20]	2021	476	970	432	398	148	741	682	558		5.6	*	4.1	*	0.001	*	54-73 [20]
71-90 [1]	59	610	1139		515	90	483	442	300		3.5	*	2.6	*	0.023	*	71-90 [1]
71-90 [10]	36	60	64	49	26	39	46	15	-137		-0.1		0.3		0.112		71-90 [10]
71-90 [20]	31	34	124	158	358	255	160	128	-23		0.8		0.9		0.796		71-90 [20]
87-106 [1]		583	746	228	1166	2317	1,008	806	825		7.8	*	5.5	*	0.000	*	87-106 [1]
87-106 [10]	2326	3773	321	1237	1788	323	1,628	1,317	1,445		12.9	*	8.9	*	0.000	*	87-106 [10]
87-106 [20]	2396	208	1837	466	458	1185	1,092	876	909		8.5	*	6.0	*	0.000	*	87-106 [20]
101-120 [1]	998	262	267		245	929	540	387	358		3.9	*	3.0	*	0.005	*	101-120 [1]
101-120 [10]	98		325	31	74	96	125	115	-58		0.5		0.7		0.543		101-120 [10]
101-120 [20]	245	359	196	99	156	81	189	103	7		1.1		1.0		0.939		101-120 [20]
116-130 [1]	216	156	114	162	173	110	155	39	-28		0.8		0.8		0.745		116-130 [1]
116-130 [10]	670		248	167	231	254	314	202	131		2.1		1.7		0.196		116-130 [10]
116-130 [20]																	116-130 [20]
126-140 [1]	1252	463	531	707	3231	699	1,147	1,058	965		9.0	*	6.3	*	0.000	*	126-140 [1]
126-140 [10]	2368	1195	2292		3919	435	2,042	1,322	1,859		16.3	*	11.2	*	0.000	*	126-140 [10]
126-140 [20]	255	1047	1952	491	1333	826	984	610	801		7.6	*	5.4	*	0.000	*	126-140 [20]
136-150 [1]	83	119	245	63	146	4088	791	1,617	608		6.0	*	4.3	*	0.070		136-150 [1]
136-150 [10]	107	173	295	81	74	83	136	86	-47		0.6		0.7		0.583		136-150 [10]
136-150 [20]	73	89	147	96	99	65	95	29	-88		0.3		0.5		0.302		136-150 [20]
146-160 [1]	728	117	126	235	598		361	284	178		2.5	*	2.0		0.104		146-160 [1]
146-160 [10]	1583	1027	1096	507	472	364	842	474	659		6.4	*	4.6	*	0.000	*	146-160 [10]
146-160 [20]		677	892	402	275	383	526	253	343		3.8	*	2.9	*	0.003	*	146-160 [20]
156-170 [1]	644	74	174	816	746	600	509	309	326		3.7	*	2.8	*	0.004	*	156-170 [1]
156-170 [10]	284	77	83	90	328	317	197	125	14		1.1		1.1		0.874		156-170 [10]
156-170 [20]	256	204	114	70	191	208	174	69	-9		0.9		1.0		0.917		156-170 [20]
166-180 [1]	49	44	67	156	80	362	126	122	-56		0.5		0.7		0.521		166-180 [1]
166-180 [10]	113	179	404	715	125	65	267	250	84		1.7		1.5		0.390		166-180 [10]
166-180 [20]	424	819	399	240	198	45	354	267	172		2.4	*	1.9		0.091		166-180 [20]
176-195 [1]	343	411	427	561	910	251	484	232	301		3.5	*	2.6	*	0.004	*	176-195 [1]
176-195 [10]	698	844	760	713		778	759	58	576		5.7	*	4.2	*	0.000	*	176-195 [10]
176-195 [20]	251	1154	313	355	1346	120	590	521	407		4.4	*	3.2	*	0.004	*	176-195 [20]
191-210 [1]	120	49	91	397	78	41	129	134	-53		0.6		0.7		0.546		191-210 [1]
191-210 [10]	262	99	56	35	86	49	98	84	-85		0.3		0.5		0.326		191-210 [10]
191-210 [20]	50	51	99	120	125	89	89	33	-94		0.2		0.5		0.272		191-210 [20]
210-229 [1]	69	779	645	950		61	501	412	318		3.6	*	2.7	*	0.013	*	210-229 [1]
210-229 [10]	489	703	1103	365	1801	81	757	616	574		5.7	*	4.1	*	0.000	*	210-229 [10]
210-229 [20]	613	1156	320	795	267	76	538	397	355		3.9	*	2.9	*	0.004	*	210-229 [20]
229-248 [1]	768	2371	379	478	659	5029	1,614	1,826	1,431		12.8	*	8.8	*	0.000	*	229-248 [1]
229-248 [10]	6600	1107	2422	1645	8728	185	3,448	3,413	3,265		27.9	*	18.9	*	0.000	*	229-248 [10]
229-248 [20]	293	1252	4141	1592	805	55	1,356	1,480	1,174		10.7	*	7.4	*	0.000	*	229-248 [20]
248-267 [1]	71	80	156	201	57	242	135	77	-48		0.6		0.7		0.574		248-267 [1]
248-267 [10]	42	70	427	170	1643	64	403	624	220		2.8	*	2.2	*	0.144		248-267 [10]
248-267 [20]	355	686	672	379	2342	1976	1,068	864	886		8.3	*	5.8	*	0.000	*	248-267 [20]
267-286 [1]	444	2992	1653	489	741	945	1,211	977	1,028		9.5	*	6.6	*	0.000	*	267-286 [1]
267-286 [10]	517	1428	461	269	1075	230	663	482	481		5.0	*	3.6	*	0.001	*	267-286 [10]
267-286 [20]	275	263	3479	781	649	89	923	1,279	740		7.1	*	5.1	*	0.008	*	267-286 [20]
287-306 [1]	132	192	164	80	87	148	134	44	-49		0.6		0.7		0.565		287-306 [1]
287-306 [10]	21	46	398	105	38	126	122	141	-60		0.5		0.7		0.497		287-306 [10]
287-306 [20]	131	266	192	92	509	51	207	166	24		1.2		1.1		0.789		287-306 [20]
307-326 [1]		687	335	460	140	292	383	205	200		2.7	*	2.1		0.054		307-326 [1]
307-326 [10]		947	286	864	777	289	633	321	450		4.7	*	3.5	*	0.000	*	307-326 [10]
307-326 [20]	1035	531	319	645	801	243	596	298	413		4.4	*	3.3	*	0.000	*	307-326 [20]
sAg 10	24	26	44	57		424	115	173	-68		0.4		0.6		0.492		sAg 10
sAg 100	30	41	64	26	48	61	45	16	-138		-0.1		0.2		0.110		sAg 100
N	84	71	45	15	63	49	55	24	-128		-0.1		0.3		0.136		N
N	67	95	39	41	142	26	68	44	-114		0.1		0.4		0.183		N
3H	44	282	266	185	176	113	178	90	-5		1.0		1.0		0.954		3H
3H	121	181	153	234	89	266	174	67	-9		0.9		1.0		0.919		3H
3H	1049	204	64	18	37	66	240	402	57		1.5		1.3		0.620		3H
3H	160	98	143	95	284	56	139	80	-43		0.6		0.8		0.613		3H
	SMC																
N	97	185	152	59	22	41	92	65	-283		0.0		0.2		0.08		N
3H	184	549	30	512	913	64	375	344	0		1.0		1.0		1.00		3H
PHA - 1	51410	51071	53703	56738	60054	77754	58,455	10,047	58,080	*	206.3	*	155.8	*	0.00	*	PHA - 1
PHA - 5	86408	55498	87513	87287	76029	120670	85,567	21,141	85,192	*	302.1	*	228.0	*	0.00	*	PHA - 5
PHA - 10	103052	83514	133905	105118	109987	125557	110,189	17,815	109,813	*	389.1	*	293.6	*	0.00	*	PHA - 10
LPS - 1	863	1256	3769	2254	3336	1721	2,200	1,154	1,824		7.4	*	5.9	*	0.00	*	LPS - 1
LPS - 5	300	688	555	4405	1076	1019	1,340	1,529	965		4.4	*	3.6	*	0.16		LPS - 5
LPS - 10	291	3461	6994	735	3372	935	2,631	2,538	2,256		9.0	*	7.0	*	0.06		LPS - 10
LPS - 20	42	430	689	887	1629	425	683	544	308		2.1		1.8		0.27		LPS - 20
LPS - 40	35	64	63	60	53	267	90	87	-285		0.0		0.2		0.08		LPS - 40
	PBMC																
N	102	30	37	41	47	80	56	28	-127		0.0		0.3		0.01	*	N
3H	76	138	269	308	219	90	183	96	0		1.0		1.0		1.00		3H
PHA - 1	8935	16258	16808	27436	34466	4715	18,103	11,164	17,920	*	142.0	*	98.9	*	0.00	*	PHA - 1
PHA - 5	15919	17486	27738	20089	24747	33697	23,279	6,760	23,096	*	182.7	*	127.2	*	0.00	*	PHA - 5
PHA - 10	10911	11862	18602	12840	22117	19372	15,951	4,659	15,768	*	125.1	*	87.2	*	0.00	*	PHA - 10
LPS - 1	114	199	277	314	346	104	226	103	43		1.3		1.2		0.48		LPS - 1
LPS - 5	52	158	393	257	283	104	208	127	25		1.2		1.1		0.71		LPS - 5
LPS - 10	87	60	174	171	176	81	124	54	-59		0.5		0.7		0.22		LPS - 10
LPS - 20	83	31	55	72	48	100	65	25	-118		0.1		0.4		0.02	*	LPS - 20
LPS - 40	36	50	177	88	67	72	81	50	-102		0.2		0.4		0.04	*	LPS - 40

Raw data for Negative control duck 2A

2A	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	86	49															
Total 3H	1159	1567								>5000		>2.1		>2.1		<0.05	
	R1	R2	R3	R4	R5	R6	Mean	SD									
1-15 [1]	825	1203	312	182	307	1351	697	503	-462		0.6		0.6		0.481		1-15 [1]
1-15 [10]	795	393	245	260	677	424	466	224	-693		0.4		0.4		0.289		1-15 [10]
1-15 [20]	1911	1140	359	168	454	12274	2718	4725	1,559		2.5	*	2.3	*	0.103		1-15 [20]
7-14W-27 [1]	228	4287	335	236	3217	382	1448	1818	289		1.3		1.2		0.680		7-14W-27 [1]
7-14W-27 [10]	4593	570	11936	17298	2445	6399	7207	6299	6,048	*	6.6	*	6.2	*	0.000	*	7-14W-27 [10]
7-14W-27 [20]	13641	1139	4433	327	551	2243	3722	5086	2,564		3.4	*	3.2	*	0.012	*	7-14W-27 [20]
7-14R-27 [1]	942	3405	435	1154	456	404	1133	1155	-26		1.0		1.0		0.969		7-14R-27 [1]
7-14R-27 [10]	473	530	384	2340	209	438	729	797	-430		0.6		0.6		0.516		7-14R-27 [10]
7-14R-27 [20]	362	5076	882	1209	937	171	1440	1823	281		1.3		1.2		0.689		7-14R-27 [20]
22-41 [1]	185	8253	1019	403	731	121	1785	3187	627		1.6		1.5		0.433		22-41 [1]
22-41 [10]	768	445	580	291	227	2411	787	819	-372		0.7		0.7		0.574		22-41 [10]
22-41 [20]	455	1656	325	291	1675	381	797	675	-361		0.7		0.7		0.583		22-41 [20]
37-56 [1]	1599	706	316	298	204	816	657	523	-502		0.5		0.6		0.444		37-56 [1]
37-56 [10]	644	432	1185	848		776	777	278	-382		0.6		0.7		0.593		37-56 [10]
37-56 [20]	12481	479	705	503	714	236	2520	4883	1,361		2.3	*	2.2	*	0.161		37-56 [20]
54-73 [1]	671	587	673	302	766	400	567	179	-592		0.4		0.5		0.364		54-73 [1]
54-73 [10]	165	282	381	84	995	3536	907	1328	-251		0.8		0.8		0.711		54-73 [10]
54-73 [20]	195	1033	226	692	436	261	474	331	-685		0.4		0.4		0.296		54-73 [20]
71-90 [1]	903	433	436	225	1163	1251	735	429	-423		0.6		0.6		0.517		71-90 [1]
71-90 [10]	287	2604	305	562	297	1725	963	977	-195		0.8		0.8		0.769		71-90 [10]
71-90 [20]	816	634	609	1192	2547	300	1016	805	-142		0.9		0.9		0.829		71-90 [20]
87-106 [1]	4942	1453	641	767	337	333	1412	1777	254		1.2		1.2		0.717		87-106 [1]
87-106 [10]	365	394	774	1311	441	538	637	362	-521		0.5		0.5		0.425		87-106 [10]
87-106 [20]	770	588	410	803	1126	752	742	239	-417		0.6		0.6		0.522		87-106 [20]
101-120 [1]	797		2884	519	723	441	1073	1023	-86		0.9		0.9		0.906		101-120 [1]
101-120 [10]	587	1948	1951	536	953	3984	1660	1303	501		1.5		1.4		0.460		101-120 [10]
101-120 [20]	304	549	1440	593	912	2232	1005	717	-154		0.9		0.9		0.815		101-120 [20]
116-130 [1]	595	533	6029	519	289	8122	2681	3469	1,523		2.4	*	2.3	*	0.069		116-130 [1]
116-130 [10]	500	801	537	439	750	389	569	168	-589		0.5		0.5		0.367		116-130 [10]
116-130 [20]	414	1019	331	468	1910	307	742	630	-417		0.6		0.6		0.526		116-130 [20]
126-140 [1]	437	1542	2945	585	536	749	1132	974	-26		1.0		1.0		0.968		126-140 [1]
126-140 [10]	471	2704	472	1485	3491	1299	1654	1219	495		1.5		1.4		0.463		126-140 [10]
126-140 [20]	4978	653	664	5709	889	2429	2554	2272	1,395		2.3	*	2.2	*	0.060		126-140 [20]
136-150 [1]	4667	461	402	189	921	2468	1518	1750	359		1.3		1.3		0.607		136-150 [1]
136-150 [10]	479	596	1848	347	469	968	785	563	-374		0.7		0.7		0.568		136-150 [10]
136-150 [20]	257	11105		1871	159	137	2706	4752	1,547		2.4	*	2.3	*	0.119		136-150 [20]
146-160 [1]	1056	272	1609	548	2195	147	1005	842	-154		0.9		0.9		0.816		146-160 [1]
146-160 [10]	1093	83	1387	1767	484	2365	1197	835	38		1.0		1.0		0.954		146-160 [10]
146-160 [20]	101	225	639	2019	924	849	793	685	-366		0.7		0.7		0.578		146-160 [20]
156-170 [1]	5522	6102	1190	1303	1079	1900	2849	2320	1,691		2.6	*	2.5	*	0.024	*	156-170 [1]
156-170 [10]	177	1215	5714	1715	1778	464	1844	2004	685		1.6		1.6		0.338		156-170 [10]
156-170 [20]	815	534	1642	1141	1826	2088	1341	609	182		1.2		1.2		0.781		156-170 [20]
166-180 [1]	355	787	2937	2090	417	5943	2088	2148	930		1.9		1.8		0.201		166-180 [1]
166-180 [10]	208	278	651	292	2779	716	821	982	-338		0.7		0.7		0.612		166-180 [10]
166-180 [20]	462	273	362	551	864	367	480	211	-679		0.4		0.4		0.299		166-180 [20]
176-195 [1]	323	4300	450	875	110	365	1071	1602	-88		0.9		0.9		0.898		176-195 [1]
176-195 [10]	75	563	144	197	1335	1308	604	581	-555		0.5		0.5		0.399		176-195 [10]
176-195 [20]	653	498	181	2068	409	622	739	673	-420		0.6		0.6		0.523		176-195 [20]
191-210 [1]	368	773		2674	312	202	866	1034	-293		0.7		0.7		0.687		191-210 [1]
191-210 [10]	352	3884	301	672	396	373	996	1421	-162		0.8		0.9		0.812		191-210 [10]
191-210 [20]	252	155	283	681	531	245	358	203	-801		0.3		0.3		0.222		191-210 [20]
210-229 [1]	186	230	454	617	2198	284	662	770	-497		0.5		0.6		0.452		210-229 [1]
210-229 [10]	2869		702	1440	4461	429	1980	1679	822		1.8		1.7		0.277		210-229 [10]
210-229 [20]	5393	1632	452	2336	899	16553	4544	6137	3,386		4.2	*	3.9	*	0.003	*	210-229 [20]
229-248 [1]		1169	544	765	259	484	644	344	-514		0.5		0.6		0.472		229-248 [1]
229-248 [10]	464	235	653	305	1205	3355	1036	1188	-122		0.9		0.9		0.855		229-248 [10]
229-248 [20]	494	445	367	392	821	527	508	165	-651		0.4		0.4		0.319		229-248 [20]
248-267 [1]	696	964	902	860	607	4308	1390	1436	231		1.2		1.2		0.735		248-267 [1]
248-267 [10]	2090	796	374	2564	9088	992	2651	3260	1,492		2.4	*	2.3	*	0.068		248-267 [10]
248-267 [20]	596	1221	534	820	5004	297	1412	1787	253		1.2		1.2		0.717		248-267 [20]
267-286 [1]	270	1124	1015	1043	2899	675	1171	904	12		1.0		1.0		0.985		267-286 [1]
267-286 [10]	8496	645	682	342	2243	430	2140	3191	981		1.9		1.8		0.222		267-286 [10]
267-286 [20]	5748	407	2768	933	1180	2106	2190	1938	1,032		2.0		1.9		0.149		267-286 [20]
287-306 [1]	272	168	6372	3457	1786	544	2100	2437	941		1.9		1.8		0.207		287-306 [1]
287-306 [10]	519	285	1318	322	311	1083	640	449	-519		0.5		0.6		0.428		287-306 [10]
287-306 [20]	2155	2600	5055	1146	442	633	2005	1717	847		1.8		1.7		0.227		287-306 [20]
307-326 [1]	820	982	218	193	577	262	509	338	-650		0.4		0.4		0.321		307-326 [1]
307-326 [10]	266	366	1949	345	1280	1397	934	704	-225		0.8		0.8		0.733		307-326 [10]
307-326 [20]	437	263	1511	220	365	532	555	482	-604		0.4		0.5		0.357		307-326 [20]
sAg 10	711	1044	815	1776	755	707	968	415	-191		0.8		0.8		0.770		sAg 10
sAg 100	272	335	274	623	1038	1993	756	674	-403		0.6		0.7		0.540		sAg 100
N	229	73	60	63	120	61	101	67	-1,058		0.0		0.1		0.108		N
N	47	87	54	94	84	59	71	20	-1,088		0.0		0.1		0.099		N
3H	1776	849	325	563	411	427	725	547	-433		0.6		0.6		0.509		3H
3H	1558	539	587	185	169	932	662	523	-497		0.5		0.6		0.449		3H
3H	183	261	127	452	253	911	365	289	-794		0.3		0.3		0.226		3H
3H	477	395	8559	216	300	1500	1908	3292	749		1.7		1.6		0.354		3H
3H	531	482	640	2531	4822	686	1615	1755	457		1.4		1.4		0.513		3H
3H	2032	444	1233	4343	634	2322	1835	1436	676		1.6		1.6		0.324		3H
3H	1053	355	272	584	2584	1159	1001	855	-157		0.9		0.9		0.812		3H
	SMC																
N	106	65	34	85	53	75	70	25	-1,399		0.0		0.0		0.00	*	N
3H	987	1354	1062	932	1305	3173	1469	852	0		1.0		1.0		1.00		3H
PHA - 1	53270	79387	61876	67069	75285	74312	68533	9759	67,064	*	48.9	*	46.7	*	0.00	*	PHA - 1
PHA - 5	55032	72197	75595	82453	79868	70031	72529	9739	71,061	*	51.8	*	49.4	*	0.00	*	PHA - 5
PHA - 10	22985	70464	61849	69735	73489	73160	61947	19546	60,478	*	44.2	*	42.2	*	0.00	*	PHA - 10
LPS - 1	3399	3819	2902	2926	3051	5864	3660	1134	2,191		2.6	*	2.5	*	0.00	*	LPS - 1
LPS - 5	3527	3688	8348	4580	3513	6903	5093	2052	3,624		3.6	*	3.5	*	0.00	*	LPS - 5
LPS - 10	3384	5088	5034	3903	5295	3483	4365	871	2,896		3.1	*	3.0	*	0.00	*	LPS - 10
LPS - 20	2458	4791	4421	5118	3164	4075	4005	1013	2,536		2.8	*	2.7	*	0.00	*	LPS - 20
LPS - 40	1608	3331	4220	4540	2903	2989	3265	1048	1,796		2.3	*	2.2	*	0.01	*	LPS - 40

Raw data for Negative control duck 2B

2B	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	116	49															
Total 3H	789	998															
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1		<0.05	
1-15 [1]	233	298	101	267	370	791	343	237	-445		0.3		0.4		0.286		1-15 [1]
1-15 [10]	163	79	411	658	1184	353	475	402	-314		0.5		0.6		0.454		1-15 [10]
1-15 [20]	1030	247	846	563	1851	855	899	542	110		1.2		1.1		0.794		1-15 [20]
7-14W-27 [1]	4855	252	418	410	839	99	1146	1834	357		1.5		1.5		0.469		7-14W-27 [1]
7-14W-27 [10]	684	7706	1465	242	261	154	1752	2957	963		2.4	*	2.2	*	0.110		7-14W-27 [10]
7-14W-27 [20]																	7-14W-27 [20]
7-14R-27 [1]	1175	556	345	604	984		733	338	-56		0.9		0.9		0.902		7-14R-27 [1]
7-14R-27 [10]	410	1052	102	2770	792	336	910	972	122		1.2		1.2		0.781		7-14R-27 [10]
7-14R-27 [20]	616	253	325	1833	798	369	699	592	-90		0.9		0.9		0.832		7-14R-27 [20]
22-41 [1]	273	497	1521	149	1280	314	672	580	-116		0.8		0.9		0.783		22-41 [1]
22-41 [10]	191	277	114	167	513	414	279	155	-509		0.2		0.4		0.223		22-41 [10]
22-41 [20]	1304	1046	214	461	904	368	716	431	-73		0.9		0.9		0.862		22-41 [20]
37-56 [1]	976	297	174	274	861	305	481	344	-308		0.5		0.6		0.462		37-56 [1]
37-56 [10]																	37-56 [10]
37-56 [20]	417	535	433	371	130	397	381	135	-408		0.4		0.5		0.327		37-56 [20]
54-73 [1]	658	919	555	3183	180	205	950	1129	161		1.2		1.2		0.717		54-73 [1]
54-73 [10]	205	486	985	258	1558	420	652	523	-137		0.8		0.8		0.745		54-73 [10]
54-73 [20]	168	164	434	442	1051	217	413	337	-376		0.4		0.5		0.369		54-73 [20]
71-90 [1]	144	651	143	519	1126	199	464	388	-325		0.5		0.6		0.438		71-90 [1]
71-90 [10]	140	126	117	356	1191	536	411	417	-378		0.4		0.5		0.368		71-90 [10]
71-90 [20]	285	88	1740	615	1207	213	691	653	-97		0.9		0.9		0.819		71-90 [20]
87-106 [1]	380	9176	733	1091	422	348	2025	3515	1,236		2.8	*	2.6	*	0.064		87-106 [1]
87-106 [10]	237	109	440	2163	413	1037	733	770	-56		0.9		0.9		0.897		87-106 [10]
87-106 [20]	389	185	198	150		453	275	136	-514		0.2		0.3		0.261		87-106 [20]
101-120 [1]	185	273	126	894	448	225	359	284	-430		0.4		0.5		0.303		101-120 [1]
101-120 [10]	125	303	148	1637	304	135	442	591	-347		0.5		0.6		0.413		101-120 [10]
101-120 [20]	147	3987	214	176	1147	236	985	1520	196		1.3		1.2		0.676		101-120 [20]
116-130 [1]	1295	682	1126	1022	1620	326	1012	457	223		1.3		1.3		0.595		116-130 [1]
116-130 [10]	490	1035	215	889	556	392	596	309	-193		0.7		0.8		0.644		116-130 [10]
116-130 [20]	439	1459	1597	590	244	320	775	597	-14		1.0		1.0		0.974		116-130 [20]
126-140 [1]	393	626	722	361	520	181	467	196	-322		0.5		0.6		0.440		126-140 [1]
126-140 [10]	234	284	282	182	759	2760	750	1007	-39		0.9		1.0		0.930		126-140 [10]
126-140 [20]	960	335	119	99	684	195	399	349	-390		0.4		0.5		0.352		126-140 [20]
136-150 [1]	303	381	952	210	202	368	403	280	-386		0.4		0.5		0.355		136-150 [1]
136-150 [10]	267	182	238	3866	385	205	857	1476	69		1.1		1.1		0.883		136-150 [10]
136-150 [20]	1743	1728	567	406	733	6494	1945	2303	1,157		2.7	*	2.5	*	0.034	*	136-150 [20]
146-160 [1]	303	843	98	151	478	612	414	286	-375		0.4		0.5		0.370		146-160 [1]
146-160 [10]	200	718	523	160	872	1095	595	372	-194		0.7		0.8		0.642		146-160 [10]
146-160 [20]	558	358	1256	1178	468	2141	993	677	205		1.3		1.3		0.631		146-160 [20]
156-170 [1]	1529	288	1788	1800	527	112	1007	782	219		1.3		1.3		0.611		156-170 [1]
156-170 [10]	322	1215	2197	237	1728	475	1029	815	240		1.4		1.3		0.577		156-170 [10]
156-170 [20]	263	389	459	247	734	342	406	179	-383		0.4		0.5		0.358		156-170 [20]
166-180 [1]	182	235	1768	892	203	1455	789	697	1		1.0		1.0		0.999		166-180 [1]
166-180 [10]	1574	274	1620	328	1761	150	951	772	163		1.2		1.2		0.705		166-180 [10]
166-180 [20]	504	791	492	321	711	547	561	168	-228		0.7		0.7		0.583		166-180 [20]
176-195 [1]	433	548	1199	312	535	1491	753	475	-36		0.9		1.0		0.932		176-195 [1]
176-195 [10]	598	479	2191	400	411		816	773	27		1.0		1.0		0.954		176-195 [10]
176-195 [20]	995	841	173	602	1264	281	693	421	-96		0.9		0.9		0.818		176-195 [20]
191-210 [1]	348	212	217	444	1167	2468	809	887	21		1.0		1.0		0.962		191-210 [1]
191-210 [10]	825	275	745	671	243	918	613	286	-176		0.7		0.8		0.673		191-210 [10]
191-210 [20]	1251	211	1276	288	1113	311	742	521	-47		0.9		0.9		0.911		191-210 [20]
210-229 [1]	202	163	1195	955	265	157	490	461	-299		0.6		0.6		0.476		210-229 [1]
210-229 [10]	1752	1893	8204	851	237	6272	3202	3243	2,413		4.6	*	4.1	*	0.000	*	210-229 [10]
210-229 [20]	396	2777	10332	2227	619	148	2750	3863	1,961		3.9	*	3.5	*	0.007	*	210-229 [20]
229-248 [1]	1830	264	669	362	268	712	684	595	-105		0.8		0.9		0.805		229-248 [1]
229-248 [10]	469	9323	1712	3038	1062	1191	2799	3312	2,011		4.0	*	3.5	*	0.003	*	229-248 [10]
229-248 [20]	1918	1018	1903	1527	860	682	1318	539	529		1.8		1.7		0.212		229-248 [20]
248-267 [1]	599	7188	216	2595	174	1261	2006	2694	1,217		2.8	*	2.5	*	0.037	*	248-267 [1]
248-267 [10]	204	383	516	467	1455	613	606	438	-182		0.7		0.8		0.663		248-267 [10]
248-267 [20]	924	483	146	278	574	86	415	313	-374		0.4		0.5		0.371		248-267 [20]
267-286 [1]	1452	381	531	588	769	292	669	418	-120		0.8		0.8		0.774		267-286 [1]
267-286 [10]	770	438	583	388	336	4397	1152	1597	363		1.5		1.5		0.445		267-286 [10]
267-286 [20]	262	544	1039	1009	1817	401	845	572	57		1.1		1.1		0.893		267-286 [20]
287-306 [1]	364	218	368	1226	714	177	511	398	-278		0.6		0.6		0.507		287-306 [1]
287-306 [10]	377	200	114	327	217	66	217	119	-572		0.2		0.3		0.172		287-306 [10]
287-306 [20]	1180	628	251	408	725	231	571	358	-218		0.7		0.7		0.601		287-306 [20]
307-326 [1]	4960	375	1841	384	164	242	1328	1886	539		1.8		1.7		0.280		307-326 [1]
307-326 [10]	106	622	470	1453	1127	240	670	523	-119		0.8		0.8		0.777		307-326 [10]
307-326 [20]	458	4420	577	391	4508	246	1767	2092	978		2.5	*	2.2	*	0.061		307-326 [20]
sAg 10	243	797	370	200	245	113	328	244	-461		0.3		0.4		0.270		sAg 10
sAg 100	147	149	149	172	271	1019	318	347	-471		0.3		0.4		0.262		sAg 100
N	61	92	103	97	67	63	81	19	-708		-0.1		0.1		0.092		N
N	91	163	223	120	160	149	151	45	-638		0.1		0.2		0.128		N
3H	597	456	962	407	831	771	671	220	-118		0.8		0.9		0.776		3H
3H	1948	652	671	3291	469	258	1215	1177	426		1.6		1.5		0.343		3H
3H	214	125	501	147	1567	1350	651	644	-138		0.8		0.8		0.745		3H
3H	134	473	5881	1187	188	1232	1516	2191	727		2.1		1.9		0.167		3H
3H	165	596	809	137	832	727	544	316	-244		0.6		0.7		0.558		3H
3H	317	258	732	201	579	91	363	243	-426		0.4		0.5		0.308		3H
3H	658	1193	499	409	175	434	561	347	-227		0.7		0.7		0.586		3H
	SMC																
N	16	22	49	88	57	35	45	26	-334		0.0		0.1		0.00	*	N
3H	302	515	210	372	450	422	379	109	0		1.0		1.0		1.00		3H
PHA - 1	5954	11940	5576	6029	9412	6702	7602	2538	7,224	*	22.6	*	20.1	*	0.00	*	PHA - 1
PHA - 5	15090	64056	51398	50517	37347	25212	40603	18239	40,225	*	121.4	*	107.3	*	0.00	*	PHA - 5
PHA - 10	30790	50320	15270	9124	9248	13186	21323	16297	20,945	*	63.7	*	56.3	*	0.01	*	PHA - 10
LPS - 1	601	605	187	307	137	189	338	213	-41		0.9		0.9		0.69		LPS - 1
LPS - 5	655	529	533	659	772	539	615	98	236		1.7		1.6		0.00	*	LPS - 5
LPS - 10	471	532	675	633	623	629	594	76	215		1.6		1.6		0.00	*	LPS - 10
LPS - 20	516	632	422	453	979	487	582	208	203		1.6		1.5		0.06		LPS - 20
LPS - 40	369	289	462	365	394	573	409	98	30		1.1		1.1		0.63		LPS - 40

Raw data for Negative control duck 2C

2C	Mean	SD												
Total N	53	25												
Total 3H	391	553							CPM-3H		S.I.		P/N	t-Test
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1 <0.05
1-15 [1]	358	191	430	116	498	96	282	170	-109		0.7		0.7	0.636
1-15 [10]	242	547	151	122	868	1333	544	480	153		1.5		1.4	0.523
1-15 [20]	194	202	132	204	691	298	287	205	-104		0.7		0.7	0.653
7-14W-27 [1]	145	194	221	1331	543	129	427	468	36		1.1		1.1	0.879
7-14W-27 [10]	225	[redacted]	247	559	602	183	363	200	-27		0.9		0.9	0.913
7-14W-27 [20]	771	334	2213	333	733	1774	1026	785	636		2.9	*	2.6	* 0.016 *
7-14R-27 [1]	160	153	492	717	[redacted]	1542	613	571	222		1.7		1.6	0.401
7-14R-27 [10]	636	188	200	1787	937	180	655	634	264		1.8		1.7	0.287
7-14R-27 [20]	163	195	250	2337	206	1119	712	877	321		1.9		1.8	0.224
22-41 [1]	375	175	128	338	1069	1089	529	436	138		1.4		1.4	0.561
22-41 [10]	256	154	285	374	218	114	234	94	-157		0.5		0.6	0.494
22-41 [20]	330	165	273	550	675	160	359	211	-32		0.9		0.9	0.890
37-56 [1]	132	98	113	235	198	330	184	89	-206		0.4		0.5	0.370
37-56 [10]	146	151	275	1198	363	1542	613	602	222		1.7		1.6	0.367
37-56 [20]	676	589	655	641	261	224	508	208	117		1.3		1.3	0.613
54-73 [1]	118	1711	163	258	169	92	419	636	28		1.1		1.1	0.910
54-73 [10]	246	290	199	2465	125	162	581	925	190		1.6		1.5	0.474
54-73 [20]	1136	147	143	269	344	819	476	408	86		1.3		1.2	0.717
71-90 [1]	193	183	347	269	218	121	222	78	-169		0.5		0.6	0.463
71-90 [10]	236	246	540	282	411	135	308	144	-82		0.8		0.8	0.720
71-90 [20]	188	400	240	331	773	131	344	231	-47		0.9		0.9	0.840
87-106 [1]	133	212	211	317	640	220	289	182	-102		0.7		0.7	0.659
87-106 [10]	115	173	1468	259	1886	1346	875	780	484		2.4	*	2.2	* 0.063
87-106 [20]	443	684	1019	557	310	372	564	260	173		1.5		1.4	0.456
101-120 [1]	175	129	118	299	448	829	333	273	-58		0.8		0.9	0.804
101-120 [10]	153	303	169	123	146	202	183	65	-208		0.4		0.5	0.366
101-120 [20]	513	663	1145	660	529	1566	846	421	455		2.3	*	2.2	* 0.059
116-130 [1]	256	123	313	333	106	934	344	304	-47		0.9		0.9	0.842
116-130 [10]	287	155	291	189	895	509	388	278	-3		1.0		1.0	0.990
116-130 [20]	199	220	1758	174	275	751	563	624	172		1.5		1.4	0.485
126-140 [1]	1592	184	1872	497	946	171	877	725	486		2.4	*	2.2	* 0.058
126-140 [10]	219	2901	129	270	235	[redacted]	751	1203	360		2.1		1.9	0.239
126-140 [20]	312	625	1290	2969	1105	617	1153	958	762		3.3	*	3.0	* 0.006 *
136-150 [1]	216	193	496	200	516	547	361	174	-29		0.9		0.9	0.899
136-150 [10]	125	106	165	170	482	1187	373	422	-18		0.9		1.0	0.939
136-150 [20]	416	273	402	168	255	97	269	126	-122		0.6		0.7	0.595
146-160 [1]	185	176	680	1010	100	109	377	379	-14		1.0		1.0	0.952
146-160 [10]	162	219	182	111	125	226	171	47	-220		0.3		0.4	0.339
146-160 [20]	125	134	330	220	2750	715	712	1022	322		2.0		1.8	0.241
156-170 [1]	150	155	169	382	124	189	195	94	-196		0.4		0.5	0.395
156-170 [10]	140	202	176	412	126	282	223	108	-168		0.5		0.6	0.466
156-170 [20]	1867	545	173	[redacted]	183	252	604	722	213		1.6		1.5	0.433
166-180 [1]	104	102	596	109	218	642	295	255	-96		0.7		0.8	0.681
166-180 [10]	1345	705	299	864	142	[redacted]	671	477	280		1.8		1.7	0.284
166-180 [20]	337	287	413	14366	1281	1863	3091	5559	2,700		9.0	*	7.9	* 0.002 *
176-195 [1]	780	204	113	697	519	206	420	284	29		1.1		1.1	0.900
176-195 [10]	126	210	250	249	780	507	354	245	-37		0.9		0.9	0.873
176-195 [20]	197	134	753	529	215	339	361	238	-30		0.9		0.9	0.899
191-210 [1]	232	201	332	180	166	198	218	60	-173		0.5		0.6	0.453
191-210 [10]	239	178	1503	223	87	370	433	532	43		1.1		1.1	0.860
191-210 [20]	2645	116	3147	477	327	151	1144	1373	753		3.2	*	2.9	* 0.016 *
210-229 [1]	138	390	[redacted]	166	293	159	229	109	-161		0.5		0.6	0.522
210-229 [10]	199	759	3472	271	356	2029	1181	1313	790		3.3	*	3.0	* 0.010 *
210-229 [20]	152	179	232	236	291	1181	379	396	-12		1.0		1.0	0.959
229-248 [1]	151	467	153	398	148	110	238	153	-153		0.5		0.6	0.507
229-248 [10]	212	473	548	542	140	292	368	177	-23		0.9		0.9	0.921
229-248 [20]	2713	647	594	306	104	738	850	943	460		2.4	*	2.2	* 0.090
248-267 [1]	138	574	351	196	119	424	300	180	-90		0.7		0.8	0.695
248-267 [10]	112	242	96	905	138	121	269	316	-122		0.6		0.7	0.603
248-267 [20]	107	502	663	278	181	1279	502	433	111		1.3		1.3	0.640
267-286 [1]	115	578	286	126	279	379	294	172	-97		0.7		0.8	0.674
267-286 [10]	384	1095	270	102	124	147	354	378	-37		0.9		0.9	0.875
267-286 [20]	911	297	236	139	843	129	426	356	35		1.1		1.1	0.881
287-306 [1]	247	620	494	[redacted]	1903	145	682	708	291		1.9		1.7	0.285
287-306 [10]	210	201	125	138	247	1677	433	611	42		1.1		1.1	0.863
287-306 [20]	400	248	224	392	1083	169	419	338	29		1.1		1.1	0.903
307-326 [1]	113	219	179	196	156	212	179	40	-212		0.4		0.5	0.358
307-326 [10]	295	647	1421	356	293	1157	695	486	304		1.9		1.8	0.208
307-326 [20]	1937	161	346	506	3209	1937	1349	1207	959		3.8	*	3.5	* 0.002 *
sAg 10	118	657	186	153	217	106	240	209	-151		0.6		0.6	0.514
sAg 100	153	207	126	182	223	137	171	39	-219		0.4		0.4	0.340
N	102	22	58	72	57	92	67	29	-324		0.0		0.2	0.162
N	45	53	26	41	32	31	38	10	-353		0.0		0.1	0.128
3H	102	102	72	99	172	182	122	45	-269		0.2		0.3	0.243
3H	183	589	217	1039	1030	194	542	411	151		1.4		1.4	0.523
3H	397	671	147	125	1463	709	585	496	195		1.6		1.5	0.419
3H	62	136	241	156	314	116	171	91	-220		0.3		0.4	0.340
3H	130	598	569	79	86	140	267	246	-124		0.6		0.7	0.594
3H	220	3306	424	127	229	298	767	1248	377		2.1	*	2.0	0.200
3H	150	230	490	139	572	104	281	200	-110		0.7		0.7	0.634
	SMC													
N	92	34	34	65	93	74	65	27	-333		0.0		0.2	0.00 *
3H	537	310	388	297	479	381	399	94	0		1.0		1.0	1.00
PHA - 1	4437	9602	12306	6352	8986	5806	7915	2907	7,516	*	23.5	*	19.9	* 0.00 *
PHA - 5	18612	35224	35498	42866	26599	29927	31454	8386	31,056	*	94.2	*	78.9	* 0.00 *
PHA - 10	1856	6933	7251	4945	20006	11252	8707	6332	8,309	*	25.9	*	21.8	* 0.01 *
LPS - 1	606	583	743	520	1310	2571	1056	796	657		3.0	*	2.6	* 0.07
LPS - 5	1421	1552	2282	3287	2165	2078	2131	664	1,732		6.2	*	5.3	* 0.00 *
LPS - 10	1408	1772	1948	1998	1911	2473	1918	345	1,520		5.6	*	4.8	* 0.00 *
LPS - 20	1012	1475	1489	1340	1258	926	1250	236	851		3.6	*	3.1	* 0.00 *
LPS - 40	753	646	845	979	869	848	823	113	425		2.3	*	2.1	0.00 *

Raw data for Negative control duck 2D

2D	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	99	65															
Total 3H	263	147															
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1		<0.05	
1-15 [1]	245	231	193	104	312	371	243	93	-21		0.9		0.9		0.741		1-15 [1]
1-15 [10]	246	219	201	268	189	169	215	37	-48		0.7		0.8		0.434		1-15 [10]
1-15 [20]	383	343	894	149	288	361	403	255	140		1.8		1.5		0.054		1-15 [20]
7-14W-27 [1]	216	190	300	385	248	123	244	91	-20		0.9		0.9		0.753		7-14W-27 [1]
7-14W-27 [10]	102	271	180	434	382	107	246	141	-17		0.9		0.9		0.788		7-14W-27 [10]
7-14W-27 [20]	950	304	183	458	175	191	377	301	114		1.7		1.4		0.134		7-14W-27 [20]
7-14R-27 [1]	113	240	173	159	586	789	343	277	80		1.5		1.3		0.275		7-14R-27 [1]
7-14R-27 [10]	81	154	519	137	478	230	267	186	3		1.0		1.0		0.961		7-14R-27 [10]
7-14R-27 [20]	265	175	291	222	416	528	316	132	53		1.3		1.2		0.409		7-14R-27 [20]
22-41 [1]	370	396	560	487	587	502	484	87	220		2.3	*	1.8		0.001	*	22-41 [1]
22-41 [10]	489	241	472	469	436	675	464	138	200		2.2	*	1.8		0.003	*	22-41 [10]
22-41 [20]	287	729	1112	438	614	296	579	314	316		2.9	*	2.2	*	0.000	*	22-41 [20]
37-56 [1]	486	150	347	338	460	362	357	119	94		1.6		1.4		0.142		37-56 [1]
37-56 [10]	223	361	366	293	485	245	329	96	66		1.4		1.2		0.297		37-56 [10]
37-56 [20]	239	266	289	430	411	212	308	91	45		1.3		1.2		0.476		37-56 [20]
54-73 [1]	286	802	1022	408	297	403	536	304	273		2.7	*	2.0		0.001	*	54-73 [1]
54-73 [10]	243	966	271	889	616	711	616	305	353		3.1	*	2.3	*	0.000	*	54-73 [10]
54-73 [20]	217	284	942	589	348	579	493	268	230		2.4	*	1.9		0.002	*	54-73 [20]
71-90 [1]	467	396	477	387	551	459	456	60	193		2.2	*	1.7		0.003	*	71-90 [1]
71-90 [10]	358	515	586	1107	142	535	541	321	277		2.7	*	2.1		0.001	*	71-90 [10]
71-90 [20]	340	486	645	671	644	416	534	139	270		2.6	*	2.0		0.000	*	71-90 [20]
87-106 [1]	400	628	567	541	424	314	479	119	216		2.3	*	1.8		0.001	*	87-106 [1]
87-106 [10]	317	235	594	386	322	949	467	265	204		2.2	*	1.8		0.007	*	87-106 [10]
87-106 [20]	415	298	798	260	457	490	453	191	190		2.2	*	1.7		0.006	*	87-106 [20]
101-120 [1]	343	704	579	596	495	542	543	120	280		2.7	*	2.1		0.000	*	101-120 [1]
101-120 [10]	316	247	514	376	520	649	437	150	174		2.1		1.7		0.010	*	101-120 [10]
101-120 [20]	585	248	421	891	575	431	525	217	262		2.6	*	2.0		0.000	*	101-120 [20]
116-130 [1]	197	292	250	312	502	288	307	104	44		1.3		1.2		0.488		116-130 [1]
116-130 [10]	197	229	474	200	552	316	328	152	65		1.4		1.2		0.320		116-130 [10]
116-130 [20]	206	584	488	257	448	823	468	225	204		2.2	*	1.8		0.005	*	116-130 [20]
126-140 [1]	171	245	695	434	481	543	428	193	165		2.0		1.6		0.017	*	126-140 [1]
126-140 [10]	176	318	337	729	399	482	407	187	144		1.9		1.5		0.035	*	126-140 [10]
126-140 [20]	183	329	181	416	391	431	322	114	59		1.4		1.2		0.355		126-140 [20]
136-150 [1]	360	511	291	317	431	291	367	88	104		1.6		1.4		0.101		136-150 [1]
136-150 [10]	381	480	605	315	271	479	422	123	159		2.0		1.6		0.016	*	136-150 [10]
136-150 [20]	256	302	375	401	267	346	325	59	61		1.4		1.2		0.322		136-150 [20]
146-160 [1]	473	173	122	571	438	276	342	179	79		1.5		1.3		0.237		146-160 [1]
146-160 [10]	548	244	226	550	387	739	449	200	186		2.1	*	1.7		0.008	*	146-160 [10]
146-160 [20]	394	253	251	460	341	362	344	82	80		1.5		1.3		0.200		146-160 [20]
156-170 [1]	278	594	388	342	344	728	446	176	182		2.1	*	1.7		0.008	*	156-170 [1]
156-170 [10]	393	365	334	259	197	544	349	120	85		1.5		1.3		0.181		156-170 [10]
156-170 [20]	304	264	316	362	504	255	334	92	71		1.4		1.3		0.258		156-170 [20]
166-180 [1]	488	264	235	248	645	530	402	175	138		1.8		1.5		0.040	*	166-180 [1]
166-180 [10]	343	182	446	275	454	288	331	106	68		1.4		1.3		0.281		166-180 [10]
166-180 [20]	188	233	384	191	181	146	221	85	-43		0.7		0.8		0.492		166-180 [20]
176-195 [1]	408	465	812	287	577	309	476	196	213		2.3	*	1.8		0.003	*	176-195 [1]
176-195 [10]	259	543	577	585	401	291	443	146	179		2.1		1.7		0.007	*	176-195 [10]
176-195 [20]	133	344	454	643	614	208	399	209	136		1.8		1.5		0.050		176-195 [20]
191-210 [1]	152	619	242	471	465	573	420	185	157		2.0		1.6		0.022	*	191-210 [1]
191-210 [10]	480	455	609	909	346	484	547	196	284		2.7	*	2.1		0.000	*	191-210 [10]
191-210 [20]	348	174	607	596	693	561	497	195	233		2.4	*	1.9		0.001	*	191-210 [20]
210-229 [1]	156	620	290	320	612	289	381	191	118		1.7		1.4		0.083		210-229 [1]
210-229 [10]	275	380	164	373	428	347	328	95	65		1.4		1.2		0.304		210-229 [10]
210-229 [20]	985	806	733	734	1212	589	843	222	580		4.5	*	3.2	*	0.000	*	210-229 [20]
229-248 [1]	266	425	426	237	421	341	353	85	89		1.5		1.3		0.155		229-248 [1]
229-248 [10]	141	141	169	113	250	164	163	47	-100		0.4		0.6		0.106		229-248 [10]
229-248 [20]	105	177	128	172	130	165	146	29	-117		0.3		0.6		0.060		229-248 [20]
248-267 [1]	300	301	272	387	576	449	381	116	118		1.7		1.4		0.068		248-267 [1]
248-267 [10]	272	314	385	499	446	347	377	84	114		1.7		1.4		0.072		248-267 [10]
248-267 [20]	403	417	446	391	470	283	402	65	138		1.8		1.5		0.029	*	248-267 [20]
267-286 [1]	438	264	500	396	502	477	430	91	166		2.0		1.6		0.010	*	267-286 [1]
267-286 [10]	[illegible]	204	442	272	339	387	329	94	66		1.4		1.2		0.338		267-286 [10]
267-286 [20]	353	330	282	430	220	322	323	70	60		1.4		1.2		0.337		267-286 [20]
287-306 [1]	720	308	318	504	504	397	459	154	195		2.2	*	1.7		0.004	*	287-306 [1]
287-306 [10]	548	743	728	478	486	320	551	162	287		2.7	*	2.1		0.000	*	287-306 [10]
287-306 [20]	674	332	246	491	377	812	489	217	225		2.4	*	1.9		0.002	*	287-306 [20]
307-326 [1]	[illegible]	390	436	257	222	406	342	96	79		1.5		1.3		0.250		307-326 [1]
307-326 [10]	344	664	402	384	527	509	472	119	208		2.3	*	1.8		0.002	*	307-326 [10]
307-326 [20]	278	210	688	645	276	470	428	205	165		2.0		1.6		0.018	*	307-326 [20]
sAg 10	205	67	268	131	139	195	168	70	-96		0.4		0.6		0.125		sAg 10
sAg 100	403	297	619	142	216	151	305	182	41		1.3		1.2		0.534		sAg 100
N	96	153	46	33	62	47	73	45	-190		-0.2		0.3		0.003	*	N
N	218	83	184	50	41	172	125	76	-139		0.2		0.5		0.029	*	N
3H	295	188	105	379	118	165	208	107	-55		0.7		0.8		0.384		3H
3H	138	126	283	323	342	429	274	120	10		1.1		1.0		0.872		3H
3H	442	319	387	522	236	233	357	116	93		1.6		1.4		0.144		3H
3H	476	151	147	180	133	316	234	136	-29		0.8		0.9		0.646		3H
3H	148	845	384	224	327	129	343	265	80		1.5		1.3		0.272		3H
3H	399	122	316	214	294	119	244	112	-19		0.9		0.9		0.760		3H
3H	216	240	217	66	121	244	184	73	-79		0.5		0.7		0.203		3H
	SMC																
N	68	28	34	32	13	44	37	18	-375		0.0		0.1		0.00	*	N
3H	344	402	721	292	307	401	411	159	0		1.0		1.0		1.00		3H
PHA - 1	454	336	7109	3425	392	583	2050	2752	1,639		5.4	*	5.0	*	0.18		PHA - 1
PHA - 5	3408	5624	8600	5863	4841	12725	6844	3345	6,432	*	18.2	*	16.6	*	0.00	*	PHA - 5
PHA - 10	1721	4266	6910	5871	7786	7203	5626	2279	5,215	*	14.9	*	13.7	*	0.00	*	PHA - 10
LPS - 1	517	491	429	238	332	1348	559	400	148		1.4		1.4		0.42		LPS - 1
LPS - 5	1762	895	889	402	739	2765	1242	871	831		3.2	*	3.0	*	0.04	*	LPS - 5
LPS - 10	1616	1337	948	634	1176	1822	1256	435	844		3.3	*	3.1	*	0.00	*	LPS - 10
LPS - 20	1170	1164	980	1175	1226	2016	1289	366	877		3.3	*	3.1	*	0.00	*	LPS - 20
LPS - 40	2022	1730	1128	1408	1457	1082	1471	359	1,060		3.8	*	3.6	*	0.00	*	LPS - 40

Raw data for Negative control duck 2E

2E	Mean	SD							CPM-3H	S.I.	P/N	t-Test	
Total N	102	34											
Total 3H	169	66											
	R1	R2	R3	R4	R5	R6	Mean	SD	>5000	>2.1	>2.1	<0.05	
1-15 [1]	154	331	295	185	130	296	232	86	63	1.9	1.4	0.039 *	1-15 [1]
1-15 [10]	93	152	117	218	192	311	181	79	12	1.2	1.1	0.687	1-15 [10]
1-15 [20]	208	221	106	62	274	219	182	80	13	1.2	1.1	0.659	1-15 [20]
7-14W-27 [1]	221	253	201	155	151	137	186	46	18	1.3	1.1	0.527	7-14W-27 [1]
7-14W-27 [10]	162	165	231	145	266	176	191	47	22	1.3	1.1	0.429	7-14W-27 [10]
7-14W-27 [20]	285	296	139	261	173	276	238	66	70	2.0	1.4	0.019 *	7-14W-27 [20]
7-14R-27 [1]	246	124	80	185	132	341	185	96	16	1.2	1.1	0.599	7-14R-27 [1]
7-14R-27 [10]	308	222	221	162	386	210	252	81	83	2.2 *	1.5	0.007 *	7-14R-27 [10]
7-14R-27 [20]	338	157	133	246	316	123	219	95	50	1.7	1.3	0.104	7-14R-27 [20]
22-41 [1]	324	127	264	269	291	256	255	67	87	2.3 *	1.5	0.004 *	22-41 [1]
22-41 [10]	265	154	174	200	220	206	203	38	35	1.5	1.2	0.217	22-41 [10]
22-41 [20]	177	116	192	213	262	129	182	54	13	1.2	1.1	0.649	22-41 [20]
37-56 [1]	203	197	274	152	143	270	207	56	38	1.6	1.2	0.186	37-56 [1]
37-56 [10]	225	138	141	163	152	242	177	45	8	1.1	1.0	0.768	37-56 [10]
37-56 [20]	255	110	170	124	112	246	170	66	1	1.0	1.0	0.975	37-56 [20]
54-73 [1]	290	189	139	255	136	364	229	91	60	1.9	1.4	0.051	54-73 [1]
54-73 [10]	337	104	229	170	221	344	234	94	66	2.0	1.4	0.035 *	54-73 [10]
54-73 [20]	234	247	133	166	167	245	199	49	30	1.4	1.2	0.288	54-73 [20]
71-90 [1]	198	172	118	90	151	161	148	39	-20	0.7	0.9	0.467	71-90 [1]
71-90 [10]	188	179	238	281	156	331	229	67	60	1.9	1.4	0.042 *	71-90 [10]
71-90 [20]	259	279	237	136	146	149	201	64	32	1.5	1.2	0.263	71-90 [20]
87-106 [1]	240	210	128	209	140	153	180	46	11	1.2	1.1	0.684	87-106 [1]
87-106 [10]	167	232	180	197	167	207	192	25	23	1.3	1.1	0.402	87-106 [10]
87-106 [20]	342	124	186	180	206	203	207	73	38	1.6	1.2	0.194	87-106 [20]
101-120 [1]	249	228	216	110	208	253	211	52	42	1.6	1.2	0.141	101-120 [1]
101-120 [10]	150	160	222	128	197	214	179	38	10	1.1	1.1	0.721	101-120 [10]
101-120 [20]	175	97	184	153	159	276	174	58	5	1.1	1.0	0.850	101-120 [20]
116-130 [1]	159	331	158	195	152	141	189	72	21	1.3	1.1	0.477	116-130 [1]
116-130 [10]	192	224	159	179	108	125	165	43	-4	0.9	1.0	0.883	116-130 [10]
116-130 [20]	187	288	204	120	97	262	193	75	24	1.4	1.1	0.407	116-130 [20]
126-140 [1]	184	178	221	119	170	193	178	34	9	1.1	1.1	0.747	126-140 [1]
126-140 [10]	187	210	102	237	146	208	182	49	13	1.2	1.1	0.642	126-140 [10]
126-140 [20]	204	183	183	204	228	205	201	17	33	1.5	1.2	0.237	126-140 [20]
136-150 [1]	172	262	95	188	188	196	184	54	15	1.2	1.1	0.599	136-150 [1]
136-150 [10]	281	321	229	364	325	211	289	59	120	2.8 *	1.7	0.000 *	136-150 [10]
136-150 [20]	224	272	269	531	202	245	291	121	122	2.8 *	1.7	0.000 *	136-150 [20]
146-160 [1]	264	240	265	286	144	224	237	50	69	2.0	1.4	0.018 *	146-160 [1]
146-160 [10]	236	236	297	229	171	153	220	52	52	1.8	1.3	0.072	146-160 [10]
146-160 [20]	193	215	303	243	247	269	245	39	76	2.1 *	1.5	0.008 *	146-160 [20]
156-170 [1]	234	300	214	357	283	216	267	56	99	2.5 *	1.6	0.001 *	156-170 [1]
156-170 [10]	309	294	234	227	287	225	263	38	94	2.4 *	1.6	0.001 *	156-170 [10]
156-170 [20]	355	259	214	235	131	174	228	77	59	1.9	1.4	0.048 *	156-170 [20]
166-180 [1]	238	160	228	230	193	234	214	31	45	1.7	1.3	0.106	166-180 [1]
166-180 [10]	164	130	237	248	218	210	201	45	33	1.5	1.2	0.248	166-180 [10]
166-180 [20]	226	364	309	269	215	201	264	63	95	2.4 *	1.6	0.002 *	166-180 [20]
176-195 [1]	158	143	179	201	142	294	186	57	18	1.3	1.1	0.538	176-195 [1]
176-195 [10]	158	173	80	258	178	166	169	57	0	1.0	1.0	0.993	176-195 [10]
176-195 [20]	86	160	69	110	125	82	105	34	-63	0.1	0.6	0.026 *	176-195 [20]
191-210 [1]	76	165	105	168	133	115	127	36	-42	0.4	0.8	0.138	191-210 [1]
191-210 [10]	96	212	187	212	256	146	185	56	16	1.2	1.1	0.568	191-210 [10]
191-210 [20]	291	147	108	70	155	166	156	75	-12	0.8	0.9	0.672	191-210 [20]
210-229 [1]	126	220	156	140	128	169	157	35	-12	0.8	0.9	0.662	210-229 [1]
210-229 [10]	105	223	112	105	108	148	134	47	-35	0.5	0.8	0.214	210-229 [10]
210-229 [20]	199	326	137	557	173	264	276	153	107	2.6 *	1.6	0.004 *	210-229 [20]
229-248 [1]	219	258	270	102	147	124	187	72	18	1.3	1.1	0.536	229-248 [1]
229-248 [10]	98	75	52	121	148	86	97	34	-72	-0.1	0.6	0.012 *	229-248 [10]
229-248 [20]	70	47	54	112	75	93	75	24	-93	-0.4	0.4	0.001 *	229-248 [20]
248-267 [1]	93	82	157	245	99	141	136	61	-32	0.5	0.8	0.260	248-267 [1]
248-267 [10]	102	102	108	298	153	251	169	85	0	1.0	1.0	0.989	248-267 [10]
248-267 [20]	137	66	110	118	103	196	122	43	-47	0.3	0.7	0.098	248-267 [20]
267-286 [1]	209	158	104	261	171	144	175	55	6	1.1	1.0	0.835	267-286 [1]
267-286 [10]	89	243	202	50	116	262	160	87	-8	0.9	1.0	0.783	267-286 [10]
267-286 [20]	128	114	126	87	357	82	149	104	-20	0.7	0.9	0.529	267-286 [20]
287-306 [1]	126	202	69	170	169	213	158	53	-10	0.8	0.9	0.712	287-306 [1]
287-306 [10]	153	130	187	215	120	270	179	57	11	1.2	1.1	0.710	287-306 [10]
287-306 [20]	114	135	112	143	130	164	133	19	-36	0.5	0.8	0.197	287-306 [20]
307-326 [1]	180	189	208	123	202	138	173	35	5	1.1	1.0	0.864	307-326 [1]
307-326 [10]	209	157	99	146	92	346	175	94	6	1.1	1.0	0.838	307-326 [10]
307-326 [20]	202	1816	126	181	201	243	462	665	293	5.4 *	2.7 *	0.005 *	307-326 [20]
sAg 10	101	211	159	225	195	179	178	44	10	1.1	1.1	0.728	sAg 10
sAg 100	180	248	161	201	172	278	207	47	38	1.6	1.2	0.179	sAg 100
N	96	165	127	92	136	146	127	29	-42	0.4	0.8	0.135	N
N	80	93	81	83	58	62	76	13	-92	-0.4	0.5	0.001 *	N
3H	226	173	159	143	111	250	177	52	8	1.1	1.0	0.766	3H
3H	189	234	326	299	361	268	280	63	111	2.7 *	1.7	0.000 *	3H
3H	102	191	253	119	144	174	164	55	-5	0.9	1.0	0.867	3H
3H	144	137	121	208	106	158	146	35	-23	0.7	0.9	0.409	3H
3H	167	197	139	182	98	224	168	45	-1	1.0	1.0	0.978	3H
3H	119	205	88	113	121	118	127	40	-41	0.4	0.8	0.143	3H
3H	110	124	99	155	113	113	119	19	-50	0.3	0.7	0.075	3H
	SMC												
N	30	24	27	11	35	51	30	13	-265	0.0	0.1	0.00 *	N
3H	222	296	354	340	223	332	295	59	0	1.0	1.0	1.00	3H
PHA - 1	9358	2839	16247	14213	5532	6167	9059	5250	8,765 *	34.1 *	30.8 *	0.00 *	PHA - 1
PHA - 5	36924	75303	47422	47048	62224	59597	54753	13657	54,459 *	206.6 *	185.9 *	0.00 *	PHA - 5
PHA - 10	47542	60962	48718	46447	45102	43640	48735	6250	48,441 *	183.9 *	165.5 *	0.00 *	PHA - 10
LPS - 1	1266	431	251	317	718	556	590	371	295	2.1 *	2.0	0.08	LPS - 1
LPS - 5	538	873	867	680	1948	892	966	501	672	3.5 *	3.3 *	0.01 *	LPS - 5
LPS - 10	1227	1565	839	1362	1778	1036	1301	344	1,007	4.8 *	4.4 *	0.00 *	LPS - 10
LPS - 20	1429	1964	779	869	1760	1662	1411	487	1,116	5.2 *	4.8 *	0.00 *	LPS - 20
LPS - 40	714	693	1206	1099	1238	909	977	241	682	3.6 *	3.3 *	0.00 *	LPS - 40

Raw data for Negative control duck 2F

2F	Mean	SD							CPM-3H		S.I.		P/N		t-Test	
Total N	79	31														
Total 3H	195	115														
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1		<0.05	
1-15 [1]	369	301	131	129	253	172	226	98	31		1.3		1.2		0.533		1-15 [1]
1-15 [10]	295	196	204	218	319	245	246	51	51		1.4		1.3		0.290		1-15 [10]
1-15 [20]	252	388	254	232	306	311	302	55	107		1.9		1.6		0.047	*	1-15 [20]
7-14W-27 [1]	298	309	117	144	189	180	206	80	11		1.1		1.1		0.816		7-14W-27 [1]
7-14W-27 [10]	255	617	396	500	528	743	507	170	312		3.7	*	2.6	*	0.000	*	7-14W-27 [10]
7-14W-27 [20]	565	1178	636	578	836	386	697	277	502		5.3	*	3.6	*	0.000	*	7-14W-27 [20]
7-14R-27 [1]	350	136	199	133	346	312	246	102	51		1.4		1.3		0.308		7-14R-27 [1]
7-14R-27 [10]	525	464	367	301	394	301	392	90	197		2.7	*	2.0		0.000	*	7-14R-27 [10]
7-14R-27 [20]	929	445	254	249	340	677	482	270	288		3.5	*	2.5	*	0.000	*	7-14R-27 [20]
22-41 [1]	470	278	221	294	378	209	308	100	114		2.0		1.6		0.027	*	22-41 [1]
22-41 [10]	605	295	204	421	517	490	422	149	227		3.0	*	2.2	*	0.000	*	22-41 [10]
22-41 [20]	614	335	437	354	315	409	411	110	216		2.9	*	2.1	*	0.000	*	22-41 [20]
37-56 [1]	241	195	262	240	270	341	258	48	63		1.5		1.3		0.193		37-56 [1]
37-56 [10]	180	246	120	269	526	249	265	139	70		1.6		1.4		0.179		37-56 [10]
37-56 [20]	225	258	184	265	311	168	235	54	40		1.3		1.2		0.405		37-56 [20]
54-73 [1]	480	394	263	280	308	179	317	106	123		2.1		1.6		0.018	*	54-73 [1]
54-73 [10]	87	145	160	115	139	161	135	29	-60		0.5		0.7		0.213		54-73 [10]
54-73 [20]	97	117	100	121	90	80	101	16	-94		0.2		0.5		0.054		54-73 [20]
71-90 [1]	65	105	92	94	357	173	148	109	-47		0.6		0.8		0.352		71-90 [1]
71-90 [10]	92	134	145	124	119	93	118	22	-77		0.3		0.6		0.113		71-90 [10]
71-90 [20]	95	190	149	93	194	104	138	47	-57		0.5		0.7		0.239		71-90 [20]
87-106 [1]	94	112	131	151	131	133	125	20	-69		0.4		0.6		0.152		87-106 [1]
87-106 [10]	105	149	302	104	103	121	147	78	-47		0.6		0.8		0.337		87-106 [10]
87-106 [20]	84	108	78	174	175	148	128	44	-67		0.4		0.7		0.169		87-106 [20]
101-120 [1]	138	154	105	117	118	122	126	17	-69		0.4		0.6		0.153		101-120 [1]
101-120 [10]	108	158	125	157	196	178	154	33	-41		0.6		0.8		0.394		101-120 [10]
101-120 [20]	142	128	90	109	133	107	118	19	-77		0.3		0.6		0.114		101-120 [20]
116-130 [1]	119	94	124	134	175	160	134	29	-60		0.5		0.7		0.212		116-130 [1]
116-130 [10]	153	127	138	175	223	117	156	39	-39		0.7		0.8		0.416		116-130 [10]
116-130 [20]	148	123	129	146	122	132	133	11	-61		0.5		0.7		0.203		116-130 [20]
126-140 [1]	156	196	210	184	112	125	164	40	-31		0.7		0.8		0.522		126-140 [1]
126-140 [10]	140	123	132	199	152	159	151	27	-44		0.6		0.8		0.362		126-140 [10]
126-140 [20]	101	117	220	150	201	143	155	47	-39		0.7		0.8		0.416		126-140 [20]
136-150 [1]	80	143	95	127	114	108	111	22	-84		0.3		0.6		0.086		136-150 [1]
136-150 [10]	159	130	241	129	169	89	153	51	-42		0.6		0.8		0.388		136-150 [10]
136-150 [20]	268	300	245	371	235	442	310	81	115		2.0		1.6		0.023	*	136-150 [20]
146-160 [1]	186	206	200	157	276	315	223	60	29		1.2		1.1		0.556		146-160 [1]
146-160 [10]	368	321	357	313	260	127	291	89	96		1.8		1.5		0.056		146-160 [10]
146-160 [20]	172	934	280	529	362	204	414	285	219		2.9	*	2.1	*	0.001	*	146-160 [20]
156-170 [1]	236	244	350	155	380	169	256	92	61		1.5		1.3		0.223		156-170 [1]
156-170 [10]	1167	233	256	339	164	203	394	383	199		2.7	*	2.0		0.009	*	156-170 [10]
156-170 [20]	231	563	1023	476	197	338	471	304	277		3.4	*	2.4	*	0.000	*	156-170 [20]
166-180 [1]	99	130	166	120	148	119	130	24	-64		0.4		0.7		0.183		166-180 [1]
166-180 [10]	95	94	101	104	100	97	99	4	-96		0.2		0.5		0.049	*	166-180 [10]
166-180 [20]	104	104	123	66	77	132	101	26	-94		0.2		0.5		0.055		166-180 [20]
176-195 [1]	136	103	138	193	103	138	135	33	-60		0.5		0.7		0.218		176-195 [1]
176-195 [10]	128	142	90	119	106	168	126	27	-69		0.4		0.6		0.153		176-195 [10]
176-195 [20]	82	116	140	121	167	90	119	32	-75		0.4		0.6		0.121		176-195 [20]
191-210 [1]	119	115	144	166	104	120	128	23	-67		0.4		0.7		0.168		191-210 [1]
191-210 [10]	103	113	118	138	116	134	120	13	-74		0.4		0.6		0.124		191-210 [10]
191-210 [20]	133	135	114	158	144	110	132	18	-62		0.5		0.7		0.196		191-210 [20]
210-229 [1]	126	136	205	145	118	194	154	37	-41		0.6		0.8		0.399		210-229 [1]
210-229 [10]	200	326	376	204	253	296	276	70	81		1.7		1.4		0.101		210-229 [10]
210-229 [20]	397	447	726	642	350	915	580	220	385		4.3	*	3.0	*	0.000	*	210-229 [20]
229-248 [1]	164	247	162	126	108	103	152	53	-43		0.6		0.8		0.376		229-248 [1]
229-248 [10]	129	109	107	132	153	119	125	17	-70		0.4		0.6		0.148		229-248 [10]
229-248 [20]	130	104	128	169	96	118	124	26	-71		0.4		0.6		0.145		229-248 [20]
248-267 [1]	107	157	94	203	116	128	134	40	-61		0.5		0.7		0.212		248-267 [1]
248-267 [10]	150	127	159	203	133	187	160	30	-35		0.7		0.8		0.468		248-267 [10]
248-267 [20]	177	126	153	134	123	156	145	21	-50		0.6		0.7		0.300		248-267 [20]
267-286 [1]	141	119	196	133	122	126	140	29	-55		0.5		0.7		0.253		267-286 [1]
267-286 [10]	212	175	145	250	177	165	187	38	-7		0.9		1.0		0.878		267-286 [10]
267-286 [20]	126	143	179	190	186	135	160	28	-35		0.7		0.8		0.468		267-286 [20]
287-306 [1]	182	144	135	150	148	103	144	26	-51		0.6		0.7		0.289		287-306 [1]
287-306 [10]	255	179	166	207	151	122	180	46	-15		0.9		0.9		0.760		287-306 [10]
287-306 [20]	231	166	281	292	139	169	213	65	18		1.2		1.1		0.707		287-306 [20]
307-326 [1]	137	143	306	125	123	195	172	71	-23		0.8		0.9		0.635		307-326 [1]
307-326 [10]	219	172	259	196	225	228	217	30	22		1.2		1.1		0.650		307-326 [10]
307-326 [20]	295	503	652	1318	393	692	642	364	447		4.9	*	3.3	*	0.000	*	307-326 [20]
sAg 10	266	290	648	130	345	292	329	172	134		2.2	*	1.7		0.016	*	sAg 10
sAg 100	246	268	295	539	414	169	322	133	127		2.1		1.7		0.017	*	sAg 100
N	134	48	51	64	53	56	68	33	-127		-0.1		0.3		0.011	*	N
N	57	86	82	71	103	138	90	28	-105		0.1		0.5		0.032	*	N
3H	120	166	208	206	592	203	249	171	54		1.5		1.3		0.314		3H
3H	365	335	293	186	327	192	283	76	88		1.8		1.5		0.077		3H
3H	143	165	117	197	159	159	157	26	-38		0.7		0.8		0.429		3H
3H	92	133	528	178	103	134	195	166	0		1.0		1.0		0.999		3H
3H	168	175	226	157	131	226	181	38	-14		0.9		0.9		0.768		3H
3H	70	84	339	214	371	154	205	127	11		1.1		1.1		0.836		3H
3H	72	111	115	112	62	90	94	23	-101		0.1		0.5		0.039	*	3H
	SMC																
N	25	55	43	65	41	36	44	14	-336		0.0		0.1		0.00	*	N
3H	503	125	243	651	356	404	380	186	0		1.0		1.0		1.00		3H
PHA - 1	30461	95722	38297	43065	47428	77330	55384	25444	55,004	*	164.6	*	145.6	*	0.00	*	PHA - 1
PHA - 5	35701	87990	72880	85003	76825	44587	67164	21806	66,784	*	199.7	*	176.6	*	0.00	*	PHA - 5
PHA - 10	43765	82234	80752	81648	75066	35388	66476	21158	66,095	*	197.6	*	174.8	*	0.00	*	PHA - 10
LPS - 1	1708	1344	1533	2810	2791	2019	2034	634	1,654		5.9	*	5.3	*	0.00	*	LPS - 1
LPS - 5	3942	5193	7210	3864	4478	3447	4689	1374	4,309		13.8	*	12.3	*	0.00	*	LPS - 5
LPS - 10	5387	6924	8139	7828	6787	5581	6774	1126	6,394	*	20.0	*	17.8	*	0.00	*	LPS - 10
LPS - 20	7433	8286	13384	10843	6907	5003	8643	3006	8,262	*	25.6	*	22.7	*	0.00	*	LPS - 20
LPS - 40	5956	7880	13745	9650	6213	5772	8203	3093	7,822	*	24.3	*	21.6	*	0.00	*	LPS - 40

Raw data for Negative control duck 2G

2G	Mean	SD							CPM-3H	S.I.	P/N	t-Test	
Total N	87	33											
Total 3H	1108	914											
	R1	R2	R3	R4	R5	R6	Mean	SD	>5000	>2.1	>2.1	<0.05	
1-15 [1]	708	420	644	452	535	635	566	115	-542	0.5	0.5	0.157	1-15 [1]
1-15 [10]	709	385	252	403	631	563	491	172	-618	0.4	0.4	0.109	1-15 [10]
1-15 [20]													1-15 [20]
7-14W-27 [1]	306	283	953	817	509	696	594	274	-514	0.5	0.5	0.181	7-14W-27 [1]
7-14W-27 [10]	683	987	546	2770	884	1497	1228	823	120	1.1	1.1	0.763	7-14W-27 [10]
7-14W-27 [20]	862	2860	802	1256	3368	819	1661	1149	553	1.5	1.5	0.185	7-14W-27 [20]
7-14R-27 [1]	689	731	492	914	558	735	687	149	-422	0.6	0.6	0.270	7-14R-27 [1]
7-14R-27 [10]	1342	438	712	440	1085	1052	845	373	-263	0.7	0.8	0.493	7-14R-27 [10]
7-14R-27 [20]	1380	494	1301	1069	937	1595	1129	388	21	1.0	1.0	0.956	7-14R-27 [20]
22-41 [1]	714	412	287	534	2439	1333	953	815	-155	0.8	0.9	0.696	22-41 [1]
22-41 [10]	406	1049	347	787	643	1241	746	353	-363	0.6	0.7	0.345	22-41 [10]
22-41 [20]	381	474	439	712	1122	4307	1239	1527	131	1.1	1.1	0.765	22-41 [20]
37-56 [1]	2433	655	460	564	1166	526	967	762	-141	0.9	0.9	0.721	37-56 [1]
37-56 [10]	711	1263	1373	501	673	1692	1036	473	-73	0.9	0.9	0.851	37-56 [10]
37-56 [20]	1421	510	584	625	1926	1404	1078	586	-30	1.0	1.0	0.939	37-56 [20]
54-73 [1]	346	406	592	480	2032	1233	848	663	-260	0.7	0.8	0.507	54-73 [1]
54-73 [10]	484	1031	484	426	1899	1640	994	645	-114	0.9	0.9	0.770	54-73 [10]
54-73 [20]	581	443	531	519	491	1262	638	309	-470	0.5	0.6	0.221	54-73 [20]
71-90 [1]	344	299	773	946	653	377	565	265	-543	0.5	0.5	0.159	71-90 [1]
71-90 [10]	532	418	681	770	2226	945	929	662	-179	0.8	0.8	0.647	71-90 [10]
71-90 [20]	350	399	1005	1012	1234	1287	881	409	-227	0.8	0.8	0.555	71-90 [20]
87-106 [1]	219	819	393	1162	571	2073	873	675	-235	0.8	0.8	0.548	87-106 [1]
87-106 [10]	843	393	525	791	623	481	609	178	-499	0.5	0.5	0.193	87-106 [10]
87-106 [20]	471	396	463	385	465	664	474	100	-634	0.4	0.4	0.099	87-106 [20]
101-120 [1]	1368	1523	811	1654	333	745	1072	520	-36	1.0	1.0	0.926	101-120 [1]
101-120 [10]	1506	441	637	626	564	1934	951	615	-157	0.8	0.9	0.687	101-120 [10]
101-120 [20]	643	571	558	582	931	578	644	144	-464	0.5	0.6	0.225	101-120 [20]
116-130 [1]	497	330	311	518	556	793	501	175	-607	0.4	0.5	0.115	116-130 [1]
116-130 [10]	928	766	1364	787	343	1022	868	336	-240	0.8	0.8	0.531	116-130 [10]
116-130 [20]	547	400	782	759	749	613	642	150	-466	0.5	0.6	0.223	116-130 [20]
126-140 [1]	881	657	660	916	646	1431	865	302	-243	0.8	0.8	0.525	126-140 [1]
126-140 [10]	1383	801	620	930	565	738	840	296	-269	0.7	0.8	0.482	126-140 [10]
126-140 [20]	470	848	739	1066	503	6768	1732	2477	624	1.6	1.6	0.235	126-140 [20]
136-150 [1]	249	1163	1594	456	760	931	859	486	-249	0.8	0.8	0.519	136-150 [1]
136-150 [10]	1472	815	1177	1054	680	3655	1476	1103	367	1.4	1.3	0.373	136-150 [10]
136-150 [20]	1847	981	705	870	397	1407	1035	518	-74	0.9	0.9	0.849	136-150 [20]
146-160 [1]	953	625	865	532	1821	1009	968	458	-141	0.9	0.9	0.715	146-160 [1]
146-160 [10]	1093	416	648	1493	963	297	818	450	-290	0.7	0.7	0.452	146-160 [10]
146-160 [20]	2349	1347	884	551	565	558	1042	711	-66	0.9	0.9	0.867	146-160 [20]
156-170 [1]	956	507	644	707	1082	904	800	216	-308	0.7	0.7	0.419	156-170 [1]
156-170 [10]	724	575	801	611	1323	968	834	278	-274	0.7	0.8	0.473	156-170 [10]
156-170 [20]	1044	912	1678	520	1528	2153	1306	591	198	1.2	1.2	0.611	156-170 [20]
166-180 [1]	1519	1149	1099	571	757	818	986	340	-123	0.9	0.9	0.748	166-180 [1]
166-180 [10]	2165	1990	3301	760	795	1060	1679	997	570	1.6	1.5	0.164	166-180 [10]
166-180 [20]	2263	1084	691	791	927	1060	1136	573	28	1.0	1.0	0.943	166-180 [20]
176-195 [1]	785	669	1146	1062	1112	904	946	193	-162	0.8	0.9	0.671	176-195 [1]
176-195 [10]	1244	999	760	467	732	374	763	325	-345	0.7	0.7	0.368	176-195 [10]
176-195 [20]	778	705	288	792	1123	573	710	275	-398	0.6	0.6	0.299	176-195 [20]
191-210 [1]	884	492	843	625	539	509	649	173	-459	0.5	0.6	0.230	191-210 [1]
191-210 [10]	860	1382	805	405	324	491	711	393	-397	0.6	0.6	0.303	191-210 [10]
191-210 [20]	697	2782	705	481	444	610	953	902	-155	0.8	0.9	0.699	191-210 [20]
210-229 [1]	630	449	562	462	539	678	553	90	-555	0.5	0.5	0.148	210-229 [1]
210-229 [10]	979	346	412	427	1221	572	660	357	-449	0.6	0.6	0.244	210-229 [10]
210-229 [20]	3781	1013	770	757	610	525	1243	1255	135	1.1	1.1	0.749	210-229 [20]
229-248 [1]	577	1314	1534	3109	2294	677	1584	974	476	1.5	1.4	0.242	229-248 [1]
229-248 [10]	998	1187	726	605	917	1664	1016	377	-92	0.9	0.9	0.810	229-248 [10]
229-248 [20]	945	1521	801	777	670	735	908	314	-200	0.8	0.8	0.601	229-248 [20]
248-267 [1]	443	563	611	517	1341	3560	1173	1215	64	1.1	1.1	0.877	248-267 [1]
248-267 [10]	466	899	807	756	809	839	763	153	-345	0.7	0.7	0.365	248-267 [10]
248-267 [20]	876	686	870	1177	913	1884	1068	430	-40	1.0	1.0	0.916	248-267 [20]
267-286 [1]	341	812	884	520	498	1077	689	279	-419	0.6	0.6	0.274	267-286 [1]
267-286 [10]	442	811	539	443	646	458	557	148	-552	0.5	0.5	0.151	267-286 [10]
267-286 [20]	514	361	539	614	505	879	569	173	-539	0.5	0.5	0.160	267-286 [20]
287-306 [1]	743	1072	877	1171	744	1121	955	192	-153	0.8	0.9	0.687	287-306 [1]
287-306 [10]	11727	696	949	518	295	887	2512	4521	1,404	2.4 *	2.3 *	0.068	287-306 [10]
287-306 [20]	559	491	939	532	1065	503	682	253	-427	0.6	0.6	0.266	287-306 [20]
307-326 [1]	594	608	569	433	5863	1434	1584	2127	475	1.5	1.4	0.332	307-326 [1]
307-326 [10]	1701	580	470	2906	1811	1334	1467	899	359	1.4	1.3	0.372	307-326 [10]
307-326 [20]	742	2591	2032	1980	1833	1082	1710	678	602	1.6	1.5	0.129	307-326 [20]
sAg 10	665	443	631	393	938	534	601	196	-507	0.5	0.5	0.186	sAg 10
sAg 100	804	520	371	1200	741	728	727	283	-381	0.6	0.7	0.320	sAg 100
N	36	51	91	85	79	94	73	24	-1,035	0.0	0.1	0.009 *	N
N	134	114	105	134	86	38	102	36	-1,006	0.0	0.1	0.010 *	N
3H	350	405	304	477	493	396	404	72	-704	0.3	0.4	0.068	3H
3H	601	746	3144	2376	1162	908	1490	1031	381	1.4	1.3	0.351	3H
3H	673	558	1027	895	1126	1922	1034	484	-75	0.9	0.9	0.847	3H
3H	862	453	601	745	939	909	752	192	-357	0.7	0.7	0.350	3H
3H	3127	1928	319	896	1131	674	1346	1026	238	1.2	1.2	0.560	3H
3H	566	618	3656	800	1260	1728	1438	1173	330	1.3	1.3	0.428	3H
3H	776	1359	681	4039	485	423	1294	1385	186	1.2	1.2	0.665	3H
	SMC												
N	30	22	24	61	95	48	47	28	-1,194	0.0	0.0	0.00 *	N
3H	1140	1178	1019	818	1370	1920	1241	379	0	1.0	1.0	1.00	3H
PHA - 1	67105	84121	75028	97130	84783	71958	80021	10853	78,780 *	67.0 *	64.5 *	0.00 *	PHA - 1
PHA - 5	54320	76028	67362	81451	94474	72605	74373	13500	73,133 *	62.2 *	59.9 *	0.00 *	PHA - 5
PHA - 10	43185	65159	52387	79424	78357	55224	62289	14649	61,049 *	52.1 *	50.2 *	0.00 *	PHA - 10
LPS - 1	5736	4313	3218	9979	4369	5879	5582	2371	4,342	4.6 *	4.5 *	0.00 *	LPS - 1
LPS - 5	10291	6024	4814	9754	9855	5266	7667	2555	6,427 *	6.4 *	6.2 *	0.00 *	LPS - 5
LPS - 10	5987	15797	7388	9773	9044	5447	8906	3770	7,665 *	7.4 *	7.2 *	0.00 *	LPS - 10
LPS - 20	10913	13577	6739	7565	6637	8656	9015	2738	7,774 *	7.5 *	7.3 *	0.00 *	LPS - 20
LPS - 40	5817	7214	5068	7044	5961	3570	5779	1348	4,538	4.8 *	4.7 *	0.00 *	LPS - 40
	PBMC												
N	114	35	11	22	10	82	46	43	-35	0.0	0.6	0.12	N
3H	65	100	63	43	108	103	80	27	0	1.0	1.0	1.00	3H
PHA - 1	21964	23759	20979	22249	6682	407	16007	9895	15,926 *	460.4 *	199.3 *	0.00 *	PHA - 1
PHA - 5	9447	11644	7861	8437	5650	6209	8208	2193	8,128 *	235.5 *	102.2 *	0.00 *	PHA - 5
PHA - 10	5418	4671	2612	3121	2933	4101	3809	1104	3,729	108.6 *	47.4 *	0.00 *	PHA - 10
LPS - 1	251	413	374	208	177	429	309	110	228	7.6 *	3.8 *	0.00 *	LPS - 1
LPS - 5	300	240	218	154	290	232	239	53	159	5.6 *	3.0 *	0.00 *	LPS - 5
LPS - 10	221	228	186	173	335	263	234	59	154	5.4 *	2.9 *	0.00 *	LPS - 10
LPS - 20	140	243	246	198	312	218	226	57	146	5.2 *	2.8 *	0.00 *	LPS - 20
LPS - 40	245	382	179	162	238	169	229	83	149	5.3 *	2.9 *	0.00 *	LPS - 40

Raw data for Negative control duck 2H

2H	Mean	SD												
Total N	67	26												
Total 3H	3550	5697							CPM-3H		S.I.		P/N	t-Test
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1	>2.1	<0.05	
1-15 [1]	1615	194	1440	9018	183	118	2095	3457	-1,455		0.6		0.6	0.547	1-15 [1]
1-15 [10]	119	108	1801	890	1136	179	706	692	-2,844		0.2		0.2	0.232	1-15 [10]
1-15 [20]	82	636	2550	1724	1287	6109	2065	2158	-1,485		0.6		0.6	0.534	1-15 [20]
7-14W-27 [1]	7553	310	9390	4904	520	2089	4128	3789	578		1.2		1.2	0.812	7-14W-27 [1]
7-14W-27 [10]	1459	456	2876	2339	2204	20227	4927	7542	1,377		1.4		1.4	0.597	7-14W-27 [10]
7-14W-27 [20]	725	443	199	1251	1788	859	878	573	-2,672		0.2		0.2	0.261	7-14W-27 [20]
7-14R-27 [1]	6887	3151	3372	7046	7259	6392	5685	1900	2,135		1.6		1.6	0.371	7-14R-27 [1]
7-14R-27 [10]	2908	8291	1751	11135	3234	1559	4813	3953	1,263		1.4		1.4	0.603	7-14R-27 [10]
7-14R-27 [20]	18336	1838	2699	1334	2844	2414	4911	6601	1,361		1.4		1.4	0.594	7-14R-27 [20]
22-41 [1]	16634	2756	2998	1967	400	10887	5940	6389	2,391		1.7		1.7	0.348	22-41 [1]
22-41 [10]	700		1940	2283	1393	1595	1582	599	-1,968		0.4		0.4	0.449	22-41 [10]
22-41 [20]	213	3230	1240	6648	11485	1163	3997	4328	447		1.1		1.1	0.855	22-41 [20]
37-56 [1]	2251	2061	3426	20484	4454	458	5522	7453	1,973		1.6		1.6	0.449	37-56 [1]
37-56 [10]	1626	5310	1663	5461	272	6340	3445	2549	-104		1.0		1.0	0.965	37-56 [10]
37-56 [20]	3703	16103	3953	1689	2852	6791	5862	5332	2,312		1.7		1.7	0.354	37-56 [20]
54-73 [1]	897	1322	20948	6093	88	469	4970	8131	1,420		1.4		1.4	0.591	54-73 [1]
54-73 [10]	161	6140	1640	118	417	436	1485	2348	-2,064		0.4		0.4	0.389	54-73 [10]
54-73 [20]	614	146	1628	1290	5754	808	1707	2049	-1,843		0.5		0.5	0.440	54-73 [20]
71-90 [1]	240	3527	952	1809	357	720	1268	1240	-2,282		0.3		0.4	0.337	71-90 [1]
71-90 [10]	6693	5822	8873	1880	680	106	4009	3605	459		1.1		1.1	0.849	71-90 [10]
71-90 [20]	751	663	17368	10741	1723	479	5288	7120	1,738		1.5		1.5	0.501	71-90 [20]
87-106 [1]	140	4094	1568	566	1799	892	1510	1408	-2,040		0.4		0.4	0.391	87-106 [1]
87-106 [10]	9870	1168	448	752	1388	4553	3030	3664	-520		0.9		0.9	0.830	87-106 [10]
87-106 [20]	1481	394	987	259	255	6433	1635	2400	-1,915		0.5		0.5	0.424	87-106 [20]
101-120 [1]	420	599	3016	1410	1425	316	1198	1014	-2,352		0.3		0.3	0.322	101-120 [1]
101-120 [10]	4288	4509	3338	1799	721	252	2485	1824	-1,065		0.7		0.7	0.654	101-120 [10]
101-120 [20]	1730	2491	157	1776	2285	781	1537	899	-2,013		0.4		0.4	0.396	101-120 [20]
116-130 [1]	155	8048	615	2752	475	343	2065	3083	-1,485		0.6		0.6	0.537	116-130 [1]
116-130 [10]	546	15228	638	360	212	654	2940	6022	-610		0.8		0.8	0.808	116-130 [10]
116-130 [20]	2507	1120	2167	1765	665	635	1477	789	-2,073		0.4		0.4	0.382	116-130 [20]
126-140 [1]	1300	1084	676	529	2431	19936	4326	7677	776		1.2		1.2	0.766	126-140 [1]
126-140 [10]	156	1855	1564	248	470	1590	981	769	-2,569		0.3		0.3	0.280	126-140 [10]
126-140 [20]	214	4373	790	579	1382	450	1298	1558	-2,252		0.4		0.4	0.345	126-140 [20]
136-150 [1]	565	482	642	1628	1870	2934	1354	972	-2,196		0.4		0.4	0.355	136-150 [1]
136-150 [10]	1247	1402	13429	2335	3851	1032	3883	4791	333		1.1		1.1	0.892	136-150 [10]
136-150 [20]	5584	654	1702	2895	4360	9323	4086	3119	537		1.2		1.2	0.823	136-150 [20]
146-160 [1]	8059	4802	1253	1062	736	4484	3399	2899	-150		1.0		1.0	0.950	146-160 [1]
146-160 [10]	771	259	11574	224	2546	8867	4040	4936	490		1.1		1.1	0.842	146-160 [10]
146-160 [20]	24973	221	98	178	2832	258	4760	9959	1,210		1.3		1.3	0.662	146-160 [20]
156-170 [1]	323	15326	2394	370	230	348	3165	6015	-384		0.9		0.9	0.879	156-170 [1]
156-170 [10]	25993	247	4043	2121	193	1372	5662	10061	2,112		1.6		1.6	0.448	156-170 [10]
156-170 [20]	553	857	544	845	510	4700	1335	1656	-2,215		0.4		0.4	0.353	156-170 [20]
166-180 [1]	311	320	4719	502	1716	428	1333	1743	-2,217		0.4		0.4	0.353	166-180 [1]
166-180 [10]	9367	1236	797	4322	9561	1166	4408	4117	858		1.2		1.2	0.725	166-180 [10]
166-180 [20]	4039	2332	10652	13663	851	2541	5680	5206	2,130		1.6		1.6	0.392	166-180 [20]
176-195 [1]	198	1334	533	1103	416	853	740	433	-2,810		0.2		0.2	0.238	176-195 [1]
176-195 [10]	9678	400	8195	2497	100	454	3554	4281	4		1.0		1.0	0.999	176-195 [10]
176-195 [20]	12766	5835	878	184	103	436	3367	5099	-183		0.9		0.9	0.941	176-195 [20]
191-210 [1]	88	211	431	653	1998	3654	1173	1397	-2,377		0.3		0.3	0.318	191-210 [1]
191-210 [10]	2130	474	965	234	245	16821	3478	6575	-72		1.0		1.0	0.978	191-210 [10]
191-210 [20]	1242	978	1246	268	103	5072	1485	1824	-2,065		0.4		0.4	0.387	191-210 [20]
210-229 [1]	2364	20883	273	1253	2636	2245	4942	7858	1,393		1.4		1.4	0.596	210-229 [1]
210-229 [10]	13871	6655	982	2232	2410	8010	5693	4861	2,144		1.6		1.6	0.386	210-229 [10]
210-229 [20]	1320	602	3315	2700	1203	17147	4381	6335	831		1.2		1.2	0.743	210-229 [20]
229-248 [1]	418	253	3741	204	903	3040	1427	1557	-2,123		0.4		0.4	0.373	229-248 [1]
229-248 [10]	13302	3624	2791	1981	495	98	3715	4883	165		1.0		1.0	0.946	229-248 [10]
229-248 [20]	13144	2819	15385	2270	19383	1362	9061	7843	5,511	*	2.6	*	2.6 *	0.040 *	229-248 [20]
248-267 [1]	4374	966	585	973	710	261	1312	1524	-2,238		0.4		0.4	0.347	248-267 [1]
248-267 [10]	45	7401	76	1077	2678	126	1901	2880	-1,649		0.5		0.5	0.492	248-267 [10]
248-267 [20]	125	144	1224	2698	2095	154	1073	1124	-2,476		0.3		0.3	0.298	248-267 [20]
267-286 [1]	1960	562	1852	2525	550	931	1397	828	-2,153		0.4		0.4	0.364	267-286 [1]
267-286 [10]	1082	1646	2018	3485	13505	8011	4958	4882	1,408		1.4		1.4	0.568	267-286 [10]
267-286 [20]	3882	220	1068	11048	4595	1461	3712	3972	163		1.0		1.0	0.947	267-286 [20]
287-306 [1]	1514	420	93	1458	998	626	852	573	-2,698		0.2		0.2	0.257	287-306 [1]
287-306 [10]	318	78	340	329	2907	6993	1828	2744	-1,722		0.5		0.5	0.473	287-306 [10]
287-306 [20]	302	754	1122	945	261	734	686	344	-2,863		0.2		0.2	0.229	287-306 [20]
307-326 [1]	2612	1176	2025	267	305	2272	1443	1014	-2,107		0.4		0.4	0.375	307-326 [1]
307-326 [10]	291	288	798	560	5065	4644	1941	2269	-1,609		0.5		0.5	0.501	307-326 [10]
307-326 [20]	1727	1089	1972	1166	390	3538	1647	1078	-1,903		0.5		0.5	0.423	307-326 [20]
sAg 10	178	72	59	510	391	1287	416	463	-3,134		0.1		0.1	0.189	sAg 10
sAg 100	72	147	110	126	984	381	303	351	-3,246		0.1		0.1	0.173	sAg 100
N	45	86	101	73	105	59	78	24	-3,472		0.0		0.0	0.146	N
N	81	39	87	23	53	54	56	24	-3,494		0.0		0.0	0.143	N
3H	184	83	263	420	780	126	309	259	-3,240		0.1		0.1	0.174	3H
3H	1238	7725	6379	1687	19063	16144	8706	7403	5,156	*	2.5	*	2.5 *	0.051	3H
3H	673	1537	727	9079	626	3849	2749	3336	-801		0.8		0.8	0.740	3H
3H	440	1345	325	23016	108	18972	7368	10640	3,818		2.1		2.1	0.180	3H
3H	293	1331	880	1593	1259	1352	1118	465	-2,432		0.3		0.3	0.306	3H
3H	4208	1279	464	4135	182	128	1733	1934	-1,817		0.5		0.5	0.446	3H
3H	1342	147	3840	2674	983	8211	2866	2926	-684		0.8		0.8	0.776	3H
	SMC														
N	40	32	40	22	45	24	34	9	-391		0.0		0.1	0.00 *	N
3H	480	567	205	395	502	397	424	126	0		1.0		1.0	1.00	3H
PHA - 1	47043	78733	76574	52740	64423	41254	60128	15602	59,704	*	153.9	*	141.7 *	0.00 *	PHA - 1
PHA - 5	47897	70099	55449	46187	62602	50479	55452	9309	55,028	*	141.9	*	130.7 *	0.00 *	PHA - 5
PHA - 10	46472	65552	59577	57384	82647	59851	61914	11931	61,490	*	158.5	*	145.9 *	0.00 *	PHA - 10
LPS - 1	3347	2333	2223	3409	3836	3232	3063	643	2,639		7.8	*	7.2 *	0.00 *	LPS - 1
LPS - 5	4495	6636	8510	8024	5733	3556	6159	1947	5,735	*	15.7	*	14.5 *	0.00 *	LPS - 5
LPS - 10	6472	5070	8579	6989	4822	5204	6189	1451	5,765	*	15.8	*	14.6 *	0.00 *	LPS - 10
LPS - 20	4236	8931	6030	7733	4630	4526	6014	1937	5,590	*	15.3	*	14.2 *	0.00 *	LPS - 20
LPS - 40	2387	2845	3668	6506	6464	3604	4246	1800	3,821		10.8	*	10.0 *	0.00 *	LPS - 40
	PBMC														
N	110	87	27	78	40	92	72	32	-18		0.0		0.8	0.29	N
3H	67	125	86	98	96	68	90	22	0		1.0		1.0	1.00	3H
PHA - 1	6186	9347	9899	8609	6666	7753	8077	1476	7,987	*	453.1	*	89.7 *	0.00 *	PHA - 1
PHA - 5	23782	39555	29298	37164	23938	31850	30931	6584	30,841	*	1746.7	*	343.7 *	0.00 *	PHA - 5
PHA - 10	9550	16620	8953	16954	15835	14552	13744	3582	13,654	*	773.9	*	152.7 *	0.00 *	PHA - 10
LPS - 1	682	1424	643	754	555	829	815	313	725		42.0	*	9.1 *	0.00 *	LPS - 1
LPS - 5	465	968	439	569	619	609	612	190	522		30.5	*	6.8 *	0.00 *	LPS - 5
LPS - 10	336	763	617	503	620	519	560	144	470		27.6	*	6.2 *	0.00 *	LPS - 10
LPS - 20	453	441	491	424	317	440	428	59	338		20.1	*	4.8 *	0.00 *	LPS - 20
LPS - 40	288	609	405	453	283	407	408	120	318		19.0	*	4.5 *	0.00 *	LPS - 40

Raw data for Negative control duck 2I

2I	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	135	126															
Total 3H	562	561								>5000		>2.1		>2.1		<0.05	
	R1	R2	R3	R4	R5	R6	Mean	SD									
1-15 [1]	276	235	288	619	231	280	322	148	-240		0.4		0.6		0.306		1-15 [1]
1-15 [10]	147	829	1894	1480	147	1465	994	739	432		2.0		1.8		0.098		1-15 [10]
1-15 [20]	2912	112	1431	401	123	674	942	1081	380		1.9		1.7		0.183		1-15 [20]
7-14W-27 [1]	172	100	327	1949	57	6169	1462	2416	900		3.1	*	2.6	*	0.040	*	7-14W-27 [1]
7-14W-27 [10]	4521	548	2059	1764	2606	9055	3426	3049	2,864		7.7	*	6.1	*	0.000	*	7-14W-27 [10]
7-14W-27 [20]	1964	8961	2563	10233	3148	2953	4970	3629	4,408		11.3	*	8.8	*	0.000	*	7-14W-27 [20]
7-14R-27 [1]	290	721	2749	6047	1940	395	2024	2194	1,462		4.4	*	3.6	*	0.001	*	7-14R-27 [1]
7-14R-27 [10]	247	252	77	1478	117	505	446	527	-116		0.7		0.8		0.637		7-14R-27 [10]
7-14R-27 [20]	174	874	172	206	755	131	385	335	-177		0.6		0.7		0.459		7-14R-27 [20]
22-41 [1]	960	191	98	548	367	813	496	343	-66		0.8		0.9		0.783		22-41 [1]
22-41 [10]	1040	201	340	1055	235	1200	679	466	117		1.3		1.2		0.631		22-41 [10]
22-41 [20]	358	211	772	24123	357	232	4342	9693	3,780		9.9	*	7.7	*	0.012	*	22-41 [20]
37-56 [1]	228	129	468	665	1606	1695	799	687	237		1.6		1.4		0.354		37-56 [1]
37-56 [10]	141	740	372	230	983	9408	1979	3653	1,417		4.3	*	3.5	*	0.020	*	37-56 [10]
37-56 [20]	1738	506	509	153	116	289	552	605	-10		1.0		1.0		0.968		37-56 [20]
54-73 [1]	1901	406	1917	1388	479	219	1052	777	490		2.1	*	1.9		0.064		54-73 [1]
54-73 [10]	266	174	250	93	351	477	269	134	-293		0.3		0.5		0.213		54-73 [10]
54-73 [20]	720	409	117	268	490	41	341	251	-221		0.5		0.6		0.350		54-73 [20]
71-90 [1]	542	363	248	208	809	829	500	273	-62		0.9		0.9		0.793		71-90 [1]
71-90 [10]	202	205	164	185	3284	1373	902	1259	340		1.8		1.6		0.257		71-90 [10]
71-90 [20]	2795	121	85	543	194	117	643	1068	81		1.2		1.1		0.775		71-90 [20]
87-106 [1]	305	396	243	339	714	224	370	180	-192		0.6		0.7		0.414		87-106 [1]
87-106 [10]	238	209	198	398	856	696	433	280	-129		0.7		0.8		0.584		87-106 [10]
87-106 [20]	750	455	127	1026	371	79	468	366	-94		0.8		0.8		0.694		87-106 [20]
101-120 [1]	397	203	145	1471	998	1079	716	543	154		1.4		1.3		0.533		101-120 [1]
101-120 [10]	1194	3948	191	790	3734	1213	1845	1591	1,283		4.0	*	3.3	*	0.000	*	101-120 [10]
101-120 [20]	818	88	4203	1356	361	1160	1331	1485	769		2.8	*	2.4	*	0.020	*	101-120 [20]
116-130 [1]	70	3391	381	427	167	1140	929	1263	367		1.9		1.7		0.222		116-130 [1]
116-130 [10]	706	137	328	273	3682	675	967	1349	405		1.9		1.7		0.191		116-130 [10]
116-130 [20]	778	768	571	1612	399	470	766	442	204		1.5		1.4		0.399		116-130 [20]
126-140 [1]	628	233	344	140	601	164	352	216	-210		0.5		0.6		0.373		126-140 [1]
126-140 [10]	316	427	578	177	384	831	452	228	-110		0.7		0.8		0.641		126-140 [10]
126-140 [20]	759	791	568	87	188	68	410	335	-152		0.6		0.7		0.524		126-140 [20]
136-150 [1]	302	433	158	385	692	686	443	212	-119		0.7		0.8		0.612		136-150 [1]
136-150 [10]	415	418	775	851	625	2147	872	650	310		1.7		1.6		0.222		136-150 [10]
136-150 [20]	892	689	452	690	342	147	535	272	-27		0.9		1.0		0.910		136-150 [20]
146-160 [1]	936	417	178	120	55	125	305	333	-257		0.4		0.5		0.283		146-160 [1]
146-160 [10]	2871	161	98	57	64	385	606	1116	44		1.1		1.1		0.877		146-160 [10]
146-160 [20]	367	1820	550	579	248	208	629	603	67		1.2		1.1		0.789		146-160 [20]
156-170 [1]	102	682	228	249	345	203	302	202	-260		0.4		0.5		0.270		156-170 [1]
156-170 [10]	414	139	98	404	127	324	251	146	-311		0.3		0.4		0.187		156-170 [10]
156-170 [20]	573	137	689	379	146	378	384	222	-178		0.6		0.7		0.449		156-170 [20]
166-180 [1]	3789	181	354	100	79	1617	1020	1477	458		2.1		1.8		0.156		166-180 [1]
166-180 [10]	1271	129	2276	312	78	149	703	892	141		1.3		1.3		0.600		166-180 [10]
166-180 [20]	542	143	231	796	585	1358	609	438	47		1.1		1.1		0.845		166-180 [20]
176-195 [1]	216	708	327	551	498	488	465	173	-97		0.8		0.8		0.678		176-195 [1]
176-195 [10]	791	355	230	290	390	210	378	214	-184		0.6		0.7		0.434		176-195 [10]
176-195 [20]	300	2697	224	623	138	37	670	1013	108		1.3		1.2		0.697		176-195 [20]
191-210 [1]	11948	307	182	227	170	81	2153	4799	1,591		4.7	*	3.8	*	0.038	*	191-210 [1]
191-210 [10]	2946	337	103	762	2100	270	1086	1165	524		2.2	*	1.9		0.075		191-210 [10]
191-210 [20]	1666	258	389	259	154	106	472	593	-90		0.8		0.8		0.718		191-210 [20]
210-229 [1]	293	346	109	295	11472	684	2200	4546	1,638		4.8	*	3.9	*	0.026	*	210-229 [1]
210-229 [10]	2383	256	633	1305	713	338	938	799	376		1.9		1.7		0.154		210-229 [10]
210-229 [20]	2766	1181	12476	17744	3422	248	6306	7109	5,744	*	14.5	*	11.2	*	0.000	*	210-229 [20]
229-248 [1]	202	526	1053	2009	603	507	817	645	255		1.6		1.5		0.314		229-248 [1]
229-248 [10]	3395	188	888	334	176	148	855	1275	293		1.7		1.5		0.331		229-248 [10]
229-248 [20]	1612	334	340	645	287	686	651	501	89		1.2		1.2		0.716		229-248 [20]
248-267 [1]	3499	257	342	194	1855	446	1099	1331	537		2.3	*	2.0		0.084		248-267 [1]
248-267 [10]	620	659	2304	303	770	1319	996	721	434		2.0		1.8		0.095		248-267 [10]
248-267 [20]	101	165	434	155	244	156	209	119	-353		0.2		0.4		0.135		248-267 [20]
267-286 [1]	327	403	168	458	188	121	278	138	-284		0.3		0.5		0.227		267-286 [1]
267-286 [10]	2059	104	162	710	163	305	584	756	22		1.1		1.0		0.932		267-286 [10]
267-286 [20]	633	961	623	370	458	119	527	284	-35		0.9		0.9		0.884		267-286 [20]
287-306 [1]	251	626	1020	160	385	809	542	335	-20		1.0		1.0		0.933		287-306 [1]
287-306 [10]	868	141	164	1755	727	326	664	612	102		1.2		1.2		0.684		287-306 [10]
287-306 [20]	261	186	806	305	1134	646	556	372	-6		1.0		1.0		0.981		287-306 [20]
307-326 [1]	198	630	408	1750	296	1365	775	635	213		1.5		1.4		0.399		307-326 [1]
307-326 [10]	235	701	143	140	68	323	268	230	-294		0.3		0.5		0.216		307-326 [10]
307-326 [20]	842	2341	312	950	910	64	903	791	341		1.8		1.6		0.194		307-326 [20]
sAg 10	351	187	172	338	776	168	332	233	-230		0.5		0.6		0.331		sAg 10
sAg 100	425	879	147	477	237	334	417	257	-145		0.7		0.7		0.538		sAg 100
N	83	61	71	60	96	74	74	14	-488		-0.1		0.1		0.041	*	N
N	27	138	251	490	142	128	196	161	-366		0.1		0.3		0.123		N
3H	354	271	92	338	163	437	276	128	-286		0.3		0.5		0.224		3H
3H	2204	670	246	980	967	605	945	673	383		1.9		1.7		0.135		3H
3H	1127	177	734	273	777	245	556	381	-6		1.0		1.0		0.979		3H
3H	102	108	377	277	279	166	218	110	-344		0.2		0.4		0.145		3H
3H	373	164	348	261	31	64	207	144	-355		0.2		0.4		0.133		3H
3H	2491	935	210	340	521	553	842	844	280		1.7		1.5		0.292		3H
3H		545		908	794	1968	1054	628	492		2.2	*	1.9		0.105		3H
	SMC																
N	177	21	17	37	90	35	63	62	-498		0.0		0.1		0.00	*	N
3H	896	488	546	426	485	525	561	169	0		1.0		1.0		1.00		3H
PHA - 1	31284	39396	56708	55868	36956	24314	40754	13109	40,193	*	81.7	*	72.6	*	0.00	*	PHA - 1
PHA - 5	52792	48493	58186	76310	51507	50055	56224	10384	55,663	*	112.7	*	100.2	*	0.00	*	PHA - 5
PHA - 10	82638	62325	68789	66943	49504	63617	65636	10733	65,075	*	131.6	*	117.0	*	0.00	*	PHA - 10
LPS - 1	1101	781	1278	1218	962	650	998	248	437		1.9		1.8		0.01	*	LPS - 1
LPS - 5	786	551	1125	822	448	702	739	236	178		1.4		1.3		0.16		LPS - 5
LPS - 10	796	1133	1377	806	585	1069	961	285	400		1.8		1.7		0.01	*	LPS - 10
LPS - 20	508	985	1856	950	719	764	964	470	403		1.8		1.7		0.08		LPS - 20
LPS - 40	1265	848	1674	807	1452	719	1128	393	567		2.1	*	2.0		0.01	*	LPS - 40
	PBMC																
N	55	31	38	22	12	11	28	17	-34		0.0		0.5		0.00	*	N
3H	64	69	48	50	81	58	62	12	0		1.0		1.0		1.00		3H
PHA - 1	1262	1010	40340	23518	29733	3583	16574	16921	16,513	*	493.9	*	268.8	*	0.04	*	PHA - 1
PHA - 5	19336	44059	47144	45234	34298	39997	38345	10373	38,283	*	1143.8	*	621.8	*	0.00	*	PHA - 5
PHA - 10	22245	33550	31440	28144	26139	39718	30206	6121	30,144	*	900.8	*	489.8	*	0.00	*	PHA - 10
LPS - 1	406	583	658	697	419	730	582	140	521		16.5	*	9.4	*	0.00	*	LPS - 1
LPS - 5	319	721	430	633	472	374	492	155	430		13.8	*	8.0	*	0.00	*	LPS - 5
LPS - 10	342	662	364	402	581	895	541	215	479		15.3	*	8.8	*	0.00	*	LPS - 10
LPS - 20	356	342	365	417	453	539	412	75	350		11.5	*	6.7	*	0.00	*	LPS - 20
LPS - 40	379	361	341	335	395	362	362	23	301		10.0	*	5.9	*	0.00	*	LPS - 40

Raw data for Negative control duck P24P53

P24P53	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	91	119															
Total 3H	1370	553															
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1		>2	
1-15 [1]	914	1054	718	303	521	970	746	290	-624		0.5		0.5		0.021	*	1-15 [1]
1-15 [10]	565	852	336	772	2107	1507	1023	661	-347		0.7		0.7		0.256		1-15 [10]
1-15 [20]	1122	1023	335	592	1190	1141	900	352	-470		0.6		0.7		0.078		1-15 [20]
7-14W-27 [1]	1421	1085	1150	1362	713	1506	1206	290	-164		0.9		0.9		0.509		7-14W-27 [1]
7-14W-27 [10]	2349	2760	2426	1945	1937	2221	2273	313	902		1.7		1.7		0.002	*	7-14W-27 [10]
7-14W-27 [20]	2514	1154	2103	5782	1767	2973	2715	1626	1345		2.1		2.0		0.018	*	7-14W-27 [20]
7-14R-27 [1]	1484	720	568	786	899	1097	925	326	-445		0.7		0.7		0.090		7-14R-27 [1]
7-14R-27 [10]	3709	2584	422	439	744	1496	1565	1333	195		1.2		1.1		0.662		7-14R-27 [10]
7-14R-27 [20]	583	1458	1385	872	582	981	977	380	-394		0.7		0.7		0.139		7-14R-27 [20]
22-41 [1]	445	769	1101	402	899	851	744	272	-626		0.5		0.5		0.020	*	22-41 [1]
22-41 [10]	984	1506	669	771	1264	2519	1285	679	-85		0.9		0.9		0.779		22-41 [10]
22-41 [20]	3392	2396	2575	2397	2193	1012	2327	768	957		1.7		1.7		0.008	*	22-41 [20]
37-56 [1]	724	1145	740	2308	898	1115	1155	593	-215		0.8		0.8		0.457		37-56 [1]
37-56 [10]	1266	720	773	739	612	564	779	252	-591		0.5		0.6		0.025	*	37-56 [10]
37-56 [20]	952	677	504	741	663	542	680	160	-690		0.5		0.5		0.009	*	37-56 [20]
54-73 [1]	787	711	543	868	422	570	650	167	-720		0.4		0.5		0.007	*	54-73 [1]
54-73 [10]	477	425	444	329	282	304	377	82	-994		0.2		0.3		0.001	*	54-73 [10]
54-73 [20]	347	360	641	338	296	344	388	126	-983		0.2		0.3		0.001	*	54-73 [20]
71-90 [1]	832	1017	502	1388	1186	907	972	305	-399		0.7		0.7		0.123		71-90 [1]
71-90 [10]	1012	711	880	500	563	1209	812	273	-558		0.6		0.6		0.035	*	71-90 [10]
71-90 [20]	361	325	258	365	632	605	424	155	-946		0.3		0.3		0.001	*	71-90 [20]
87-106 [1]	1294	1023	593	599	1059	1917	1081	494	-289		0.8		0.8		0.295		87-106 [1]
87-106 [10]	1171	832	503	1214	1796	1474	1165	457	-205		0.8		0.9		0.445		87-106 [10]
87-106 [20]	920	612	785	933	1087	1483	970	298	-401		0.7		0.7		0.120		87-106 [20]
101-120 [1]	1305	669	333	962	1085	938	882	340	-488		0.6		0.6		0.067		101-120 [1]
101-120 [10]	1053	746	308	1002	1190	2154	1075	613	-295		0.8		0.8		0.318		101-120 [10]
101-120 [20]	500	644	387	427	402	463	470	94	-900		0.3		0.3		0.001	*	101-120 [20]
116-130 [1]	650	648	717	1650	1236	800	950	407	-420		0.7		0.7		0.120		116-130 [1]
116-130 [10]	299	396	805	1513	1070	1226	885	476	-486		0.6		0.6		0.086		116-130 [10]
116-130 [20]	212	195	284	297	282	493	294	106	-1077		0.2		0.2		0.000	*	116-130 [20]
126-140 [1]	361	379	251	363	411	376	357	55	-1014		0.2		0.3		0.000	*	126-140 [1]
126-140 [10]	329	208	126	206	128	256	209	78	-1162		0.1		0.2		0.000	*	126-140 [10]
126-140 [20]	279	204	157	235	130	320	221	72	-1150		0.1		0.2		0.000	*	126-140 [20]
136-150 [1]	692	461	431	580	419	423	501	111	-870		0.3		0.4		0.002	*	136-150 [1]
136-150 [10]	556	266	349	388	327	303	365	102	-1006		0.2		0.3		0.000	*	136-150 [10]
136-150 [20]	913	228	181	329	322	288	377	269	-994		0.2		0.3		0.001	*	136-150 [20]
146-160 [1]	578	394	834	860	784	728	696	179	-674		0.5		0.5		0.011	*	146-160 [1]
146-160 [10]	443	286	278	404	247	350	335	77	-1036		0.2		0.2		0.000	*	146-160 [10]
146-160 [20]	241	201	238	326	416	370	298	85	-1072		0.2		0.2		0.000	*	146-160 [20]
156-170 [1]	485	591	454	348	488	251	436	120	-934		0.3		0.3		0.001	*	156-170 [1]
156-170 [10]	713	436	290	359	428	384	435	146	-936		0.3		0.3		0.001	*	156-170 [10]
156-170 [20]	436	220	330	220	172	482	310	127	-1060		0.2		0.2		0.000	*	156-170 [20]
166-180 [1]	566	877	939	825	1241	918	894	217	-476		0.6		0.7		0.062		166-180 [1]
166-180 [10]	595	800	543	591	585	460	596	112	-775		0.4		0.4		0.004	*	166-180 [10]
166-180 [20]	678	462	524	408	794	947	636	209	-735		0.4		0.5		0.007	*	166-180 [20]
176-195 [1]	549	183	220	238	198	689	346	217	-1024		0.2		0.3		0.001	*	176-195 [1]
176-195 [10]	385	232	261	278	274	694	354	175	-1016		0.2		0.3		0.001	*	176-195 [10]
176-195 [20]	332	332	364	363	485	538	402	87	-968		0.2		0.3		0.001	*	176-195 [20]
191-210 [1]	474	12	309	289	412	382	313	162	-1057		0.2		0.2		0.000	*	191-210 [1]
191-210 [10]	718	466	162	166	283	180	329	223	-1041		0.2		0.2		0.000	*	191-210 [10]
191-210 [20]	358	237	124	253	254	220	241	75	-1129		0.1		0.2		0.000	*	191-210 [20]
210-229 [1]	414	229	398	581	368	333	387	116	-983		0.2		0.3		0.001	*	210-229 [1]
210-229 [10]	545	558	372	666	415	11	428	230	-942		0.3		0.3		0.001	*	210-229 [10]
210-229 [20]	1176	2151	2786	1856	1486	13	1578	947	208		1.2		1.2		0.561		210-229 [20]
229-248 [1]	816	822	486	455	695	1252	754	290	-616		0.5		0.6		0.022	*	229-248 [1]
229-248 [10]	838	1190	544	783	1251	1396	1000	328	-370		0.7		0.7		0.153		229-248 [10]
229-248 [20]	3553	2653	1821	1848	2626	3710	2702	807	1331		2.0		2.0		0.001	*	229-248 [20]
248-267 [1]	462	137	193	153	202	188	222	120	-1148		0.1		0.2		0.000	*	248-267 [1]
248-267 [10]	191	139	165	212	216	217	190	32	-1181		0.1		0.1		0.000	*	248-267 [10]
248-267 [20]	161	154	181	147	93	167	150	30	-1220		0.0		0.1		0.000	*	248-267 [20]
267-286 [1]	221	428	191	286	367	438	322	105	-1048		0.2		0.2		0.000	*	267-286 [1]
267-286 [10]	855	484	396	1276	775	738	754	311	-616		0.5		0.6		0.023	*	267-286 [10]
267-286 [20]	795	1096	1301	1894	2020	2195	1550	565	180		1.1		1.1		0.527		267-286 [20]
287-306 [1]	1001	842	679	293	858	299	662	301	-708		0.4		0.5		0.010	*	287-306 [1]
287-306 [10]	885	589	442	338	465	329	508	208	-863		0.3		0.4		0.002	*	287-306 [10]
287-306 [20]	643	562	469	605	401	378	509	110	-861		0.3		0.4		0.002	*	287-306 [20]
307-326 [1]	2972	239	230	247	500	568	793	1078	-578		0.5		0.6		0.147		307-326 [1]
307-326 [10]	451	341	516	289	394	760	459	168	-912		0.3		0.3		0.001	*	307-326 [10]
307-326 [20]	6421	444	400	429	670	10	1396	2471	25		1.0		1.0		0.973		307-326 [20]
sAg 10	1400	1532	1864	290	2693	2909	1781	953	411		1.3		1.3		0.259		sAg 10
sAg 100	1319	1311	1326	1058	2127	1310	1408	367	38		1.0		1.0		0.882		sAg 100
N	79	102	92	83	91	116	94	14	-1277		0.0		0.1		0.000	*	N
N	31	24	37	38	92	43	44	24	-1326		0.0		0.0		0.000	*	N
N	101	612	19	30	51	95	151	228	-1219		0.0		0.1		0.000	*	N
N	203	25	21	35	68	103	76	70	-1294		0.0		0.1		0.000	*	N
3H	2604	1224	911	1086	2295	1743	1643	690	273		1.2		1.2		0.375		3H
3H	1187	941	995	1110	1049	1300	1097	132	-273		0.8		0.8		0.257		3H
	SMC																
N	43	36	38	29	41	35	37	5	-789		0.0		0.0		0.00	*	N
3H	731	730	706	1403	798	586	825	291	0		1.0		1.0		1.00		3H
PHA - 1	3908	3776	4311	4364	2797	2326	3580	834	2755		4.5	*	4.3	*	0.00	*	PHA - 1
PHA - 5	7250	8200	5720	6649	4469	5771	6343	1311	5518	*	8.0	*	7.7	*	0.00	*	PHA - 5
PHA - 10	6785	6579	6749	6673	7716	3946	6408	1275	5583	*	8.1	*	7.8	*	0.00	*	PHA - 10
LPS - 1	1469	1069	1561	1816	1406	710	1338	392	513		1.7		1.6		0.03	*	LPS - 1
LPS - 5	1038	1529	1520	1564	1297	1174	1353	218	528		1.7		1.6		0.01	*	LPS - 5
LPS - 10	2375	2667	2327	2422	2191	1444	2237	419	1412		2.8	*	2.7	*	0.00	*	LPS - 10
LPS - 20	1717	2979	1604	1639	1706	884	1754	678	929		2.2	*	2.1	*	0.01	*	LPS - 20
LPS - 40	2053	2503	2293	1982	2293	806	1988	609	1163		2.5	*	2.4	*	0.00	*	LPS - 40
	PBMC																
N	38	22	21	24	18	28	25	7	-316		0.0		0.1		0.01	*	N
3H	537	675	333	174	164	163	341	220	0		1.0		1.0		1.00		3H
PHA - 1	1668	1576	611	389	325	445	836	617	495		2.6	*	2.5	*	0.09		PHA - 1
PHA - 5	4220	4099	2559	1406	1542	1458	2547	1319	2206		8.0	*	7.5	*	0.00	*	PHA - 5
PHA - 10	4792	3191	2468	1411	2344	2235	2740	1154	2399		8.6	*	8.0	*	0.00	*	PHA - 10
LPS - 1	604	407	214	210	270	227	322	157	-19		0.9		0.9		0.87		LPS - 1
LPS - 5	236	105	44	84	98	88	109	66	-232		0.3		0.3		0.03	*	LPS - 5
LPS - 10	252	187	51	41	355	51	156	130	-185		0.4		0.5		0.11		LPS - 10
LPS - 20	230	168	76	44	54	117	115	73	-226		0.3		0.3		0.04	*	LPS - 20
LPS - 40	225	456	111	60	113	65	171	152	-169		0.5		0.5		0.15		LPS - 40

Raw data for Negative control duck V2T

V2T	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	118	42															
Total 3H	730	644															
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1		<0.05	
1-15 [1]	823	1076	606	772	816	503	766	198	36		1.1		1.0		0.893		1-15 [1]
1-15 [10]	878	428	582	1394	389	1006	780	388	50		1.1		1.1		0.856		1-15 [10]
1-15 [20]	304	375	876	1140	1672	361	788	548	58		1.1		1.1		0.835		1-15 [20]
7-14W-27 [1]	2052	1089	462	1517	406	400	988	690	258		1.4		1.4		0.368		7-14W-27 [1]
7-14W-27 [10]	1131	289	216	637	497	1511	714	508	-16		1.0		1.0		0.953		7-14W-27 [10]
7-14W-27 [20]	601	472	1191	472	737	894	728	279	-2		1.0		1.0		0.994		7-14W-27 [20]
7-14R-27 [1]	897	522	3603	3209	2531	705	1911	1367	1,181		2.9	*	2.6	*	0.001	*	7-14R-27 [1]
7-14R-27 [10]	373	2553	1331	1065	719	3271	1552	1125	822		2.3	*	2.1	*	0.011	*	7-14R-27 [10]
7-14R-27 [20]	3161	857	2637	793	2207	2058	1952	954	1,222		3.0	*	2.7	*	0.000	*	7-14R-27 [20]
22-41 [1]	471	1114	424	439	940	775	694	294	-36		0.9		1.0		0.894		22-41 [1]
22-41 [10]	1560	360	592	472	1733	1108	971	585	241		1.4		1.3		0.392		22-41 [10]
22-41 [20]	354	1877	477	567	1221	739	873	577	143		1.2		1.2		0.611		22-41 [20]
37-56 [1]	971	739	1281	526	2546	544	1101	763	371		1.6		1.5		0.203		37-56 [1]
37-56 [10]	364	386	632	464	1073		584	293	-146		0.8		0.8		0.621		37-56 [10]
37-56 [20]	1250	1173	658	2394	603	1127	1201	646	471		1.8		1.6		0.101		37-56 [20]
54-73 [1]	645	3823	1726	338	381	957	1312	1331	582		2.0		1.8		0.082		54-73 [1]
54-73 [10]	1071	447	1280	358	190	1186	755	476	25		1.0		1.0		0.927		54-73 [10]
54-73 [20]	484		834	368	1889	1065	928	605	198		1.3		1.3		0.517		54-73 [20]
71-90 [1]	751	878	188	412	1560	669	743	471	13		1.0		1.0		0.962		71-90 [1]
71-90 [10]	305	243	1478	167	317	715	538	499	-192		0.7		0.7		0.488		71-90 [10]
71-90 [20]	1095	1224	401	1127	85	2395	1055	800	325		1.5		1.4		0.268		71-90 [20]
87-106 [1]	1897	246	1561	585	527	3273	1348	1142	618		2.0		1.8		0.054		87-106 [1]
87-106 [10]	538	2084	482	266	624	1709	951	752	221		1.4		1.3		0.446		87-106 [10]
87-106 [20]	984	702	1201	837	842	1543	1018	308	288		1.5		1.4		0.290		87-106 [20]
101-120 [1]	1006	233	501	2111	275	2048	1029	859	299		1.5		1.4		0.313		101-120 [1]
101-120 [10]	449	809	530	2345	1771	983	1148	753	418		1.7		1.6		0.152		101-120 [10]
101-120 [20]	1031	744	385	272	851	1456	790	434	60		1.1		1.1		0.827		101-120 [20]
116-130 [1]	885	613	1510	638	1255	2326	1205	652	475		1.8		1.7		0.099		116-130 [1]
116-130 [10]	1713	1121	665	560	1034	689	964	429	234		1.4		1.3		0.396		116-130 [10]
116-130 [20]	748	378	956	273	598	3805	1126	1335	396		1.6		1.5		0.233		116-130 [20]
126-140 [1]	481	269	193	718	872	5352	1314	1995	584		2.0		1.8		0.142		126-140 [1]
126-140 [10]	420	550	462	340	323	382	413	84	-317		0.5		0.6		0.239		126-140 [10]
126-140 [20]	245	140	683	371	191	600	372	224	-358		0.4		0.5		0.187		126-140 [20]
136-150 [1]	1324	753	980	563	432	547	767	334	37		1.1		1.1		0.893		136-150 [1]
136-150 [10]	1008	556	620	1554	811	848	900	360	170		1.3		1.2		0.534		136-150 [10]
136-150 [20]	1027	1251	1658	727	383	320	894	519	164		1.3		1.2		0.554		136-150 [20]
146-160 [1]	1142	3928	482	1007	313	236	1185	1394	455		1.7		1.6		0.178		146-160 [1]
146-160 [10]	881	2051	381	405	1743	918	1063	691	333		1.5		1.5		0.246		146-160 [10]
146-160 [20]	1574	265	409	214	1939	419	803	752	73		1.1		1.1		0.799		146-160 [20]
156-170 [1]	809	5572	2340	756	941	300	1786	1979	1,056		2.7	*	2.4	*	0.009	*	156-170 [1]
156-170 [10]	3805	350	1090	2780	584	2669	1880	1399	1,150		2.9	*	2.6	*	0.001	*	156-170 [10]
156-170 [20]	936	1278	458	495	247	820	706	377	-24		1.0		1.0		0.929		156-170 [20]
166-180 [1]	641	501	1170	2080	1053	1392	1140	568	410		1.7		1.6		0.147		166-180 [1]
166-180 [10]	2109	1175	744	1286	1217	2585	1519	685	789		2.3	*	2.1		0.008	*	166-180 [10]
166-180 [20]	1461	4464	1178	906	1696	368	1679	1440	949		2.6	*	2.3	*	0.007	*	166-180 [20]
176-195 [1]	4015	1740	249	1494	2394	613	1751	1353	1,021		2.7	*	2.4	*	0.003	*	176-195 [1]
176-195 [10]	642	603	511	372	2580	1231	990	833	260		1.4		1.4		0.377		176-195 [10]
176-195 [20]	392	440	1023	1496	544	341	706	459	-24		1.0		1.0		0.931		176-195 [20]
191-210 [1]	224	355	246	180	310	536	309	128	-421		0.3		0.4		0.120		191-210 [1]
191-210 [10]	662	198	376	165	128	440	328	205	-402		0.3		0.4		0.139		191-210 [10]
191-210 [20]	259	499	346	513	409	264	382	111	-348		0.4		0.5		0.197		191-210 [20]
210-229 [1]	2402	446	1360	1215	575	823	1137	714	407		1.7		1.6		0.160		210-229 [1]
210-229 [10]	2676	2704	4804	1323	3696	2427	2938	1187	2,208		4.6	*	4.0	*	0.000	*	210-229 [10]
210-229 [20]	2123	5525	10331	2649		4861	5098	3258	4,368		8.1	*	7.0	*	0.000	*	210-229 [20]
229-248 [1]	2224	421	2418	1148	823	531	1261	861	531		1.9		1.7		0.077		229-248 [1]
229-248 [10]	815	284	196	500	296	976	511	318	-219		0.6		0.7		0.421		229-248 [10]
229-248 [20]	619	237	243	171	264	356	315	160	-415		0.3		0.4		0.126		229-248 [20]
248-267 [1]	514	920	1034	525	1044	1180	870	283	140		1.2		1.2		0.606		248-267 [1]
248-267 [10]	751	598	302	1213	540	2217	937	696	207		1.3		1.3		0.470		248-267 [10]
248-267 [20]	554	839	433	152	640	1283	650	385	-80		0.9		0.9		0.770		248-267 [20]
267-286 [1]	886	162	471	736	455	1767	746	559	16		1.0		1.0		0.954		267-286 [1]
267-286 [10]	1500	312	889	1453	2056	1409	1270	598	540		1.9		1.7		0.059		267-286 [10]
267-286 [20]	1000	2428	1359	2002	2379	797	1661	706	931		2.5	*	2.3	*	0.002	*	267-286 [20]
287-306 [1]	1162	948	825	1330	2048	1144	1243	432	513		1.8		1.7		0.066		287-306 [1]
287-306 [10]	762	786	510	1821	734	1795	1068	582	338		1.6		1.5		0.231		287-306 [10]
287-306 [20]	972	1907	921	476	806	1186	1045	483	315		1.5		1.4		0.257		287-306 [20]
307-326 [1]	555	774	446	804	1375	750	784	322	54		1.1		1.1		0.842		307-326 [1]
307-326 [10]	1475	352	523	448	585	738	687	407	-43		0.9		0.9		0.875		307-326 [10]
307-326 [20]	1150	797	525	1419	975	731	933	320	203		1.3		1.3		0.455		307-326 [20]
sAg 10	816	1084	1676	1344	1800	1087	1301	379	571		1.9		1.8		0.041	*	sAg 10
sAg 100	224	713	564	278	1085	2057	820	682	90		1.1		1.1		0.751		sAg 100
N	188	74	88	139	135	177	134	46	-596		0.0		0.2		0.030	*	N
N	109	69	134	154	77	74	103	35	-627		0.0		0.1		0.022	*	N
3H	265	483	802	511	568	2130	793	677	63		1.1		1.1		0.824		3H
3H	302	2890	557	892	1734	776	1192	963	462		1.8		1.6		0.130		3H
3H	387	790	263	544	368	1359	619	406	-111		0.8		0.8		0.684		3H
3H	235	152	212	189	161	2213	527	827	-203		0.7		0.7		0.489		3H
3H	664	126	726	346	343	656	477	240	-253		0.6		0.7		0.349		3H
3H	310	280	892	614	1343	264	617	432	-113		0.8		0.8		0.681		3H
3H	1830	592	201	640	1678	370	885	693	155		1.3		1.2		0.587		3H
	SMC																
N	58	30	23	28	73	59	45	21	-612		0.0		0.1		0.00	*	N
3H	661	542	446	974	639	681	657	178	0		1.0		1.0		1.00		3H
PHA - 1	44988	40198	41999	20404	33905	44330	37637	9331	36,980	*	61.4	*	57.3	*	0.00	*	PHA - 1
PHA - 5	37396	47292	46660	45193	32161	50128	43138	6873	42,481	*	70.4	*	65.6	*	0.00	*	PHA - 5
PHA - 10	44581	33172	32666	40280	32841	49029	38762	7001	38,104	*	63.3	*	59.0	*	0.00	*	PHA - 10
LPS - 1	1321	1557	1846	1168	2222	1807	1654	385	996		2.6	*	2.5	*	0.00	*	LPS - 1
LPS - 5	2175	2457	3158	1697	1090	1247	1971	783	1,314		3.1	*	3.0	*	0.00	*	LPS - 5
LPS - 10	1818	1112	2590	3668	2578	1500	2211	924	1,554		3.5	*	3.4	*	0.00	*	LPS - 10
LPS - 20	965	348	440	1791	436	464	741	560	84		1.1		1.1		0.74		LPS - 20
LPS - 40	385	374	515	394	445	444	426	53	-231		0.6		0.6		0.01	*	LPS - 40
	PBMC																
N	44	45	20	22	20	32	31	12	-19		0.0		0.6		0.17		N
3H	51	32	25	26	63	101	50	29	0		1.0		1.0		1.00		3H
PHA - 1	3522	1935	380	10892	928	2316	3329	3864	3,279		172.1	*	67.0	*	0.06		PHA - 1
PHA - 5	21863	30496	26000	27343	33614	24264	27263	4255	27,214	*	1420.8	*	548.9	*	0.00	*	PHA - 5
PHA - 10	35398	41919	33231	32767	35215	27351	34314	4730	34,264	*	1788.7	*	690.9	*	0.00	*	PHA - 10
LPS - 1	529	593	970	438	629	411	595	202	545		29.5	*	12.0	*	0.00	*	LPS - 1
LPS - 5	485	544	384	409	455	449	454	57	405		22.1	*	9.1	*	0.00	*	LPS - 5
LPS - 10	440	369	512	269	282	280	359	100	309		17.1	*	7.2	*	0.00	*	LPS - 10
LPS - 20	381	395	202	434	315	323	342	82	292		16.2	*	6.9	*	0.00	*	LPS - 20
LPS - 40	249	292	368	319	378	468	346	77	296		16.4	*	7.0	*	0.00	*	LPS - 40

Raw data for Negative control duck V2U

V2U	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	149	69															
Total 3H	12650	15000								>5000		>2.1		>2.1		<0.05	
	R1	R2	R3	R4	R5	R6	Mean	SD									
1-15 [1]	2168	298	2904	2539	552	892	1559	1113	-11,091		0.1		0.1		0.079		1-15 [1]
1-15 [10]	876	1365	1975	2750	3471	1672	2018	949	-10,632		0.1		0.2		0.092		1-15 [10]
1-15 [20]	37197	1660	1926	1705	25045	405	11323	15819	-1,327		0.9		0.9		0.841		1-15 [20]
7-14W-27 [1]	9224	6385	3206	1900	4574	8578	5645	2935	-7,005		0.4		0.4		0.264		7-14W-27 [1]
7-14W-27 [10]	7231	852	3556	7340	1074	912	3494	3107	-9,156		0.3		0.3		0.146		7-14W-27 [10]
7-14W-27 [20]	34381	52801	29347	10305	6385	35498	28120	17286	15,470	*	2.2	*	2.2	*	0.025	*	7-14W-27 [20]
7-14R-27 [1]	27186	1623	55167	3085	17458	6596	18519	20421	5,869	*	1.5		1.5		0.396		7-14R-27 [1]
7-14R-27 [10]	49031	67589	27264	23555	8653	21200	32882	21482	20,232	*	2.6	*	2.6	*	0.005	*	7-14R-27 [10]
7-14R-27 [20]	47175	59775	65219	31192	43518	3862	41790	22159	29,140	*	3.3	*	3.3	*	0.000	*	7-14R-27 [20]
22-41 [1]	11545	14271	20405	9290	1920	26115	13924	8509	1,274		1.1		1.1		0.841		22-41 [1]
22-41 [10]	16491	11382	355	3291	745	2018	5714	6651	-6,936		0.4		0.5		0.273		22-41 [10]
22-41 [20]	3788	4151	1843	1111	1101	13312	4218	4645	-8,432		0.3		0.3		0.182		22-41 [20]
37-56 [1]	27965	4692	1174	576	2711	501	6270	10747	-6,380		0.5		0.5		0.322		37-56 [1]
37-56 [10]	502	13033	681	226	3958	1768	3361	4932	-9,289		0.3		0.3		0.142		37-56 [10]
37-56 [20]	4817	261	23214	40084	11388	1405	13528	15508	878		1.1		1.1		0.894		37-56 [20]
54-73 [1]	31066	10984	19604	6293	10428	18260	16106	8893	3,456		1.3		1.3		0.587		54-73 [1]
54-73 [10]	3044	22661	2242	4239	11599	1728	7586	8223	-5,064		0.6		0.6		0.425		54-73 [10]
54-73 [20]	13093	2615	658	4125	9981	52427	13817	19486	1,167		1.1		1.1		0.864		54-73 [20]
71-90 [1]	1942	1325	410	5632	4424	12919	4442	4593	-8,208		0.3		0.4		0.193		71-90 [1]
71-90 [10]	531	3571	748	397	4035	586	1645	1682	-11,005		0.1		0.1		0.082		71-90 [10]
71-90 [20]	35962	395	256	1245	3073	1118	7009	14220	-5,642		0.5		0.6		0.391		71-90 [20]
87-106 [1]	1452	305	1007	3602	645	765	1296	1193	-11,354		0.1		0.1		0.073		87-106 [1]
87-106 [10]	2232	134	414	665	783	1239	911	746	-11,739		0.1		0.1		0.064		87-106 [10]
87-106 [20]	11497	289	1412	593	1406	1374	2762	4306	-9,888		0.2		0.2		0.118		87-106 [20]
101-120 [1]	65486	44841	6100	7587	283	3361	21276	27163	8,626	*	1.7		1.7		0.244		101-120 [1]
101-120 [10]	2525	1828	6505	18294	4366	9785	7217	6148	-5,433		0.6		0.6		0.389		101-120 [10]
101-120 [20]	5696	30018	10118	7019	880	15543	11546	10273	-1,104		0.9		0.9		0.863		101-120 [20]
116-130 [1]	6556	1695	450	345	1515	58020	11430	22939	-1,220		0.9		0.9		0.863		116-130 [1]
116-130 [10]	975	845	1791	558	1498	7281	2158	2549	-10,492		0.2		0.2		0.097		116-130 [10]
116-130 [20]	2885	513	505	1183	2609	605	1383	1089	-11,267		0.1		0.1		0.075		116-130 [20]
126-140 [1]	399	7817	4437	367	268	28687	6996	11049	-5,654		0.5		0.6		0.380		126-140 [1]
126-140 [10]	9938	3321	735	231	13743	5474	5574	5345	-7,076		0.4		0.4		0.262		126-140 [10]
126-140 [20]	1846	627	2577	4408	2896	8379	3456	2714	-9,194		0.3		0.3		0.144		126-140 [20]
136-150 [1]	18719	2299	591	1286	1219	1513	4271	7099	-8,379		0.3		0.3		0.188		136-150 [1]
136-150 [10]	50364	1401	5575	1993	1321	2102	10459	19613	-2,191		0.8		0.8		0.749		136-150 [10]
136-150 [20]	711	10524	5112	2128	30329	704	8251	11435	-4,399		0.6		0.7		0.495		136-150 [20]
146-160 [1]	17617	44222	4452	6779	45290	57345	29284	22477	16,634	*	2.3	*	2.3	*	0.021	*	146-160 [1]
146-160 [10]	1075	17561	423	41501	3731	34257	16425	17894	3,775		1.3		1.3		0.576		146-160 [10]
146-160 [20]	6350	12121	58131	2792	651	9533	14930	21579	2,280		1.2		1.2		0.743		146-160 [20]
156-170 [1]	58624	13946	26860	1279	1507	6580	18133	22033	5,483	*	1.4		1.4		0.434		156-170 [1]
156-170 [10]	3710	10857	701	10648	719	21640	8046	8073	-4,604		0.6		0.6		0.468		156-170 [10]
156-170 [20]	10339	1752	10169	2692	19102	13512	9594	6569	-3,056		0.8		0.8		0.627		156-170 [20]
166-180 [1]	1604	3911	1717	2943	6937	6122	3872	2241	-8,778		0.3		0.3		0.163		166-180 [1]
166-180 [10]	7866	8904	722	1119	356	11394	5060	4884	-7,590		0.4		0.4		0.229		166-180 [10]
166-180 [20]	763	185	573	849	8675	698	1957	3299	-10,693		0.1		0.2		0.091		166-180 [20]
176-195 [1]	8394	2684	2240	32033	15851	582	10297	12035	-2,353		0.8		0.8		0.716		176-195 [1]
176-195 [10]	511	512	983	2314	243	20481	4174	8023	-8,476		0.3		0.3		0.184		176-195 [10]
176-195 [20]	1973	9200	1433	1812	1247	2062	2955	3076	-9,695		0.2		0.2		0.125		176-195 [20]
191-210 [1]	734	1323	855	164	2651	12887	3102	4866	-9,548		0.2		0.2		0.132		191-210 [1]
191-210 [10]	329	326	762	2868	939	3555	1463	1392	-11,187		0.1		0.1		0.077		191-210 [10]
191-210 [20]	1135	16588	5326	1771	958	3346	4854	5978	-7,796		0.4		0.4		0.218		191-210 [20]
210-229 [1]	9021	59884	21376	847	7968	4771	17311	21970	4,661		1.4		1.4		0.505		210-229 [1]
210-229 [10]	6738	15054	6938	2144	6971	53379	15204	19161	2,554		1.2		1.2		0.708		210-229 [10]
210-229 [20]	47485	55666	5441	8584	6880	48388	28741	24039	16,091	*	2.3	*	2.3	*	0.028	*	210-229 [20]
229-248 [1]	65138	29403	16416	2985	73240	1989	31529	30950	18,879	*	2.5	*	2.5	*	0.017	*	229-248 [1]
229-248 [10]	53354	25983	7899	12663	21162	4993	21009	17714	8,359	*	1.7		1.7		0.218		229-248 [10]
229-248 [20]	8154	32458	5107	4773	2564	29567	13771	13505	1,121		1.1		1.1		0.863		229-248 [20]
248-267 [1]	8622	64068	3033	1156	215	17762	15809	24517	3,159		1.3		1.2		0.659		248-267 [1]
248-267 [10]	37289	18217	928	472	1010	52783	18450	22218	5,800	*	1.5		1.5		0.409		248-267 [10]
248-267 [20]	1164	557	424	1141	11649	16678	5269	7077	-7,381		0.4		0.4		0.245		248-267 [20]
267-286 [1]	1740	4720	757	1212	2511	2545	2248	1402	-10,402		0.2		0.2		0.099		267-286 [1]
267-286 [10]	3007	1580	6555	649	11801	2764	4393	4149	-8,257		0.3		0.3		0.190		267-286 [10]
267-286 [20]	14622	4779	800	444	6680	7795	5853	5240	-6,797		0.5		0.5		0.281		267-286 [20]
287-306 [1]	10264	48733	9169	25549	13754	50281	26292	18905	13,642	*	2.1		2.1		0.049	*	287-306 [1]
287-306 [10]	1946	33975	521	791	4599	2191	7337	13130	-5,313		0.6		0.6		0.415		287-306 [10]
287-306 [20]	1231	2914	675	234	1356	2863	1546	1115	-11,104		0.1		0.1		0.079		287-306 [20]
307-326 [1]	1218	983	3348	1695	584	725	1426	1020	-11,224		0.1		0.1		0.076		307-326 [1]
307-326 [10]	556	3177	767	318	18377	6440	4939	6984	-7,711		0.4		0.4		0.224		307-326 [10]
307-326 [20]	3276	389	1659	2898	550	27354	6021	10517	-6,629		0.5		0.5		0.303		307-326 [20]
sAg 10	2491	7759	2081	14569	36979	6099	11663	13202	-987		0.9		0.9		0.879		sAg 10
sAg 100	584	11230	1902	928	21533	42159	13056	16427	406		1.0		1.0		0.951		sAg 100
N	124	112	102	94	110	236	130	53	-12,520		0.0		0.0		0.049	*	N
N	250	294	88	142	122	118	169	83	-12,481		0.0		0.0		0.049	*	N
3H	8749	4055	698	4891	3593	17580	6594	5974	-6,056		0.5		0.5		0.337		3H
3H	804	33785	29425	1421	480	5870	11964	15400	-686		0.9		0.9		0.917		3H
3H	7179	28852	54612	37319	6787	8479	23871	19789	11,221	*	1.9		1.9		0.106		3H
3H	11475	7611	23194	4366	3746	49325	16620	17529	3,970		1.3		1.3		0.555		3H
3H	2818	1259	4894	698	3656	49148	10412	19039	-2,238		0.8		0.8		0.742		3H
3H	355	4848	7419	11140	15820	2599	7030	5714	-5,620		0.6		0.6		0.372		3H
3H	41758	1181	8411	5529	6579	8890	12058	14808	-592		1.0		1.0		0.928		3H
	SMC																
N	39	69	61	42	43	20	46	17	-239		0.0		0.2		0.00	*	N
3H.	192	296	314	218	495	195	285	115	0		1.0		1.0		1.00		3H
PHA - 1	36317	36974	42764	38594	40223	49446	40720	4868	40,435	*	169.9	*	142.9	*	0.00	*	PHA - 1
PHA - 5	28998	24960	30995	26133	27344	39552	29664	5291	29,379	*	123.8	*	104.1	*	0.00	*	PHA - 5
PHA - 10	30438	18500	19993	19768	24087	24927	22952	4471	22,667	*	95.7	*	80.5	*	0.00	*	PHA - 10
LPS - 1	4448	2643	6268	4435	4585	4586	4494	1149	4,209		18.6	*	15.8	*	0.00	*	LPS - 1
LPS - 5	25065	21703	19884	22901	17574	8950	19346	5699	19,061	*	80.6	*	67.9	*	0.00	*	LPS - 5
LPS - 10	8992	42138	21667	27078	9666	20714	21709	12279	21,424	*	90.5	*	76.2	*	0.00	*	LPS - 10
LPS - 20	33619	16739	18213	30484	25820	36723	26933	8177	26,648	*	112.3	*	94.5	*	0.00	*	LPS - 20
LPS - 40	30241	22752	22870	35667	26951	22012	26749	5389	26,464	*	111.6	*	93.9	*	0.00	*	LPS - 40
	PBMC																
N	20	48	39	87	40	35	45	23	-23		0.0		0.7		0.05	*	N
3H	66	72	77	73	66	51	68	9	0		1.0		1.0		1.00		3H
PHA - 1	3571	2681	7221	4045	6339	55932	13298	20957	13,231	*	584.7	*	197.0	*	0.15		PHA - 1
PHA - 5	24930	63003	50426	61050	71080	60321	55135	16202	55,068	*	2430.4	*	816.8	*	0.00	*	PHA - 5
PHA - 10	29996	49689	47289	54677	58500	54347	49083	10155	49,016	*	2163.4	*	727.2	*	0.00	*	PHA - 10
LPS - 1	522	1803	741	355	1165	2183	1128	733	1,061		47.8	*	16.7	*	0.01	*	LPS - 1
LPS - 5	413	662	1446	1153	2235	2017	1321	724	1,254		56.3	*	19.6	*	0.00	*	LPS - 5
LPS - 10	912	1269	880	1435	2377	2109	1497	621	1,430		64.1	*	22.2	*	0.00	*	LPS - 10
LPS - 20	679	1277	1090	941	1183	1713	1147	347	1,080		48.6	*	17.0	*	0.00	*	LPS - 20
LPS - 40	671	888	1018	1090	1263	1261	1032	228	964		43.5	*	15.3	*	0.00	*	LPS - 40

Raw data for Protein vaccinated duck G51

G51	Mean	SD														
Total N	200	106														
Total 3H	4108	4007							CPM-3H		S.I.		P/N		t-Test	
	R1	R2	R3	R4	R5	R6	Mean	SD	>5000		>2.1		>2.1		<0.05	
1-15 [1]	3598	1579	4240	4304	2891	1672	3047	1215	-1061		0.7		0.7		0.92	1-15 [1]
1-15 [10]	3624	4265	3144	3275	4994	4590	3982	749	-127		1.0		1.0		0.42	1-15 [10]
1-15 [20]	3897	2576	1641	11767	3592	919	4065	3939	-43		1.0		1.0		0.54	1-15 [20]
7-14W-27 [1]	6085	3568	1854	1645	1917	14306	4896	4908	787		1.2		1.2		0.32	7-14W-27 [1]
7-14W-27 [10]	1869	24118	14073	16515	2343	12039	11826	8570	7718	*	3.0	*	2.9	*	0.00	* 7-14W-27 [10]
7-14W-27 [20]	4090	1845	19729	9434	4709	28101	11318	10408	7209	*	2.8	*	2.8	*	0.02	* 7-14W-27 [20]
7-14R-27 [1]	1062	3604	3314	1741	4136	1869	2621	1226	-1488		0.6		0.6		0.61	7-14R-27 [1]
7-14R-27 [10]	2877	9720	11597		4649	3744	6517	3889	2409		1.6		1.6		0.04	* 7-14R-27 [10]
7-14R-27 [20]	25919	20846	13665	7495	14507	5881	14719	7674	10610	*	3.7	*	3.6	*	0.00	* 7-14R-27 [20]
22-41 [1]	1967	7362	4802	2407	1399	4415	3725	2240	-383		0.9		0.9		0.63	22-41 [1]
22-41 [10]	2562	2331	5990	7034	10324	5182	5570	2987	1462		1.4		1.4		0.08	22-41 [10]
22-41 [20]	7879	12809	12217	5203	9681	3594	8563	3717	4455		2.1	*	2.1		0.00	* 22-41 [20]
37-56 [1]	6217	3424	11649	9604	2954	4903	6458	3487	2350		1.6		1.6		0.03	* 37-56 [1]
37-56 [10]	6679	2632	14388	9574	9230	4707	7868	4149	3760		2.0		1.9		0.01	* 37-56 [10]
37-56 [20]	5438	3346	8919	2529	2578	17627	6739	5857	2631		1.7		1.6		0.08	37-56 [20]
54-73 [1]	1048	9723	8259	14281	6680	4211	7367	4572	3258		1.8		1.8		0.02	* 54-73 [1]
54-73 [10]	1122	13669	6178	2388	4049	7381	5798	4498	1689		1.4		1.4		0.12	54-73 [10]
54-73 [20]	1966	4412	6690	1499	7632	5488	4614	2487	506		1.1		1.1		0.24	54-73 [20]
71-90 [1]	5020	8706	10278	5759	3454	5622	6473	2527	2364		1.6		1.6		0.01	* 71-90 [1]
71-90 [10]	23454	7889	17460	8067	8536	3825	11538	7361	7430	*	2.9	*	2.8	*	0.00	* 71-90 [10]
71-90 [20]	6939	5578	26715	28037	14488	13734	15915	9569	11806	*	4.0	*	3.9	*	0.00	* 71-90 [20]
87-106 [1]	1940	4955	8097	6736	26344	6110	9030	8733	4922		2.3	*	2.2	*	0.04	* 87-106 [1]
87-106 [10]	2601	5622	8025	5918	3861	8472	5750	2285	1641		1.4		1.4		0.04	* 87-106 [10]
87-106 [20]	5582	1213	5054	6155	2853	2601	3910	1962	-199		0.9		1.0		0.51	87-106 [20]
101-120 [1]	8574	2932	5135	4689	6752	9282	6227	2430	2119		1.5		1.5		0.02	* 101-120 [1]
101-120 [10]	10182	20049	7409	5221	5029	5452	8890	5806	4782		2.2	*	2.2	*	0.01	* 101-120 [10]
101-120 [20]	5298	4967	4219	9277	11818	2212	6298	3553	2190		1.6		1.5		0.04	* 101-120 [20]
116-130 [1]	8670	4144	2932	5819	2488	6658	5118	2372	1010		1.3		1.2		0.11	116-130 [1]
116-130 [10]	10789	1460	4236	5128	11771	2040	5904	4390	1795		1.5		1.4		0.10	116-130 [10]
116-130 [20]	6710	17873	11358	6071	14693	2677	9897	5756	5788	*	2.5	*	2.4	*	0.00	* 116-130 [20]
126-140 [1]	1583	3906	1404	1384	1476	1394	1858	1006	-2251		0.4		0.5		0.21	126-140 [1]
126-140 [10]	6466	1454	5824	16803	5551	1437	6256	5626	2147		1.5		1.5		0.11	126-140 [10]
126-140 [20]	2033	4051	1395	4634	1783	3287	2864	1322	-1245		0.7		0.7		0.78	126-140 [20]
136-150 [1]	2364	9884	5392	1733	18449	15340	8860	6928	4752		2.2	*	2.2	*	0.02	* 136-150 [1]
136-150 [10]	2188	4654	3745	4626	9901	15352	6744	4953	2636		1.7		1.6		0.05	* 136-150 [10]
136-150 [20]	4792	4211	7657	2168	19510		7668	6905	3559		1.9		1.9		0.06	136-150 [20]
146-160 [1]	4226	2246	2785	3095	3115	7906	3895	2069	-213		0.9		0.9		0.52	146-160 [1]
146-160 [10]	2632	2546	4359	9933	3474	6049	4832	2816	724		1.2		1.2		0.20	146-160 [10]
146-160 [20]	4968	2001	3440	5643	3725	5854	4272	1484	163		1.0		1.0		0.30	146-160 [20]
156-170 [1]	13352	5773	4954	5826	3078	7057	6673	3526	2565		1.7		1.6		0.02	* 156-170 [1]
156-170 [10]	7333	7025	15101	3265	4923	4783	7071	4216	2963		1.8		1.7		0.02	* 156-170 [10]
156-170 [20]	6214	3012	1780	15145	4412		6112	5312	2004		1.5		1.5		0.12	156-170 [20]
166-180 [1]	11006	2997	2582	17219	5905	2325	7005	5984	2897		1.7		1.7		0.06	166-180 [1]
166-180 [10]	2057	2909	5770	3285	7222	7812	4842	2419	734		1.2		1.2		0.17	166-180 [10]
166-180 [20]	5759	3449	5769	6477	4879	2488	4803	1539	695		1.2		1.2		0.14	166-180 [20]
176-195 [1]	2869	5031	17199	2817	12156	12118	8698	5966	4589		2.2	*	2.1	*	0.01	* 176-195 [1]
176-195 [10]	5020	5707	4458	3239	12700	2073	5533	3743	1424		1.4		1.3		0.11	176-195 [10]
176-195 [20]	4319	4733	7293	7351	2769	8108	5762	2120	1653		1.4		1.4		0.04	* 176-195 [20]
191-210 [1]	3437	4323	3458	7660	1287	1642	3634	2290	-474		0.9		0.9		0.69	191-210 [1]
191-210 [10]	3393	8552	5332	2722	5914	5061	5162	2058	1054		1.3		1.3		0.09	191-210 [10]
191-210 [20]	6808	2361	2187	6422	1763	4084	3937	2223	-171		1.0		1.0		0.51	191-210 [20]
210-229 [1]	5875	4469	4174	5661	21767	6553	8083	6763	3975		2.0		2.0		0.03	* 210-229 [1]
210-229 [10]	16884	25376	3200	6898	12567	3346	11378	8708	7270	*	2.9	*	2.8	*	0.01	* 210-229 [10]
210-229 [20]	5588	7784	12390	35634	21190	8641	15204	11417	11096	*	3.8	*	3.7	*	0.00	* 210-229 [20]
229-248 [1]	2590	1472	408	1053	820	823	1194	767	-2914		0.3		0.3		0.06	229-248 [1]
229-248 [10]	4121	6866	8878	4897	3896	1771	5071	2487	963		1.2		1.2		0.13	229-248 [10]
229-248 [20]	3320	26602	29646	30359	42382	18912	25203	13129	21095	*	6.4	*	6.1	*	0.00	* 229-248 [20]
248-267 [1]	3897	9426	4815	3719	4172	10700	6121	3102	2013		1.5		1.5		0.04	* 248-267 [1]
248-267 [10]	9539	8332	6983	6757	4030	2057	6283	2773	2174		1.6		1.5		0.02	* 248-267 [10]
248-267 [20]	3269	3277	7450	17326	10284	34166	12628	11776	8520	*	3.2	*	3.1	*	0.01	* 248-267 [20]
267-286 [1]	1707	2285	8363	6856	2677	13436	5887	4578	1779		1.5		1.4		0.11	267-286 [1]
267-286 [10]	3972	3553	1839	2326	5938	4136	3627	1457	-481		0.9		0.9		0.66	267-286 [10]
267-286 [20]	3708	5985	6139	7584	3637	9633	6114	2302	2006		1.5		1.5		0.02	* 267-286 [20]
287-306 [1]	2943	2798	2653	3269	3988	6155	3634	1323	-474		0.9		0.9		0.65	287-306 [1]
287-306 [10]	1000	2947	7596	1847	6779	2492	3777	2734	-332		0.9		0.9		0.62	287-306 [10]
287-306 [20]	1958	8318	1778	18462	3162	12630	7718	6769	3609		1.9		1.9		0.05	* 287-306 [20]
307-326 [1]	3512	2403	6256	4658	6919	3234	4497	1785	388		1.1		1.1		0.24	307-326 [1]
307-326 [10]	2900	6844	38509	5071	17459	7634	13069	13432	8961	*	3.3	*	3.2	*	0.02	* 307-326 [10]
307-326 [20]	13365	15480	9163	16128	6361	7105	11267	4280	7158	*	2.8	*	2.7	*	0.00	* 307-326 [20]
sAg 10	1562	5618	2252	4915	1594	1746	2948	1827	-1161		0.7		0.7		0.85	sAg 10
sAg 100	5150	5864	7127	3029	1227	1321	3953	2465	-156		1.0		1.0		0.51	sAg 100
N	115	98	169	184	464	273	217	136	-3891		0.0		0.1		0.01	* N
N	182	124	103	214	163	316	184	76	-3925		0.0		0.0		0.01	* N
N	5477	20388	2845	5325	6671	2517	7204	6657	3095		1.8		1.8		0.07	N
N	1531	3853	5110	3633	1442	1927	2916	1502	-1193		0.7		0.7		0.82	N
3H	2093	4200	2243	9915	2449	1020	3653	3235	-455		0.9		0.9		0.71	3H
3H	2892	2019	2833	3662	3423	1142	2662	937	-1447		0.6		0.6		0.63	3H
	SMC															
N	51	33	21	55	35	154	58	48	-2370		0.0		0.0		0.00	* N
3H	2134	3026	2571	2222	1850	2767	2428	437	0		1.0		1.0		1.00	3H
PHA - 1	7440	9345	8959	7043	6124	9624	8089	1419	5661	*	3.4	*	3.3	*	0.00	* PHA - 1
PHA - 5	20690	27718	26991	24640	21306	25575	24486	2914	22058	*	10.3	*	10.1	*	0.00	* PHA - 5
PHA - 10	33564	46737	38825	45452	39828	48172	42096	5628	39668	*	17.7	*	17.3	*	0.00	* PHA - 10
LPS - 1	2553	2977	2842	2853	2250	2782	2709	265	281		1.1		1.1		0.21	LPS - 1
LPS - 5	3880	4314	4498	3429	3099	4493	3952	588	1524		1.6		1.6		0.00	* LPS - 5
LPS - 10	5091	6532	5571	4605	4540	4721	5177	767	2749		2.2	*	2.1	*	0.00	* LPS - 10
LPS - 20	6668	7350	7638	6488	5439	7867	6908	899	4480		2.9	*	2.8	*	0.00	* LPS - 20
LPS - 40	9250	9781	9879	9430	8502	9956	9466	545	7038	*	4.0	*	3.9	*	0.00	* LPS - 40
	PBMC															
N	135	257	44	43	74	67	103	82	-900		0.0		0.1		0.00	* N
3H	501	924	1058	1343	1163	1031	1003	284	0		1.0		1.0		1.00	3H
PHA - 1	4511	5130	9251	11147	14130	9684	8975	3649	7972	*	9.9	*	8.9	*	0.00	* PHA - 1
PHA - 5	6960	8850	17664	21653	17316	19181	15270	5937	14268	*	16.9	*	15.2	*	0.00	* PHA - 5
PHA - 10	8865	10883	21538	29694	25488	21992	19743	8212	18740	*	21.8	*	19.7	*	0.00	* PHA - 10
LPS - 1	895	1110	1270	1312	1650	1272	1251	249	248		1.3		1.2		0.14	LPS - 1
LPS - 5	697	809	982	1172	1065	1211	989	203	-14		1.0		1.0		0.92	LPS - 5
LPS - 10	620	788	753	1171	1152	1086	928	236	-75		0.9		0.9		0.63	LPS - 10
LPS - 20	519	717	912	832	951	788	786	156	-217		0.8		0.8		0.13	LPS - 20
LPS - 40	326	564	783	860	962	668	694	228	-309		0.7		0.7		0.06	LPS - 40

Raw data for Protein vaccinated duck G53

G53	Mean	SD													
Total N	161	151													
Total 3H	2177	535							CPM-3H		S.I.		P/N	t-Test	
	R1	R2	R3	R4	R5	R6	Mean	SD	>5000		>2.1		>2.1	<0.05	
1-15 [1]	13969	1645	1054	3135	2468	2221	4082	4896	1904		1.9		1.9	0.19	1-15 [1]
1-15 [10]	1535	1425	1902	1234	2726	5585	2401	1647	224		1.1		1.1	0.67	1-15 [10]
1-15 [20]	729	13553	4260	6443	3242	8348	6096	4497	3918		2.9	*	2.8 *	0.01 *	1-15 [20]
7-14W-27 [1]	998	844	1265	1073	2118	2414	1452	652	-726		0.6		0.7	0.02 *	7-14W-27 [1]
7-14W-27 [10]	3724	4573	1424	3148	1862	943	2612	1423	435		1.2		1.2	0.35	7-14W-27 [10]
7-14W-27 [20]	4409	1876	2610	1411	5104	2279	2948	1473	771		1.4		1.4	0.12	7-14W-27 [20]
7-14R-27 [1]	1263	1470	1385	2044	940	1855	1492	401	-685		0.7		0.7	0.01 *	7-14R-27 [1]
7-14R-27 [10]	1479	4322	2755	1338	1064	3942	2483	1408	306		1.2		1.1	0.51	7-14R-27 [10]
7-14R-27 [20]	1970	8202	4160	1815	3688	2350	3697	2402	1520		1.8		1.7	0.05 *	7-14R-27 [20]
22-41 [1]	6543	1967	2600	2378	3305	2080	3145	1731	968		1.5		1.4	0.09	22-41 [1]
22-41 [10]	4082	7871	2152	3735	8552	6304	5449	2526	3271		2.6	*	2.5 *	0.00 *	22-41 [10]
22-41 [20]	28046	9251	2710	1830	3743	2847	8071	10140	5894	*	3.9	*	3.7 *	0.05	22-41 [20]
37-56 [1]	137	776	463	487	1572	197	605	526	-1572		0.2		0.3	0.00 *	37-56 [1]
37-56 [10]	322	1578	521	1165	710	132	738	543	-1440		0.3		0.3	0.00 *	37-56 [10]
37-56 [20]	2757	1690	1148	897	3057	2948	2083	958	-95		1.0		1.0	0.79	37-56 [20]
54-73 [1]	803	510	4349	1112	2449	2752	1996	1465	-182		0.9		0.9	0.70	54-73 [1]
54-73 [10]	5559	574	1660	1139	1471	1364	1961	1802	-217		0.9		0.9	0.70	54-73 [10]
54-73 [20]	2346	866	1729	1164	1453	1536	1515	507	-662		0.7		0.7	0.02 *	54-73 [20]
71-90 [1]	1751	1460	1316	2148	979	1349	1500	403	-677		0.7		0.7	0.02 *	71-90 [1]
71-90 [10]	1181	1505	948	1795	798	1045	1212	373	-966		0.5		0.6	0.00 *	71-90 [10]
71-90 [20]	3028	2882	1043	1374	1030	5184	2423	1621	246		1.1		1.1	0.63	71-90 [20]
87-106 [1]	1415	1115	1504	1525	767	861	1197	333	-980		0.5		0.5	0.00 *	87-106 [1]
87-106 [10]	1498	2271	2659	1754	480	535	1533	891	-645		0.7		0.7	0.07	87-106 [10]
87-106 [20]	1018	975	1240	934	877	1837	1147	360	-1031		0.5		0.5	0.00 *	87-106 [20]
101-120 [1]	2392	1347	721	1223	2388	853	1487	736	-690		0.7		0.7	0.04 *	101-120 [1]
101-120 [10]	1058	1550	1762	1510	1266	1112	1376	276	-801		0.6		0.6	0.00 *	101-120 [10]
101-120 [20]	2325	1901	2157	1164	795	685	1504	714	-673		0.7		0.7	0.04 *	101-120 [20]
116-130 [1]	817	1874	1095	2776	971	931	1411	769	-767		0.6		0.6	0.02 *	116-130 [1]
116-130 [10]	776	2718	2540	2203	1218	1759	1869	763	-309		0.8		0.9	0.33	116-130 [10]
116-130 [20]	2976	2162	1173	1179	756	2318	1761	854	-417		0.8		0.8	0.22	116-130 [20]
126-140 [1]	2211	1478	1032	1891	957	938	1418	538	-760		0.6		0.7	0.01 *	126-140 [1]
126-140 [10]	1189	612	937	998	1300	677	952	272	-1226		0.4		0.4	0.00 *	126-140 [10]
126-140 [20]	2048	1204	2348	1271	699	1551	1520	600	-657		0.7		0.7	0.03 *	126-140 [20]
136-150 [1]	645	471	842	302	691	619	595	187	-1583		0.2		0.3	0.00 *	136-150 [1]
136-150 [10]	894	437	17	920	576	2022	811	680	-1367		0.3		0.4	0.00 *	136-150 [10]
136-150 [20]	1088	435	986	812	1101	1341	960	310	-1217		0.4		0.4	0.00 *	136-150 [20]
146-160 [1]	247	2954	830	794	1279	4116	1703	1502	-474		0.8		0.8	0.33	146-160 [1]
146-160 [10]	2388	1477	2447	1635	2949	2020	2152	551	-25		1.0		1.0	0.93	146-160 [10]
146-160 [20]	1435	3439	3879	1511	1422	1281	2161	1171	-16		1.0		1.0	0.97	146-160 [20]
156-170 [1]	1213	1507	1492	1790	2308	1481	1632	378	-546		0.7		0.7	0.04 *	156-170 [1]
156-170 [10]	1989	1013	893	5006	3052	1740	2282	1545	104		1.1		1.0	0.83	156-170 [10]
156-170 [20]	1930	2171	2351	1227	5349	1408	2417	1683	239		1.1		1.1	0.65	156-170 [20]
166-180 [1]	4930	3597	2059	1426	2042	1244	2550	1430	372		1.2		1.2	0.43	166-180 [1]
166-180 [10]	1038	1331	1010	646	1470	605	1016	350	-1161		0.4		0.5	0.00 *	166-180 [10]
166-180 [20]	1312	2370	2089	1477	316	1333	1482	717	-695		0.7		0.7	0.03 *	166-180 [20]
176-195 [1]	2926	1640	1267	1530	1178	1081	1603	682	-574		0.7		0.7	0.07	176-195 [1]
176-195 [10]	1602	1849	1251	1113	1828	553	1366	498	-812		0.6		0.6	0.01 *	176-195 [10]
176-195 [20]	315	399	1071	668	982	269	617	347	-1560		0.2		0.3	0.00 *	176-195 [20]
191-210 [1]	2357	1548	2312	1641	1205	1848	1818	450	-359		0.8		0.8	0.18	191-210 [1]
191-210 [10]	2650	1900	3006	1276	926	3166	2154	932	-24		1.0		1.0	0.95	191-210 [10]
191-210 [20]	1814	2745	1952	827	1404	495	1539	815	-638		0.7		0.7	0.06	191-210 [20]
210-229 [1]	1685	853	533	1197	301	1054	937	493	-1240		0.4		0.4	0.00 *	210-229 [1]
210-229 [10]	2733	1595	1493	2452	1370	1717	1893	561	-284		0.9		0.9	0.31	210-229 [10]
210-229 [20]	1560	3015	1252	4914	1473	2264	2413	1386	235		1.1		1.1	0.61	210-229 [20]
229-248 [1]	1574	1426	1030	1751	3679	4504	2327	1411	150		1.1		1.1	0.75	229-248 [1]
229-248 [10]	590	432	1100	629	2113	917	963	613	-1214		0.4		0.4	0.00 *	229-248 [10]
229-248 [20]	415	504	981	583	1360	683	754	355	-1424		0.3		0.3	0.00 *	229-248 [20]
248-267 [1]	3820	1049	3910	2355	2455	1882	2578	1114	401		1.2		1.2	0.31	248-267 [1]
248-267 [10]	1508	1916	1815	965	2956	2071	1872	660	-306		0.8		0.9	0.30	248-267 [10]
248-267 [20]	1657	1859	1210	1323	2199	972	1537	454	-641		0.7		0.7	0.02 *	248-267 [20]
267-286 [1]	3857	1137	1022	926	2433	1329	1784	1154	-394		0.8		0.8	0.33	267-286 [1]
267-286 [10]	1072	1399	2345	3317	952	861	1658	976	-520		0.7		0.8	0.16	267-286 [10]
267-286 [20]	1545	1056	1945	2387	1773	3041	1958	689	-220		0.9		0.9	0.46	267-286 [20]
287-306 [1]	1143	1858	969	2052	2781	2120	1820	671	-357		0.8		0.8	0.24	287-306 [1]
287-306 [10]	1837	2410	1598	1776	2634	2325	2096	414	-81		1.0		1.0	0.75	287-306 [10]
287-306 [20]	1364	1789	830	1402	2172	7266	2470	2392	293		1.1		1.1	0.68	287-306 [20]
307-326 [1]	1297	947	873	1106	439	417	846	355	-1331		0.3		0.4	0.00 *	307-326 [1]
307-326 [10]	365	459	441	1163	536	260	537	321	-1640		0.2		0.2	0.00 *	307-326 [10]
307-326 [20]	947	5064	910	1042	1047	1453	1744	1638	-434		0.8		0.8	0.41	307-326 [20]
sAg 10	4520	1352	1688	2233	1831	2211	2305	1134	128		1.1		1.1	0.75	sAg 10
sAg 100	1298	1029	3973	1402	845	2558	1851	1200	-327		0.8		0.8	0.43	sAg 100
N	238	697	229	207	429	302	350	188	-1827		0.1		0.2	0.00 *	N
N	262	110	101	46	121	104	124	72	-2054		0.0		0.1	0.00 *	N
N	53	48	71	98	151	228	108	70	-2070		0.0		0.0	0.00 *	N
N	31	52	64	56	90	89	64	23	-2114		0.0		0.0	0.00 *	N
3H	2191	2026	2434	2358	2737	2564	2385	255	207		1.1		1.1	0.39	3H
3H	2609	2572	951	2353	1381	1956	1970	679	-207		0.9		0.9	0.49	3H
	SMC														
N	47	44	22	23	30	39	34	11	-1099		0.0		0.0	0.00 *	N
3H	1418	1801	1034	715	749	1082	1133	415	0		1.0		1.0	1.00	3H
PHA - 1	4121	4826	3522	3812	2869	3749	3816	648	2683		3.4	*	3.4 *	0.00 *	PHA - 1
PHA - 5	10297	12128	7612	10178	8313	8998	9588	1624	8455	*	8.7	*	8.5 *	0.00 *	PHA - 5
PHA - 10	11511	13560	13046	11010	8336	13317	11797	1983	10664	*	10.7	*	10.4 *	0.00 *	PHA - 10
LPS - 1	1660	1475	1483	1338	1503	1871	1555	185	422		1.4		1.4	0.05 *	LPS - 1
LPS - 5	2323	2737	2065	2162	1941	2530	2293	299	1160		2.1		2.0	0.00 *	LPS - 5
LPS - 10	2664	3022	2380	2199	2070	3037	2562	414	1429		2.3	*	2.3 *	0.00 *	LPS - 10
LPS - 20	3054	2944	2815	2915	2219	3059	2834	315	1701		2.5	*	2.5 *	0.00 *	LPS - 20
LPS - 40	3114	3995	3526	2959	2667	3003	3210	475	2077		2.9	*	2.8 *	0.00 *	LPS - 40
	PBMC														
N	90	57	42	39	46	79	59	21	-293		0.0		0.2	0.00 *	N
3H	568	356	287	285	381	236	352	118	0		1.0		1.0	1.00	3H
PHA - 1	4341	2676	3543	4715	3033	4227	3756	803	3404		12.6	*	10.7 *	0.00 *	PHA - 1
PHA - 5	11526	7869	6388	10955	7295	5917	8325	2366	7973	*	28.2	*	23.7 *	0.00 *	PHA - 5
PHA - 10	12148	7906	8064	10873	6120	7079	8698	2321	8346	*	29.4	*	24.7 *	0.00 *	PHA - 10
LPS - 1	1243	811	1125	1008	1047	741	996	190	644		3.2	*	2.8 *	0.00 *	LPS - 1
LPS - 5	1162	680	770	808	828	740	831	170	479		2.6	*	2.4 *	0.00 *	LPS - 5
LPS - 10	1448	898	1032	1055	852	815	1016	232	664		3.3	*	2.9 *	0.00 *	LPS - 10
LPS - 20	958	639	636	623	649	650	692	130	340		2.2	*	2.0	0.00 *	LPS - 20
LPS - 40	684	402	425	446	456	412	471	106	119		1.4		1.3	0.10	LPS - 40

Raw data for Protein vaccinated duck G63

G63	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	237	559															
Total 3H	1669	2402								>5000		>2.1		>2.1		<0.05	
	R1	R2	R3	R4	R5	R6	Mean	SD									
1-15 [1]	480	298	394	577	250	670	445	162	-1024		0.2		0.3		0.32		1-15 [1]
1-15 [10]	403	701	767	733	559	660	637	135	-832		0.3		0.4		0.42		1-15 [10]
1-15 [20]	1084	1385	793	821	1933	1150	1194	423	-275		0.8		0.8		0.79		1-15 [20]
7-14W-27 [1]	379	652	552	656	323	556	519	139	-950		0.2		0.4		0.35		7-14W-27 [1]
7-14W-27 [10]	1592	507	978	618	587	626	818	413	-652		0.5		0.6		0.52		7-14W-27 [10]
7-14W-27 [20]	239	574	222	251	315	475	346	145	-1123		0.1		0.2		0.28		7-14W-27 [20]
7-14R-27 [1]	427	423	433	384	274	279	370	74	-1099		0.1		0.3		0.29		7-14R-27 [1]
7-14R-27 [10]	301	311	446	296	195	483	338	107	-1131		0.1		0.2		0.27		7-14R-27 [10]
7-14R-27 [20]	222	169	406	392	239	523	325	136	-1144		0.1		0.2		0.27		7-14R-27 [20]
22-41 [1]	205	189	167	331	367	777	339	229	-1130		0.1		0.2		0.27		22-41 [1]
22-41 [10]	265	305	185	281	1075	249	393	337	-1076		0.1		0.3		0.30		22-41 [10]
22-41 [20]	395	306	221	488	749	387	424	183	-1045		0.2		0.3		0.31		22-41 [20]
37-56 [1]	449	244	539	396	233	339	367	119	-1103		0.1		0.2		0.28		37-56 [1]
37-56 [10]	368	375	393	201	518	686	423	164	-1046		0.2		0.3		0.31		37-56 [10]
37-56 [20]	633	566	384	208	244	402	406	169	-1063		0.1		0.3		0.30		37-56 [20]
54-73 [1]	451	349	255	302	305	165	304	95	-1165		0.1		0.2		0.26		54-73 [1]
54-73 [10]	285	228	437	651	222	252	346	169	-1123		0.1		0.2		0.28		54-73 [10]
54-73 [20]	738	235	255	147	291	550	369	226	-1100		0.1		0.3		0.29		54-73 [20]
71-90 [1]	556	287	501	383	282	175	364	145	-1105		0.1		0.2		0.28		71-90 [1]
71-90 [10]	475	434	423	496	269	298	399	94	-1070		0.1		0.3		0.30		71-90 [10]
71-90 [20]	413	210	186	224	316	263	268	84	-1201		0.0		0.2		0.25		71-90 [20]
87-106 [1]	503	269	453	273	462	619	430	137	-1039		0.2		0.3		0.31		87-106 [1]
87-106 [10]	443	302	367	352	335	358	359	47	-1110		0.1		0.2		0.28		87-106 [10]
87-106 [20]	362	306	474	417	560	270	398	108	-1071		0.1		0.3		0.30		87-106 [20]
101-120 [1]	511	225	247	293	350	621	374	158	-1095		0.1		0.3		0.29		101-120 [1]
101-120 [10]	302	333	291	398	542	522	398	111	-1072		0.1		0.3		0.30		101-120 [10]
101-120 [20]	507	478	691	405	205	445	455	157	-1014		0.2		0.3		0.32		101-120 [20]
116-130 [1]	523	847	692	299	486	362	534	205	-935		0.2		0.4		0.36		116-130 [1]
116-130 [10]	413	466	1089	749	352	376	574	290	-895		0.3		0.4		0.38		116-130 [10]
116-130 [20]	951	523	461	543	389	414	547	207	-923		0.3		0.4		0.37		116-130 [20]
126-140 [1]	897	380	454	606	192	256	464	258	-1005		0.2		0.3		0.33		126-140 [1]
126-140 [10]	649	447	259	533	284	219	398	172	-1071		0.1		0.3		0.30		126-140 [10]
126-140 [20]	640	435	366	283	210	457	398	150	-1071		0.1		0.3		0.30		126-140 [20]
136-150 [1]	436	267	229	285	192	187	266	92	-1203		0.0		0.2		0.24		136-150 [1]
136-150 [10]	641	227	228	136	147	227	268	188	-1202		0.0		0.2		0.25		136-150 [10]
136-150 [20]	703	427	543	402	278	259	435	168	-1034		0.2		0.3		0.31		136-150 [20]
146-160 [1]	444	371	242	286	243	258	307	83	-1162		0.1		0.2		0.26		146-160 [1]
146-160 [10]	468	445	220	289	127	208	293	137	-1177		0.0		0.2		0.25		146-160 [10]
146-160 [20]	649	489	616	529	173	150	434	219	-1035		0.2		0.3		0.31		146-160 [20]
156-170 [1]	404	605	326	377	321	373	401	105	-1069		0.1		0.3		0.30		156-170 [1]
156-170 [10]	218	252	312	501	520	218	337	139	-1133		0.1		0.2		0.27		156-170 [10]
156-170 [20]	726	712	641	686	166	220	525	259	-944		0.2		0.4		0.36		156-170 [20]
166-180 [1]	322	290	295	824	7543	2735	2001	2874	532		1.4		1.4		0.68		166-180 [1]
166-180 [10]	319	240	202	315	8149	1178	1734	3164	265		1.2		1.2		0.85		166-180 [10]
166-180 [20]	433	290	330	322	8322	1598	1882	3195	413		1.3		1.3		0.76		166-180 [20]
176-195 [1]	294	301	342	312	200	161	268	71	-1201		0.0		0.2		0.25		176-195 [1]
176-195 [10]	392	379	179	296	183	110	256	116	-1213		0.0		0.2		0.24		176-195 [10]
176-195 [20]	372	542	241	225	314	160	309	136	-1160		0.1		0.2		0.26		176-195 [20]
191-210 [1]	326	247	304	245	180	203	251	56	-1218		0.0		0.2		0.24		191-210 [1]
191-210 [10]	333	350	351	345	305	401	347	31	-1122		0.1		0.2		0.28		191-210 [10]
191-210 [20]	622	259	361	247	216	287	332	150	-1137		0.1		0.2		0.27		191-210 [20]
210-229 [1]	615	760	302	573	683	345	546	184	-923		0.3		0.4		0.37		210-229 [1]
210-229 [10]	508	383	538	354	383	458	437	75	-1032		0.2		0.3		0.32		210-229 [10]
210-229 [20]	897	1154	1367	1578	1053	801	1141	292	-328		0.7		0.8		0.75		210-229 [20]
229-248 [1]	285	282	397	292	549	603	401	143	-1068		0.1		0.3		0.30		229-248 [1]
229-248 [10]	316	295	385	296	481	403	363	74	-1107		0.1		0.2		0.28		229-248 [10]
229-248 [20]	248	339	556	240	330	546	376	141	-1093		0.1		0.3		0.29		229-248 [20]
248-267 [1]	449	348	229	198	269	210	284	97	-1186		0.0		0.2		0.25		248-267 [1]
248-267 [10]	250	203	566	300	179	168	277	149	-1192		0.0		0.2		0.25		248-267 [10]
248-267 [20]	440	386	188	516	122	267	320	153	-1150		0.1		0.2		0.27		248-267 [20]
267-286 [1]	701	558	321	8012	7436	1402	3072	3626	1602		2.3	*	2.1		0.28		267-286 [1]
267-286 [10]	720	162	239	1460	5552	1338	1578	2020	109		1.1		1.1		0.93		267-286 [10]
267-286 [20]	766	612	852	4358	6745	1107	2407	2555	937		1.8		1.6		0.46		267-286 [20]
287-306 [1]	567	626	663	229	239	397	453	193	-1016		0.2		0.3		0.32		287-306 [1]
287-306 [10]	741	913	960	545	824	341	720	237	-749		0.4		0.5		0.46		287-306 [10]
287-306 [20]	1180	855	836	599	325	365	693	327	-776		0.4		0.5		0.45		287-306 [20]
307-326 [1]	445	323	399	358	702	269	416	153	-1053		0.1		0.3		0.31		307-326 [1]
307-326 [10]	489	348	566	559	359	360	447	103	-1023		0.2		0.3		0.32		307-326 [10]
307-326 [20]	823	735	561	704	407	464	615	164	-854		0.3		0.4		0.40		307-326 [20]
sAg 10	215	184	277	286	219	265	241	41	-1228		0.0		0.2		0.24		sAg 10
sAg 100	202	242	248	219	264	255	238	24	-1231		0.0		0.2		0.23		sAg 100
N	212	621	232	263	162	39	255	196	-1215		0.0		0.2		0.24		N
N	47	80	89	86	593	2750	607	1070	-862		0.3		0.4		0.42		N
N	53	102	44	54	26	17	49	30	-1420		-0.2		0.0		0.17		N
N	49	35	72	33	22	16	38	20	-1431		-0.2		0.0		0.17		N
3H	453	793	430	784	304	379	524	211	-945		0.2		0.4		0.36		3H
3H	339	268	326	1480	8496	3580	2415	3240	945		1.8		1.6		0.49		3H
	SMC																
N	76	47	47	53	51	64	56	11	-1049		0.0		0.1		0.00	*	N
3H	1322	1473	1137	1009	706	984	1105	271	0		1.0		1.0		1.00		3H
PHA - 1	2830	2883	3206	3972	1598	2270	2793	809	1688		2.6	*	2.5	*	0.00	*	PHA - 1
PHA - 5	10651	7720	6754	6415	3606	3255	6400	2744	5295	*	6.0	*	5.8	*	0.00	*	PHA - 5
PHA - 10	11396	12307	10457	4756	3918	5801	8106	3690	7001	*	7.7	*	7.3	*	0.00	*	PHA - 10
LPS - 1	1327	1123	1148	1003	775	885	1043	198	-62		0.9		0.9		0.66		LPS - 1
LPS - 5	1288	902	1017	907	661	767	923	217	-182		0.8		0.8		0.23		LPS - 5
LPS - 10	864	1083	1047	915	832	865	934	105	-171		0.8		0.8		0.18		LPS - 10
LPS - 20	1046	1348	1313	1276	1149	1140	1212	118	107		1.1		1.1		0.40		LPS - 20
LPS - 40	1784	1583	1483	1366	1326	1050	1432	249	327		1.3		1.3		0.05		LPS - 40
	PBMC																
N	42	30	14	16	29	116	41	38	-1461		0.0		0.0		0.00	*	N
3H	1366	1340	1864	1433	1716	1293	1502	232	0		1.0		1.0		1.00		3H
PHA - 1	2856	4261	4985	3515	5249	4975	4306	952	2805		2.9	*	2.9	*	0.00	*	PHA - 1
PHA - 5	8076	7964	8955	6965	11075	9751	8797	1462	7296	*	6.0	*	5.9	*	0.00	*	PHA - 5
PHA - 10	8454	12552	17	9923	16934	13980	10310	5864	8808	*	7.0	*	6.9	*	0.00	*	PHA - 10
LPS - 1	252	363	244	272	475	271	313	90	-1189		0.2		0.2		0.00	*	LPS - 1
LPS - 5	227	307	271	234	322	203	260	47	-1241		0.2		0.2		0.00	*	LPS - 5
LPS - 10	261	193	228	310	225	252	245	40	-1257		0.1		0.2		0.00	*	LPS - 10
LPS - 20	222	141	235	258	272	276	234	50	-1268		0.1		0.2		0.00	*	LPS - 20
LPS - 40	398	233	203	213	242	828	353	244	-1149		0.2		0.2		0.00	*	LPS - 40

Raw data for Protein vaccinated duck G99

G99	Mean	SD													
Total N	688	675													
Total 3H	70788	14636							CPM-3H		S.I.		P/N	t-Test	
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1	<0.05	
1-15 [1]	58016	67963	65124	57203	56249	72447	62833	6667	-7954		0.9		0.9		0.23	1-15 [1]
1-15 [10]	42092	76919	67766	59941	65805	76881	64901	12976	-5887		0.9		0.9		0.42	1-15 [10]
1-15 [20]	80081	77417	68371	49103	71573	72198	69790	10982	-997		1.0		1.0		0.89	1-15 [20]
7-14W-27 [1]	43982	61077	52083	52173	75557	52994	56311	10873	-14477		0.8		0.8		0.05	* 7-14W-27 [1]
7-14W-27 [10]	42681	65440	73935	72247	81645	63419	66561	13384	-4227		0.9		0.9		0.56	7-14W-27 [10]
7-14W-27 [20]	67410	80632	82727	91842	105855	92462	86821	13048	16033	*	1.2		1.2		0.04	* 7-14W-27 [20]
7-14R-27 [1]	63893	58767	67614	92108	80344	91507	75705	14368	4918		1.1		1.1		0.51	7-14R-27 [1]
7-14R-27 [10]	65311	61513	65548	86568	94117	94465	77920	15441	7133	*	1.1		1.1		0.35	7-14R-27 [10]
7-14R-27 [20]	61811	61237	71014	72822	81622	86628	72522	10259	1734		1.0		1.0		0.80	7-14R-27 [20]
22-41 [1]	54495	70320	74159	78772	79269	86238	73875	10902	3088		1.0		1.0		0.66	22-41 [1]
22-41 [10]	33624	71995	65391	91033	81294	69574	68952	19730	-1836		1.0		1.0		0.83	22-41 [10]
22-41 [20]	48750	70838	77647	77883	63681	58367	66194	11500	-4594		0.9		0.9		0.51	22-41 [20]
37-56 [1]	77176	65108	71323	91469	64411	97420	77818	13816	7030	*	1.1		1.1		0.34	37-56 [1]
37-56 [10]	70961	76198	78575	81755	108503	70098	81015	14182	10227	*	1.1		1.1		0.18	37-56 [10]
37-56 [20]	81482	56052	56872	51088	65819	55078	61065	11113	-9723		0.9		0.9		0.17	37-56 [20]
54-73 [1]	99049	63067	55694	79807	84555	85144	77886	15880	7098	*	1.1		1.1		0.36	54-73 [1]
54-73 [10]	84161	55696	81481	76001	73364	79564	75044	10228	4257		1.1		1.1		0.53	54-73 [10]
54-73 [20]	75862	69178	90373	60594	90964	77711	77447	11872	6659	*	1.1		1.1		0.35	54-73 [20]
71-90 [1]	53890	80916	64285	71599	85300	58017	69001	12535	-1787		1.0		1.0		0.80	71-90 [1]
71-90 [10]	58376	98936	61017	86372	78493	65856	74841	15936	4054		1.1		1.1		0.60	71-90 [10]
71-90 [20]	65078	73906	76907	85686	72628	78192	75400	6817	4612		1.1		1.1		0.48	71-90 [20]
87-106 [1]	72878	77205	56859	66286	75073	126275	79096	24260	8308	*	1.1		1.1		0.37	87-106 [1]
87-106 [10]	53522	79696	56342	63419	86688	91757	71904	16282	1116		1.0		1.0		0.88	87-106 [10]
87-106 [20]	65197	82798	61977	84776	73110	87556	75902	10756	5114	*	1.1		1.1		0.46	87-106 [20]
101-120 [1]	69890	89940	87009	90845	85975	84556	84702	7636	13915	*	1.2		1.2		0.05	* 101-120 [1]
101-120 [10]	66248	91276	85440	100690	79733	99912	87216	13100	16429	*	1.2		1.2		0.03	* 101-120 [10]
101-120 [20]	83031	85163	83048	99468	87934	85592	87372	6200	16585	*	1.2		1.2		0.02	* 101-120 [20]
116-130 [1]	71376	58315	82655	74392	99924	102518	81530	17164	10742	*	1.2		1.2		0.18	116-130 [1]
116-130 [10]	75077	70011	77511	77132	70086	80630	75074	4279	4287		1.1		1.1		0.50	116-130 [10]
116-130 [20]	68781	92260	73313	61265	71405	75393	73736	10311	2948		1.0		1.0		0.67	116-130 [20]
126-140 [1]	48593	69340	65844	54476	61526	71972	61958	8996	-8829		0.9		0.9		0.20	126-140 [1]
126-140 [10]	51346	22700	41771	32286	58933	39853	41148	12971	-29640		0.6		0.6		0.00	* 126-140 [10]
126-140 [20]	62507	36391	44066	49699	31457	58007	47021	12097	-23767		0.7		0.7		0.00	* 126-140 [20]
136-150 [1]	71569	66628	76022	51672	89966	78977	72472	12873	1685		1.0		1.0		0.81	136-150 [1]
136-150 [10]	63353	22655	52223	32896	79289	73486	53983	22529	-16804		0.8		0.8		0.07	136-150 [10]
136-150 [20]	60165	70532	36189	66273	78709	40111	58663	17042	-12125		0.8		0.8		0.14	136-150 [20]
146-160 [1]	79927	85735	75206	81183	84771	82210	81505	3777	10718	*	1.2		1.2		0.10	146-160 [1]
146-160 [10]	58702	86441	28225	69498	69515	72692	64179	19736	-6609		0.9		0.9		0.43	146-160 [10]
146-160 [20]	57003	90247	63770	62806	65026	67229	67680	11572	-3108		1.0		1.0		0.66	146-160 [20]
156-170 [1]	47087	71780	72059	57486	62173	56414	61166	9661	-9621		0.9		0.9		0.17	156-170 [1]
156-170 [10]	53808	68849	66650	58305	49111	62854	59929	7621	-10858		0.8		0.8		0.11	156-170 [10]
156-170 [20]	56315	74310	67148	70104	73403	74407	69281	6956	-1507		1.0		1.0		0.82	156-170 [20]
166-180 [1]	78603	89420	76566	89355	71129	80684	80959	7262	10172	*	1.1		1.1		0.13	166-180 [1]
166-180 [10]	83425	73383	102266	82126	67563	86072	82473	11922	11685	*	1.2		1.2		0.11	166-180 [10]
166-180 [20]	88339	70342	72812	71461	69160	60898	72168	8964	1381		1.0		1.0		0.84	166-180 [20]
176-195 [1]	112234	90871	65165	52732	98522	91007	85088	22044	14301	*	1.2		1.2		0.12	176-195 [1]
176-195 [10]	89931	93074	62763	76196	98588	92255	85468	13397	14680	*	1.2		1.2		0.06	176-195 [10]
176-195 [20]	93553	75825	86137	64132	77162	89567	81062	10802	10275	*	1.1		1.1		0.15	176-195 [20]
191-210 [1]	43384	43859	21568	49813	64956	34451	43005	14573	-27783		0.6		0.6		0.00	* 191-210 [1]
191-210 [10]	46139	57681	63589	60501	58232	55149	56882	5983	-13906		0.8		0.8		0.04	* 191-210 [10]
191-210 [20]	49663	37347	50675	61220	46149	54595	49942	8031	-20846		0.7		0.7		0.01	* 191-210 [20]
210-229 [1]	47771	72904	62509	80197	82758	64061	68366	12998	-2421		1.0		1.0		0.74	210-229 [1]
210-229 [10]	71226	58201	52657	40943	79143	38897	56844	16115	-13943		0.8		0.8		0.08	210-229 [10]
210-229 [20]	102385	56917	69742	45415	55692	55793	64324	20188	-6464		0.9		0.9		0.45	210-229 [20]
229-248 [1]	58475	85939	54464	81398	90669	92681	77271	16629	6483	*	1.1		1.1		0.41	229-248 [1]
229-248 [10]	81992	94669	63654	93795	95034	106665	89301	14796	18514	*	1.3		1.3		0.02	* 229-248 [10]
229-248 [20]	86217	82691	92054	103253	97384	100595	93699	8152	22911	*	1.3		1.3		0.00	* 229-248 [20]
248-267 [1]	86367	71352	69548	65043	68478	79248	73372	7918	2585		1.0		1.0		0.69	248-267 [1]
248-267 [10]	84937	49570	49237	65813	89936	91364	71809	19613	1022		1.0		1.0		0.90	248-267 [10]
248-267 [20]	78662	50143	56871	60362	66809	77624	65078	11468	-5709		0.9		0.9		0.42	248-267 [20]
267-286 [1]	80597	79942	72201	72968	76794	87245	78291	5584	7503	*	1.1		1.1		0.25	267-286 [1]
267-286 [10]	68787	58693	71205	86630	62001	66889	69034	9751	-1754		1.0		1.0		0.80	267-286 [10]
267-286 [20]	42328	51111	37832	77867	84098	14	48875	30458	-21913		0.7		0.7		0.05	267-286 [20]
287-306 [1]	79531	48697	74211	64605	61233	74760	67173	11341	-3615		0.9		0.9		0.60	287-306 [1]
287-306 [10]	66476	64763	79621	81187	66713	60293	69842	8514	-946		1.0		1.0		0.89	287-306 [10]
287-306 [20]	77092	53099	71442	65332	76549	52502	66002	11073	-4785		0.9		0.9		0.49	287-306 [20]
307-326 [1]	37806	37926	49534	22305	31717	6358	30941	14974	-39847		0.4		0.4		0.00	* 307-326 [1]
307-326 [10]	110669	82696	52741	67716	46731	26551	64517	29554	-6270		0.9		0.9		0.55	307-326 [10]
307-326 [20]	75345	73854	77004	84020	95895	78532	80775	8195	9987	*	1.1		1.1		0.14	307-326 [20]
sAg 10	29301	48940	41355	82702	75733	63227	56876	20626	-13912		0.8		0.8		0.12	sAg 10
sAg 100	39873	49633	78494	69818	94364	75804	67997	19989	-2790		1.0		1.0		0.74	sAg 100
N	161	353	1550	2173	1943	2498	1446	974	-69342		0.0		0.0		0.00	* N
N	508	305	37	566	581	527	421	213	-70367		0.0		0.0		0.00	* N
N	1084	268	124	513	686	350	504	344	-70284		0.0		0.0		0.00	* N
N	708	282	61	604	366	278	383	236	-70405		0.0		0.0		0.00	* N
3H	35153	66850	71939	73441	76529	82410	67720	16765	-3067		1.0		1.0		0.69	3H
3H	57126	77509	63443	88663	68721	87670	73855	12947	3067		1.0		1.0		0.67	3H
	SMC															
N	26	35	31	33	19	51	32	11	-1096		0.0		0.0		0.00	* N
3H	915	1575	1393	1054	895	939	1128	286	0		1.0		1.0		1.00	3H
PHA - 1	5122	7299	7250	4862	4262	3474	5378	1575	4250		4.9	*	4.8	*	0.00	* PHA - 1
PHA - 5	8586	11302	7996	6125	3920	5590	7253	2603	6125	*	6.6	*	6.4	*	0.00	* PHA - 5
PHA - 10	6256	9362	7621	6046	4157	4424	6311	1965	5183	*	5.7	*	5.6	*	0.00	* PHA - 10
LPS - 1	1327	2075	1664	1317	1085	1059	1421	387	293		1.3		1.3		0.17	LPS - 1
LPS - 5	1318	1487	1625	1282	1390	1337	1406	129	278		1.3		1.2		0.06	LPS - 5
LPS - 10	1259	1740	1589	1193	1178	1155	1352	249	224		1.2		1.2		0.18	LPS - 10
LPS - 20	1249	1745	1580	970	980	889	1235	356	107		1.1		1.1		0.58	LPS - 20
LPS - 40	961	1677	1522	903	961	856	1146	356	18		1.0		1.0		0.92	LPS - 40
	PBMC															
N	32	30	35	31	37	50	35	7	-870		0.0		0.0		0.00	* N
3H	1261	716	1156	873	616	812	906	253	0		1.0		1.0		1.00	3H
PHA - 1	4939	2887	2812	2212	2350	2053	2875	1063	1970		3.3	*	3.2	*	0.00	* PHA - 1
PHA - 5	7987	6261	5057	3988	4328	3572	5199	1661	4293		5.9	*	5.7	*	0.00	* PHA - 5
PHA - 10	10444	6861	5564	4698	6071	4484	6354	2188	5448	*	7.3	*	7.0	*	0.00	* PHA - 10
LPS - 1	851	534	415	353	404	378	489	188	-417		0.5		0.5		0.01	* LPS - 1
LPS - 5	432	274	224	174	244	154	250	99	-656		0.2		0.3		0.00	* LPS - 5
LPS - 10	430	237	223	205	243	433	295	106	-610		0.3		0.3		0.00	* LPS - 10
LPS - 20	423	287	228	188	248	153	254	95	-652		0.3		0.3		0.00	* LPS - 20
LPS - 40	311	266	223	193	233	174	233	50	-673		0.2		0.3		0.00	* LPS - 40

Raw data for Protein vaccinated duck P63

P63	Mean	SD													
Total N	97	76													
Total 3H	9822	5838							CPM-3H		S.I.		P/N	t-Test	
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1	<0.05	
1-15 [1]	6426	3095	3495	4592	3641	4332	4263	1194	-5559		0.4		0.4	0.04	*	1-15 [1]
1-15 [10]	3945	2084	3044	3699	6115	5231	4019	1460	-5803		0.4		0.4	0.03	*	1-15 [10]
1-15 [20]	4263	9476	4963	4481	4359	5420	5494	1999	-4329		0.6		0.6	0.10		1-15 [20]
7-14W-27 [1]	3600	6037	10603	10290	7532	9832	7982	2786	-1840		0.8		0.8	0.48		7-14W-27 [1]
7-14W-27 [10]	6255	12672	9683	6398	5991	9549	8424	2669	-1398		0.9		0.9	0.59		7-14W-27 [10]
7-14W-27 [20]	5072	9070	7895	15649	23905	16754	13057	6991	3235		1.3		1.3	0.31		7-14W-27 [20]
7-14R-27 [1]	8927	18273	10139	12176	14863	11776	12692	3396	2870		1.3		1.3	0.29		7-14R-27 [1]
7-14R-27 [10]	18278	10405	12711	11741	7180	8621	11489	3889	1667		1.2		1.2	0.54		7-14R-27 [10]
7-14R-27 [20]	21384	18860	22072	11831	12634	8973	15959	5515	6137	*	1.6		1.6	0.05	*	7-14R-27 [20]
22-41 [1]	5162	9693	3325	4900	6814	10821	6786	2929	-3037		0.7		0.7	0.25		22-41 [1]
22-41 [10]	8996	6928	4452	5019	4324	5616	5889	1793	-3934		0.6		0.6	0.13		22-41 [10]
22-41 [20]	5587	6716	6148	6413	11243	16833	8823	4425	-999		0.9		0.9	0.72		22-41 [20]
37-56 [1]	10020	6217	9788	9610	7690	17947	10212	4068	390		1.0		1.0	0.89		37-56 [1]
37-56 [10]	5147	6398	7501	6930	15120	7692	8131	3544	-1691		0.8		0.8	0.53		37-56 [10]
37-56 [20]	8891	7415	3792	8111	14494	18548	10208	5348	386		1.0		1.0	0.89		37-56 [20]
54-73 [1]	9555	5663	5523	6857	14547	19279	10237	5574	415		1.0		1.0	0.89		54-73 [1]
54-73 [10]	7654	6447	4895	9692	12227	11166	8680	2834	-1142		0.9		0.9	0.66		54-73 [10]
54-73 [20]	11663	8811	6014	12814	11571	20734	11934	4964	2112		1.2		1.2	0.46		54-73 [20]
71-90 [1]	15630	6396	10798	10390	10827	11002	10840	2930	1018		1.1		1.1	0.70		71-90 [1]
71-90 [10]	9565	9238	6596	14013	10038	16789	11040	3690	1217		1.1		1.1	0.65		71-90 [10]
71-90 [20]	12021	5419	5495	7623	10134	12970	8943	3261	-879		0.9		0.9	0.74		71-90 [20]
87-106 [1]	8301	6244	5250	7600	6867	5962	6704	1119	-3118		0.7		0.7	0.22		87-106 [1]
87-106 [10]	5747	8587	4676	5537	4309	6916	5962	1576	-3860		0.6		0.6	0.14		87-106 [10]
87-106 [20]	8353	8953	5057	5080	3802	12867	7352	3377	-2471		0.7		0.7	0.36		87-106 [20]
101-120 [1]	8646	9786	8577	6957	11982	8632	9096	1677	-726		0.9		0.9	0.77		101-120 [1]
101-120 [10]	10443	8279	8828	11114	15599	9337	10600	2661	778		1.1		1.1	0.76		101-120 [10]
101-120 [20]	12205	10257	14993	13139	12311	19597	13750	3249	3928		1.4		1.4	0.15		101-120 [20]
116-130 [1]	4983	4691	6285	13072	9810	10633	8246	3422	-1577		0.8		0.8	0.55		116-130 [1]
116-130 [10]	1454	2848	6251	9915	4029	6640	5189	3044	-4633		0.5		0.5	0.09		116-130 [10]
116-130 [20]	6575	5749	8007	13796	4718	9232	8013	3255	-1810		0.8		0.8	0.49		116-130 [20]
126-140 [1]	4851	4319	5772	7894	6328	7530	6115	1425	-3707		0.6		0.6	0.15		126-140 [1]
126-140 [10]	3780	3623	3247	5126	7519	8874	5361	2326	-4461		0.5		0.5	0.09		126-140 [10]
126-140 [20]	6418	11893	5412	6379	7067	7280	7408	2292	-2414		0.8		0.8	0.35		126-140 [20]
136-150 [1]	6271	3862	9831	5934	8482	8677	7176	2207	-2646		0.7		0.7	0.31		136-150 [1]
136-150 [10]	8618	7799	5762	5803	6970	9187	7356	1431	-2466		0.7		0.7	0.33		136-150 [10]
136-150 [20]	7987	5699	4237	9007	8812	4098	6640	2247	-3182		0.7		0.7	0.22		136-150 [20]
146-160 [1]	7179	5920	3898	6443	5448	4681	5595	1189	-4228		0.6		0.6	0.10		146-160 [1]
146-160 [10]	8576	4764	5897	6727	9104	6940	7001	1625	-2821		0.7		0.7	0.27		146-160 [10]
146-160 [20]	5910	5901	6340	3924	3586	3877	4923	1251	-4899		0.5		0.5	0.06		146-160 [20]
156-170 [1]	4728	2456	1812	13057	2259	5312	4937	4224	-4885		0.5		0.5	0.09		156-170 [1]
156-170 [10]	1571	1819	4939	10168	7199	4530	5037	3275	-4785		0.5		0.5	0.08		156-170 [10]
156-170 [20]	12528	7438	10308	12499	16393	6915	11013	3564	1191		1.1		1.1	0.66		156-170 [20]
166-180 [1]	6609	7294	5686	10571	8422	9945	8088	1914	-1735		0.8		0.8	0.49		166-180 [1]
166-180 [10]	6427	7970	11529	8313	7545	8751	8422	1716	-1400		0.9		0.9	0.58		166-180 [10]
166-180 [20]	6692	3638	5982	3836	5333	4119	4933	1257	-4889		0.5		0.5	0.06		166-180 [20]
176-195 [1]	10600	11276	6925	9212	10092	8883	9498	1537	-325		1.0		1.0	0.90		176-195 [1]
176-195 [10]	7669	9393	6173	11539	8264	12452	9248	2386	-574		0.9		0.9	0.82		176-195 [10]
176-195 [20]	11690	12058	9894	7254	13052	13481	11238	2319	1416		1.1		1.1	0.58		176-195 [20]
191-210 [1]	3136	3846	7001	5153	6256	4029	4903	1503	-4919		0.5		0.5	0.06		191-210 [1]
191-210 [10]	6890	5241	6576	9394	2522	5702	6054	2253	-3768		0.6		0.6	0.15		191-210 [10]
191-210 [20]	6402	2823	3799	6312	4333	9161	5472	2293	-4351		0.6		0.6	0.10		191-210 [20]
210-229 [1]	13293	8928	11216	3706	3741	8484	8228	3891	-1595		0.8		0.8	0.56		210-229 [1]
210-229 [10]	9488	8516	11096	9242	15992	7965	10383	2946	561		1.1		1.1	0.83		210-229 [10]
210-229 [20]	8643	16523	15635	12510	12782	11975	13011	2817	3189		1.3		1.3	0.23		210-229 [20]
229-248 [1]	9688	4697	7545	8169	2541	12340	7497	3496	-2326		0.8		0.8	0.39		229-248 [1]
229-248 [10]	6903	10916	7442	8196	10551	4991	8166	2256	-1656		0.8		0.8	0.52		229-248 [10]
229-248 [20]	11640	9696	8114	12520	13020	16525	11919	2908	2097		1.2		1.2	0.42		229-248 [20]
248-267 [1]	11438	5968	9499	6490	7135	12347	8813	2689	-1010		0.9		0.9	0.70		248-267 [1]
248-267 [10]	5701	8456	11890	10919	13199	10011	10029	2665	207		1.0		1.0	0.94		248-267 [10]
248-267 [20]	13184	9203	5265	13330	7606	9104	9615	3160	-207		1.0		1.0	0.94		248-267 [20]
267-286 [1]	7144	8395	7975	11762	11550	14273	10183	2771	360		1.0		1.0	0.89		267-286 [1]
267-286 [10]	5888	6133	9303	15400	10388	9914	9504	3470	-318		1.0		1.0	0.90		267-286 [10]
267-286 [20]	4545	15171	7343	24674	8592	8924	11541	7318	1719		1.2		1.2	0.60		267-286 [20]
287-306 [1]	6941	8598	14407	9151	18631	12972	11783	4375	1961		1.2		1.2	0.48		287-306 [1]
287-306 [10]	10696	8382	9371	5628	10051	16446	10096	3581	273		1.0		1.0	0.92		287-306 [10]
287-306 [20]	17855	9071	7756	9867	14528	10001	11513	3857	1691		1.2		1.2	0.53		287-306 [20]
307-326 [1]	12202	7983	6190	14287	10691	12248	10600	3006	778		1.1		1.1	0.77		307-326 [1]
307-326 [10]	15066	17849	19065	9405	7846	10531	13293	4683	3471		1.4		1.4	0.23		307-326 [10]
307-326 [20]	25137	17455	13371	7666	17266	15641	16089	5721	6267	*	1.6		1.6	0.05	*	307-326 [20]
sAg 10	8399	4199	5970	10542	5266	8367	7124	2375	-2699		0.7		0.7	0.30		sAg 10
sAg 100	4721	5740	8666	7997	20142	6936	9033	5629	-789		0.9		0.9	0.79		sAg 100
N	256	155	301	238	37	47	172	112	-9650		0.0		0.0	0.00	*	N
N	86	131	92	76	42	22	75	38	-9748		0.0		0.0	0.00	*	N
N	71	33	76	151	81	19	72	46	-9751		0.0		0.0	0.00	*	N
N	83	141	76	62	39	23	70	41	-9752		0.0		0.0	0.00	*	N
3H	13330	4501	6468	7270	7292	24992	10642	7627	820		1.1		1.1	0.80		3H
3H	8421	6086	11947	14480	9403	3681	9003	3899	-820		0.9		0.9	0.76		3H
	SMC															
N	45	32	29	22	20	74	37	20	-1113		0.0		0.0	0.00	*	N
3H	1449	1022	1150	1191	1031	1058	1150	161	0		1.0		1.0	1.00		3H
PHA - 1	2980	2624	2639	2128	1964	3616	2658	598	1508		2.4	*	2.3	* 0.00	*	PHA - 1
PHA - 5	4630	4185	4873	3187	3831	2195	3817	994	2667		3.4	*	3.3	* 0.00	*	PHA - 5
PHA - 10	5055	4695	3711	3700	4640	3770	4262	603	3112		3.8	*	3.7	* 0.00	*	PHA - 10
LPS - 1	1552	1181	1289	978	1073	1026	1183	213	33		1.0		1.0	0.77		LPS - 1
LPS - 5	1490	1156	1334	1143	1264	1070	1243	153	93		1.1		1.1	0.33		LPS - 5
LPS - 10	1373	1478	1172	1290	1074	1007	1232	181	82		1.1		1.1	0.43		LPS - 10
LPS - 20	1743	1557	1599	1617	1542	1435	1582	101	432		1.4		1.4	0.00	*	LPS - 20
LPS - 40	1841	1567	1597	1677	1599	1574	1642	105	492		1.4		1.4	0.00	*	LPS - 40
	PBMC															
N	127	106	234	31	89	209	133	76	-679		0.0		0.2	0.00	*	N
3H	789	673	1001	689	948	768	811	135	0		1.0		1.0	1.00		3H
PHA - 1	2479	2803	2801	2961	2588	2627	2710	176	1899		3.8	*	3.3	* 0.00	*	PHA - 1
PHA - 5	4100	4483	4569	4669	5077	5063	4660	372	3849		6.7	*	5.7	* 0.00	*	PHA - 5
PHA - 10	3679	5303	5654	5115	5922	6126	5300	878	4489		7.6	*	6.5	* 0.00	*	PHA - 10
LPS - 1	351	254	258	277	234	446	303	81	-508		0.3		0.4	0.00	*	LPS - 1
LPS - 5	312	227	329	239	362	611	346	140	-465		0.3		0.4	0.00	*	LPS - 5
LPS - 10	245	289	399	291	438	747	401	184	-410		0.4		0.5	0.00	*	LPS - 10
LPS - 20	206	281	317	246	436	620	351	153	-460		0.3		0.4	0.00	*	LPS - 20
LPS - 40	151	222	178	205	330	370	243	88	-568		0.2		0.3	0.00	*	LPS - 40

Raw data for Protein vaccinated duck W45

W45	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	86	62															
Total 3H	2052	782															
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1		<0.05	
1-15 [1]	2269	3987	3330	1307	3112	6548	3425	1789	1373		1.7		1.7		0.03	*	1-15 [1]
1-15 [10]	1619	1323	1885	697	1448	732	1284	480	-768		0.6		0.6		0.04	*	1-15 [10]
1-15 [20]	1070	1690	1860	1267	1957	534	1396	544	-656		0.7		0.7		0.09		1-15 [20]
7-14W-27 [1]	73	101	187	104	36	52	92	54	-1960		0.0		0.0		0.00	*	7-14W-27 [1]
7-14W-27 [10]	3439	3053	3946	5705	3857	12204	5367	3471	3315		2.7	*	2.6	*	0.01	*	7-14W-27 [10]
7-14W-27 [20]	13230	1186	39410	26772	6036	36946	20597	16140	18545	*	10.4	*	10.0	*	0.00	*	7-14W-27 [20]
7-14R-27 [1]	1522	1990	6262	4521	2566	2599	3243	1797	1191		1.6		1.6		0.06		7-14R-27 [1]
7-14R-27 [10]	7393	3445	5436	37891	34289	10010	16411	15440	14359	*	8.3	*	8.0	*	0.00	*	7-14R-27 [10]
7-14R-27 [20]	29411	16916	65365	23082	30018	51848	36106	18567	34055	*	18.3	*	17.6	*	0.00	*	7-14R-27 [20]
22-41 [1]	7718	1756	2730	1887	2360	1164	2936	2403	884		1.4		1.4		0.25		22-41 [1]
22-41 [10]	10989	2863	6234	6182	4516	1725	5418	3264	3366		2.7	*	2.6	*	0.00	*	22-41 [10]
22-41 [20]	3520	5214	6896	4269	6724	7093	5619	1511	3567		2.8	*	2.7	*	0.00	*	22-41 [20]
37-56 [1]	1259	1254	1273	2894	1432	3438	1925	979	-127		0.9		0.9		0.77		37-56 [1]
37-56 [10]	1202	3586	1521	2183	2630	3710	2472	1039	420		1.2		1.2		0.35		37-56 [10]
37-56 [20]	1865	2907	662	2010	4964	1750	2360	1463	308		1.2		1.1		0.56		37-56 [20]
54-73 [1]	1602	1647	3871	1922	1744	1264	2008	938	-44		1.0		1.0		0.92		54-73 [1]
54-73 [10]	1620	2255	1709	2002	1999	1161	1791	384	-261		0.9		0.9		0.46		54-73 [10]
54-73 [20]	3673	2488	2320	1989	1172	9516	3526	3044	1474		1.7		1.7		0.12		54-73 [20]
71-90 [1]	2025	1652	2410	1517	1926	2559	2015	410	-37		1.0		1.0		0.91		71-90 [1]
71-90 [10]	2371	1421	859	3578	3548	2132	2318	1102	266		1.1		1.1		0.56		71-90 [10]
71-90 [20]	4113	6999	2152	2173	930	1822	3031	2204	979		1.5		1.5		0.18		71-90 [20]
87-106 [1]	1569	1223	1620	4759	2165	2082	2236	1284	184		1.1		1.1		0.71		87-106 [1]
87-106 [10]	2137	4371	1381	985	2417	1946	2206	1181	154		1.1		1.1		0.74		87-106 [10]
87-106 [20]	2138	1887	2213	1402	1018	1519	1696	464	-356		0.8		0.8		0.32		87-106 [20]
101-120 [1]	2168	5579	1870	3610	1327	2003	2759	1577	707		1.4		1.3		0.21		101-120 [1]
101-120 [10]	1206	1317	1563	1224	5079	1901	2048	1508	-4		1.0		1.0		0.99		101-120 [10]
101-120 [20]	2261	1761	6903	1430	1441	981	2463	2216	411		1.2		1.2		0.56		101-120 [20]
116-130 [1]	6669	1802	1799	1594	2229	1981	2679	1966	627		1.3		1.3		0.34		116-130 [1]
116-130 [10]	2929	1493	2052	2050	2055	1774	2059	491	7		1.0		1.0		0.98		116-130 [10]
116-130 [20]	5902	1596	1865	2102	5339	2404	3201	1901	1149		1.6		1.6		0.08		116-130 [20]
126-140 [1]	2847	2005	2205	4689	2226	2154	2688	1022	636		1.3		1.3		0.16		126-140 [1]
126-140 [10]	1026	1087	1456	2047	1385	1439	1407	364	-645		0.7		0.7		0.08		126-140 [10]
126-140 [20]	1716	1263	3278	1661	2847	1816	2097	783	45		1.0		1.0		0.91		126-140 [20]
136-150 [1]	2596	2561	1311	1629	1858	2205	2026	518	-26		1.0		1.0		0.94		136-150 [1]
136-150 [10]	2578	1738	1176	1957	1836	1054	1723	556	-329		0.8		0.8		0.37		136-150 [10]
136-150 [20]	1411	1735	2210	1243	1866	1262	1621	384	-431		0.8		0.8		0.22		136-150 [20]
146-160 [1]	1311	1229	2350	1468	1030	1807	1532	478	-520		0.7		0.7		0.16		146-160 [1]
146-160 [10]	1467	1244	952	2025	1759	2306	1625	503	-427		0.8		0.8		0.24		146-160 [10]
146-160 [20]	2592	2361	1460	2351	2698	848	2051	734	-1		1.0		1.0		1.00		146-160 [20]
156-170 [1]	5813	1041	2850	1966	857	1683	2368	1832	316		1.2		1.2		0.61		156-170 [1]
156-170 [10]	1393	1254	1642	1403	1035	2603	1555	551	-497		0.7		0.8		0.18		156-170 [10]
156-170 [20]	922	1852	1830	10069	9304	1076	4175	4293	2123		2.1		2.0		0.11		156-170 [20]
166-180 [1]	1551	11751	1592	1136	977	1404	3068	4260	1016		1.5		1.5		0.42		166-180 [1]
166-180 [10]	2011	2809	2254	1276	5008	1524	2480	1351	428		1.2		1.2		0.40		166-180 [10]
166-180 [20]	1442	2018	2313	1138	1124	808	1474	580	-578		0.7		0.7		0.13		166-180 [20]
176-195 [1]	1812	2193	3250	1843	2010	2000	2184	539	132		1.1		1.1		0.72		176-195 [1]
176-195 [10]	2491	5570	2730	1728	1143	2277	2656	1537	604		1.3		1.3		0.28		176-195 [10]
176-195 [20]	1063	1697	2104	963	3584	1815	1871	949	-181		0.9		0.9		0.67		176-195 [20]
191-210 [1]	1225	2474	2457	1650	1603	2411	1970	544	-82		1.0		1.0		0.82		191-210 [1]
191-210 [10]	932	1098	1093	1913	1100	2731	1478	706	-574		0.7		0.7		0.15		191-210 [10]
191-210 [20]	4719	1210	1065	2144	1914	1373	2071	1362	19		1.0		1.0		0.97		191-210 [20]
210-229 [1]	1265	8824	1036	1967	2715	1431	2873	2977	821		1.4		1.4		0.37		210-229 [1]
210-229 [10]	1732	2865	3973	3542	5685	6568	4061	1792	2009		2.0		2.0		0.00	*	210-229 [10]
210-229 [20]	4903	21327	5709	2078	7193	16792	9667	7600	7615	*	4.9	*	4.7	*	0.00	*	210-229 [20]
229-248 [1]	1945	3707	1725	2110	3847	5026	3060	1329	1008		1.5		1.5		0.06		229-248 [1]
229-248 [10]	4659	6976	2281	2129	5531	3189	4127	1931	2075		2.1		2.0		0.00	*	229-248 [10]
229-248 [20]	11149	7531	7939	46839	13943	7440	15807	15418	13755	*	8.0	*	7.7	*	0.01	*	229-248 [20]
248-267 [1]	5907	879	1498	2043	1874	12249	4075	4382	2023		2.0		2.0		0.13		248-267 [1]
248-267 [10]	1723	1091	2063	1399	3463	2856	2099	904	47		1.0		1.0		0.91		248-267 [10]
248-267 [20]	5817	2122	3723	2394	1194	561	2635	1898	583		1.3		1.3		0.36		248-267 [20]
267-286 [1]	2190	2739	1651	2658	2851	2628	2453	453	401		1.2		1.2		0.27		267-286 [1]
267-286 [10]	2388	7814	2929	1903	2498	5785	3886	2369	1834		1.9		1.9		0.02	*	267-286 [10]
267-286 [20]	1925	11426	2794	2169	11002	3408	5454	4493	3402		2.7	*	2.7	*	0.02	*	267-286 [20]
287-306 [1]	1600	3353	7358	1757	2543	880	2922	2332	870		1.4		1.4		0.25		287-306 [1]
287-306 [10]	3108	1466	3189	1490	869	2720	2140	987	88		1.0		1.0		0.84		287-306 [10]
287-306 [20]	4340	3800	5432	1538	2952	5702	3961	1565	1909		2.0		1.9		0.00	*	287-306 [20]
307-326 [1]	270	148	122	51	28	19	106	96	-1946		0.0		0.1		0.00	*	307-326 [1]
307-326 [10]	1152	1632	2461	1578	1926	7205	2659	2269	607		1.3		1.3		0.41		307-326 [10]
307-326 [20]	1777	7236	3486	2806	2453	4058	3636	1934	1584		1.8		1.8		0.02	*	307-326 [20]
sAg 10	1477	2185	1530	1733	1435	1316	1613	312	-439		0.8		0.8		0.21		sAg 10
sAg 100	2265	1466	1843	2942	1898	2209	2104	501	52		1.0		1.0		0.89		sAg 100
N	226	152	177	127	82	51	136	64	-1916		0.0		0.1		0.00	*	N
N	185	72	63	115	39	23	83	59	-1969		0.0		0.0		0.00	*	N
N	148	169	116	61	23	28	91	62	-1961		0.0		0.0		0.00	*	N
N	62	38	37	29	23	20	34	15	-2018		0.0		0.0		0.00	*	N
3H	1301	1545	2488	2170	2093	2341	1989	466	-62		1.0		1.0		0.86		3H
3H	1264	1286	1594	2187	2273	4084	2114	1057	63		1.0		1.0		0.89		3H
	BMC																
N	24	26	27	39	22	38	29	7	-1187		0.0		0.0		0.00	*	N
3H	1009	1456	1250	1522	1199	861	1216	253	0		1.0		1.0		1.00		3H
PHA - 1	8136	7744	8967	10178	9962	8208	8866	1016	7650	*	7.4	*	7.3	*	0.00	*	PHA - 1
PHA - 5	16390	23567	34521	29359	26027	18249	24685	6810	23469	*	20.8	*	20.3	*	0.00	*	PHA - 5
PHA - 10	17529	25057	33149	36589	29902	20305	27088	7436	25872	*	22.8	*	22.3	*	0.00	*	PHA - 10
LPS - 1	767	1356	1211	1251	1248	1053	1147	211	-69		0.9		0.9		0.62		LPS - 1
LPS - 5	1196	1566	1330	1842	1655	1371	1493	238	277		1.2		1.2		0.08		LPS - 5
LPS - 10	1656	1646	1213	1178	1423	1580	1449	214	233		1.2		1.2		0.12		LPS - 10
LPS - 20	1779	2325	2207	2039	2164	2006	2086	190	871		1.7		1.7		0.00	*	LPS - 20
LPS - 40	2131	2507	1681	2395	2345	2340	2233	297	1017		1.9		1.8		0.00	*	LPS - 40
	PBMC																
N	67	73	111	160	35	34	80	49	-847		0.0		0.1		0.00	*	N
3H	981	753	777	1313	1175	563	927	282	0		1.0		1.0		1.00		3H
PHA - 1	21	4449	2563	4808	3891	4049	3296	1778	2370		3.8	*	3.6	*	0.01	*	PHA - 1
PHA - 5	7239	6678	7306	8797	8668	7604	7715	844	6789	*	9.0	*	8.3	*	0.00	*	PHA - 5
PHA - 10	10661	8177	9453	11360	8631	17282	10927	3336	10001	*	12.8	*	11.8	*	0.00	*	PHA - 10
LPS - 1	354	298	329	436	506	639	427	129	-500		0.4		0.5		0.00	*	LPS - 1
LPS - 5	507	205	182	242	244	293	279	118	-648		0.2		0.3		0.00	*	LPS - 5
LPS - 10	389	212	205	422	392	322	323	95	-603		0.3		0.3		0.00	*	LPS - 10
LPS - 20	384	263	205	292	413	263	303	80	-624		0.3		0.3		0.00	*	LPS - 20
LPS - 40	627	283	270	336	355	260	355	138	-572		0.3		0.4		0.00	*	LPS - 40

Raw data for Protein vaccinated duck V2J

V2J	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	77	38															
Total 3H	1197	1074															
	R1	R2	R3	R4	R5	R6	Mean	SD	>5000		>2.1		>2.1		>2		
1-15 [1]	486	183	1528	641	552	170	593	497	-604		0.5		0.5		0.18		1-15 [1]
1-15 [10]	325	356	1832	603	1109	1065	882	575	-316		0.7		0.7		0.49		1-15 [10]
1-15 [20]	1993	1033	5590	747	634	2850	2141	1890	944		1.8		1.8		0.08		1-15 [20]
7-14W-27 [1]	967	91	1703	932	284	8403	2063	3158	866		1.8		1.7		0.18		7-14W-27 [1]
7-14W-27 [10]	2500	767	1659	1950	494	10346	2953	3698	1,755		2.6	*	2.5	*	0.01	*	7-14W-27 [10]
7-14W-27 [20]	2534	8049	49093	42221	3832	6142	18645	21121	17,448	*	16.6	*	15.6	*	0.00	*	7-14W-27 [20]
7-14R-27 [1]	904	131	817	1336	1549	4625	1560	1579	363		1.3		1.3		0.47		7-14R-27 [1]
7-14R-27 [10]	292	1657	3643	2463	767	7160	2664	2508	1,466		2.3	*	2.2	*	0.01	*	7-14R-27 [10]
7-14R-27 [20]	1475	1537	3653	1154	771	3477	2011	1235	814		1.7		1.7		0.09		7-14R-27 [20]
22-41 [1]	2035	347	1159	4618	838	1962	1827	1514	629		1.6		1.5		0.21		22-41 [1]
22-41 [10]	1458	1134	1095	1055	2437	1184	1394	531	197		1.2		1.2		0.66		22-41 [10]
22-41 [20]	525	1663	845	478	558	1021	848	452	-349		0.7		0.7		0.44		22-41 [20]
37-56 [1]	885	1132	1163	803	888	1449	1053	242	-144		0.9		0.9		0.75		37-56 [1]
37-56 [10]	640	592	442	1520	293	1595	847	564	-350		0.7		0.7		0.44		37-56 [10]
37-56 [20]	1711	1211	791	2097	1658	1533	1500	450	303		1.3		1.3		0.50		37-56 [20]
54-73 [1]	635	466	4994	2921	1293	637	1824	1800	627		1.6		1.5		0.23		54-73 [1]
54-73 [10]	1682	814	368	630	453	347	716	505	-482		0.6		0.6		0.29		54-73 [10]
54-73 [20]	1550	911	2553	1600	786	391	1299	770	101		1.1		1.1		0.83		54-73 [20]
71-90 [1]	1200	2233	800	2022	757	1046	1343	632	146		1.1		1.1		0.75		71-90 [1]
71-90 [10]	2709	685	1066	915	902	331	1101	828	-96		0.9		0.9		0.84		71-90 [10]
71-90 [20]	7274	1195	1079	343	337	1267	1916	2658	719		1.6		1.6		0.23		71-90 [20]
87-106 [1]	1804	1489	2614	1393	1543	1454	1716	462	519		1.5		1.4		0.25		87-106 [1]
87-106 [10]	942	686	388	1067	3201	4326	1768	1603	571		1.5		1.5		0.26		87-106 [10]
87-106 [20]	2405	1433	1934	1346	4071	2071	2210	995	1,013		1.9		1.8		0.03	*	87-106 [20]
101-120 [1]	3529	1416	2410	2663	1631	1501	2192	831	994		1.9		1.8		0.04	*	101-120 [1]
101-120 [10]	2481	901	1196	456	1790	677	1250	760	53		1.0		1.0		0.91		101-120 [10]
101-120 [20]	654	642	772	631	935	1120	792	198	-405		0.6		0.7		0.37		101-120 [20]
116-130 [1]	1234	5665	112	672	888	4827	2233	2377	1,036		1.9		1.9		0.07		116-130 [1]
116-130 [10]	5074	337	847	959	2953	5016	2531	2142	1,334		2.2	*	2.1	*	0.02	*	116-130 [10]
116-130 [20]	661	1029	700	1047	661	388	748	251	-450		0.6		0.6		0.32		116-130 [20]
126-140 [1]	1216	1197	1443	2653	1182	3662	1892	1035	695		1.6		1.6		0.14		126-140 [1]
126-140 [10]	1922	528	1614	2613	1203	7081	2494	2353	1,296		2.2	*	2.1		0.02	*	126-140 [10]
126-140 [20]	1021	374	1575	766	3299	1688	1454	1030	257		1.2		1.2		0.59		126-140 [20]
136-150 [1]	1491	9220	715	2108	3177	2167	3180	3064	1,982		2.8	*	2.7	*	0.00	*	136-150 [1]
136-150 [10]	8894	2215	1855	3012	2468	4158	3767	2637	2,570		3.3	*	3.1	*	0.00	*	136-150 [10]
136-150 [20]	2709	1178	705	3682	26186	7001	6910	9706	5,713	*	6.1	*	5.8	*	0.00	*	136-150 [20]
146-160 [1]	2672	972	1587	845	2058	1723	1643	682	446		1.4		1.4		0.33		146-160 [1]
146-160 [10]	2854	3773	3309	303	321	6915	2913	2468	1,715		2.5	*	2.4	*	0.00	*	146-160 [10]
146-160 [20]	2399	533	1877	2849	370	285	1386	1130	188		1.2		1.2		0.69		146-160 [20]
156-170 [1]	854	4332	710	726	677	6052	2225	2362	1,028		1.9		1.9		0.07		156-170 [1]
156-170 [10]	776	[illegible]	805	831	1160	260	766	323	-431		0.6		0.6		0.38		156-170 [10]
156-170 [20]	3399	1554	642	150	630	6399	2129	2390	932		1.8		1.8		0.10		156-170 [20]
166-180 [1]	1754	1265	2518	1524	1325	2792	1863	643	666		1.6		1.6		0.15		166-180 [1]
166-180 [10]	605	2421	868	436	844	3772	1491	1325	294		1.3		1.2		0.55		166-180 [10]
166-180 [20]	316	3061	1430	370	189	1685	1175	1117	-22		1.0		1.0		0.96		166-180 [20]
176-195 [1]	3137	2377	8368	1375	601	2093	2992	2773	1,795		2.6	*	2.5	*	0.00	*	176-195 [1]
176-195 [10]	98	985	7292	1040	962	1369	1958	2647	760		1.7		1.6		0.20		176-195 [10]
176-195 [20]	2877	176	556	2200	1159	128	1183	1134	-15		1.0		1.0		0.98		176-195 [20]
191-210 [1]	1977	2511	1336	726	597	445	1265	834	68		1.1		1.1		0.88		191-210 [1]
191-210 [10]	120	383	712	327	236	1044	470	344	-727		0.4		0.4		0.11		191-210 [10]
191-210 [20]	269	1411	490	1224	340	1260	832	519	-365		0.7		0.7		0.42		191-210 [20]
210-229 [1]	3222	938	349	1170	810	511	1167	1049	-31		1.0		1.0		0.95		210-229 [1]
210-229 [10]	7821	3266	627	1834	2140	1855	2924	2542	1,727		2.5	*	2.4	*	0.00	*	210-229 [10]
210-229 [20]	4846	267	983	2531	2002	3489	2353	1667	1,156		2.0		2.0		0.03	*	210-229 [20]
229-248 [1]	1970	1532	257	884	1863	5216	1954	1723	756		1.7		1.6		0.14		229-248 [1]
229-248 [10]	777	627	312	2664	571	322	879	893	-318		0.7		0.7		0.49		229-248 [10]
229-248 [20]	483	3107	272	136	1522	210	955	1172	-242		0.8		0.8		0.61		229-248 [20]
248-267 [1]	297	327	430	647	530	720	492	171	-705		0.4		0.4		0.12		248-267 [1]
248-267 [10]	1186	1243	706	3343	1560	704	1457	982	260		1.2		1.2		0.58		248-267 [10]
248-267 [20]	2070	864	345	790	850	1194	1019	582	-178		0.8		0.9		0.69		248-267 [20]
267-286 [1]	787	658	698	1372	1124	271	818	385	-379		0.7		0.7		0.40		267-286 [1]
267-286 [10]	1007	201	403	373	1111	1650	791	559	-406		0.6		0.7		0.37		267-286 [10]
267-286 [20]	1015	734	1778	1694	3704	1785	1785	1038	588		1.5		1.5		0.21		267-286 [20]
287-306 [1]	1234	1906	582	1070	262	4333	1565	1469	367		1.3		1.3		0.46		287-306 [1]
287-306 [10]	139	4981	1414	562	238	5058	2065	2332	868		1.8		1.7		0.12		287-306 [10]
287-306 [20]	2075	686	368	1355	2485	562	1255	870	58		1.1		1.0		0.90		287-306 [20]
307-326 [1]	7378	1562	6446	464	1131	454	2906	3145	1,708		2.5	*	2.4	*	0.01	*	307-326 [1]
307-326 [10]	4977	1165	863	2026	1560	1217	1968	1527	771		1.7		1.6		0.13		307-326 [10]
307-326 [20]	5738	2638	942	2630	609	1120	2280	1905	1,082		2.0		1.9		0.04	*	307-326 [20]
sAg 10	933	277	149	646	1433	826	711	467	-487		0.6		0.6		0.28		sAg 10
sAg 100	576	97	228	150	479	1026	426	349	-771		0.3		0.4		0.09		sAg 100
N	121	76	47	38	77	82	74	29	-1,124		0.0		0.1		0.01	*	N
N	46	170	62	39	81	83	80	47	-1,117		0.0		0.1		0.02	*	N
3H	187	47	66	99	109	116	104	49	-1,093		0.0		0.1		0.02	*	3H
3H	1777	861	4194	4347	992	1720	2315	1560	1,118		2.0		1.9		0.03	*	3H
3H	666	1454	824	1569	547	542	934	461	-264		0.8		0.8		0.56		3H
3H	982	1923	1318	1033	804	662	1120	452	-77		0.9		0.9		0.86		3H
3H	3583	480	250	462	138	487	900	1322	-297		0.7		0.8		0.54		3H
3H	972	1107	1620	3459	1818	319	1549	1074	352		1.3		1.3		0.46		3H
3H	2065	1818	1768	596	1657	845	1458	592	261		1.2		1.2		0.57		3H
	SMC																
N	24	41	78	44	42	21	42	20	-1,181		0.0		0.0		0.00	*	N
3H	1323	1710	1350	703	1034	1213	1222	337	0		1.0		1.0		1.00		3H
PHA - 1	51130	46163	22047	38933	63777	83253	50884	21027	49,662	*	43.1	*	41.6	*	0.00	*	PHA - 1
PHA - 5	61807	54118	67327	59702	71055	89413	67237	12367	66,015	*	56.9	*	55.0	*	0.00	*	PHA - 5
PHA - 10	73494	37072	65459	57011	60215	82447	62616	15552	61,394	*	53.0	*	51.2	*	0.00	*	PHA - 10
LPS - 1	1499	1603	2764	2208	1089	2186	1892	605	669		1.6		1.5		0.04	*	LPS - 1
LPS - 5	4316	4078	4736	7482	5886	6294	5465	1320	4,243		4.6	*	4.5	*	0.00	*	LPS - 5
LPS - 10	4194	8072	3713	4421	6268	6144	5469	1654	4,247		4.6	*	4.5	*	0.00	*	LPS - 10
LPS - 20	5130	3425	6430	12183	7084	15554	8301	4615	7,079	*	7.0	*	6.8	*	0.00	*	LPS - 20
LPS - 40	6711	7881	9431	7773	493	9943	7039	3416	5,817	*	5.9	*	5.8	*	0.00	*	LPS - 40

Raw data for Protein vaccinated duck V2K

V2K	Mean	SD														
Total N	81	25														
Total 3H	981	1660							CPM-3H		S.I.		P/N		t-Test	
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1		>2
1-15 [1]	1329	662	734	1362	272	1092	909	427	-72		0.9		0.9		0.92		1-15 [1]
1-15 [10]	197	383	1792	552	1614	922	910	662	-71		0.9		0.9		0.92		1-15 [10]
1-15 [20]	469	661	748	[illegible]	944	525	669	189	-311		0.7		0.7		0.68		1-15 [20]
7-14W-27 [1]	513	345	790	1618	424	703	732	465	-248		0.7		0.7		0.72		7-14W-27 [1]
7-14W-27 [10]	4352	5609	2853	9380	2386	9716	5716	3181	4,735		6.3	*	5.8	*	0.00	*	7-14W-27 [10]
7-14W-27 [20]	34377	2755	21324	20670	18022	10248	17899	10759	16,919	*	19.8	*	18.3	*	0.00	*	7-14W-27 [20]
7-14R-27 [1]	1351	1926	917	13386	2678	1175	3572	4849	2,591		3.9	*	3.6	*	0.01	*	7-14R-27 [1]
7-14R-27 [10]	21017	12303	13868	8324	9038	1320	10978	6557	9,998	*	12.1	*	11.2	*	0.00	*	7-14R-27 [10]
7-14R-27 [20]	5331	29067	11331	50164	44899	5283	24346	20028	23,365	*	27.0	*	24.8	*	0.00	*	7-14R-27 [20]
22-41 [1]	426	5101	776	598	779	1710	1565	1789	584		1.6		1.6		0.43		22-41 [1]
22-41 [10]	1299	830	22108	8935	3263	1611	6341	8282	5,360	*	7.0	*	6.5	*	0.00	*	22-41 [10]
22-41 [20]	2097	19492	698	6942	1236	1723	5365	7278	4,384		5.9	*	5.5	*	0.00	*	22-41 [20]
37-56 [1]	930	730	515	503	13260	3716	3276	5043	2,295		3.6	*	3.3	*	0.03	*	37-56 [1]
37-56 [10]	3308	1380	501	500	16943	1324	3993	6427	3,012		4.3	*	4.1	*	0.01	*	37-56 [10]
37-56 [20]	32638	1136	523	641	3664	666	6545	12838	5,564	*	7.2	*	6.7	*	0.01	*	37-56 [20]
54-73 [1]	430	492	701	667	697	506	582	120	-398		0.6		0.6		0.56		54-73 [1]
54-73 [10]	2055	3593	539	1193	2064	1358	1800	1049	820		1.9		1.8		0.25		54-73 [10]
54-73 [20]	13520	1014	595	1608	498	2144	3214	5090	2,233		3.5		3.3	*	0.03	*	54-73 [20]
71-90 [1]	2106	2546	501	1107	603	639	1250	870	270		1.3		1.3		0.70		71-90 [1]
71-90 [10]	4339	8550	1276	43706	743	335	9825	16884	8,844	*	10.8	*	10.0	*	0.00	*	71-90 [10]
71-90 [20]	957	1947	4345	579	863	687	1563	1448	582		1.6		1.6		0.42		71-90 [20]
87-106 [1]	3708	1150	481	709	722	988	1293	1206	312		1.3		1.3		0.66		87-106 [1]
87-106 [10]	7292	548	10977	1728	1060	815	3737	4358	2,756		4.1	*	3.8	*	0.01	*	87-106 [10]
87-106 [20]	690	805	265	1017	630	904	719	263	-262		0.7		0.7		0.70		87-106 [20]
101-120 [1]	3957	1113	482	765	921	887	1354	1292	374		1.4		1.4		0.60		101-120 [1]
101-120 [10]	2384	702	731	1082	1836	2603	1556	836	576		1.6		1.6		0.41		101-120 [10]
101-120 [20]	886	495	322	484	575	689	575	194	-405		0.5		0.6		0.56		101-120 [20]
116-130 [1]	476	300	606	5914	424	723	1407	2213	427		1.5		1.4		0.58		116-130 [1]
116-130 [10]	2001	562	1199	706	315	360	857	644	-123		0.9		0.9		0.86		116-130 [10]
116-130 [20]	276	342	351	563	361	441	389	100	-592		0.3		0.4		0.39		116-130 [20]
126-140 [1]	682	1904	541	540	417	335	737	584	-244		0.7		0.8		0.73		126-140 [1]
126-140 [10]	1201	762	670	613	1068	501	803	274	-178		0.8		0.8		0.80		126-140 [10]
126-140 [20]	1242	4045	615	597	250	330	1180	1446	199		1.2		1.2		0.78		126-140 [20]
136-150 [1]	354	6667	1430	453	444	400	1625	2504	644		1.7		1.7		0.41		136-150 [1]
136-150 [10]	615	1085	3800	1136	810	381	1305	1255	324		1.4		1.3		0.65		136-150 [10]
136-150 [20]	653	628	916	403	776	861	706	186	-274		0.7		0.7		0.69		136-150 [20]
146-160 [1]	209	708	1821	762	955	401	809	563	-171		0.8		0.8		0.81		146-160 [1]
146-160 [10]	543	1277	3594	3356	684	481	1656	1439	675		1.8		1.7		0.35		146-160 [10]
146-160 [20]	375	631	2920	768	693	939	1054	932	74		1.1		1.1		0.92		146-160 [20]
156-170 [1]	773	854	1595	410	693	674	833	402	-147		0.8		0.8		0.83		156-170 [1]
156-170 [10]	689	2094	269	3386	907	1912	1543	1148	562		1.6		1.6		0.43		156-170 [10]
156-170 [20]	556	537	475	3253	949	803	1096	1072	115		1.1		1.1		0.87		156-170 [20]
166-180 [1]	835	1786	1193	1493	397	413	1020	571	39		1.0		1.0		0.96		166-180 [1]
166-180 [10]	971	2028	478	7143	606	823	2008	2575	1,028		2.1	*	2.0		0.20		166-180 [10]
166-180 [20]	540	469	1021	355	337	2631	892	886	-88		0.9		0.9		0.90		166-180 [20]
176-195 [1]	587	471	294	883	1734	297	711	547	-270		0.7		0.7		0.70		176-195 [1]
176-195 [10]	372	1283	919	560	874	329	723	369	-258		0.7		0.7		0.71		176-195 [10]
176-195 [20]	1534	898	313	1075	419	668	818	452	-163		0.8		0.8		0.81		176-195 [20]
191-210 [1]	1467	1523	937	4425	482	435	1545	1485	564		1.6		1.6		0.44		191-210 [1]
191-210 [10]	1841	689	435	360	305	226	643	608	-338		0.6		0.7		0.63		191-210 [10]
191-210 [20]	772	682	413	475	1056	763	694	232	-287		0.7		0.7		0.68		191-210 [20]
210-229 [1]	1916	664	418	586	699	890	862	539	-118		0.9		0.9		0.86		210-229 [1]
210-229 [10]	819	3485	1027	1114	1287	2780	1752	1103	771		1.9		1.8		0.28		210-229 [10]
210-229 [20]	6898	32829	2379	3666	1684	1791	8208	12217	7,227	*	9.0	*	8.4	*	0.00	*	210-229 [20]
229-248 [1]	1736	983	399	1445	989	587	1023	504	43		1.0		1.0		0.95		229-248 [1]
229-248 [10]	788	525	440	1920	617	360	775	580	-206		0.8		0.8		0.77		229-248 [10]
229-248 [20]	1144	1719	399	539	560	606	828	506	-153		0.8		0.8		0.83		229-248 [20]
248-267 [1]	1350	1671	443	380	367	3080	1215	1069	235		1.3		1.2		0.74		248-267 [1]
248-267 [10]	1402	1039	473	816	595	759	847	334	-133		0.9		0.9		0.85		248-267 [10]
248-267 [20]	275	1093	715	1682	527	475	795	515	-186		0.8		0.8		0.79		248-267 [20]
267-286 [1]	345	734	348	601	566	604	533	155	-448		0.5		0.5		0.52		267-286 [1]
267-286 [10]	430	2481	1103	2066	543	404	1171	900	191		1.2		1.2		0.79		267-286 [10]
267-286 [20]	1394	1655	728	642	448	1326	1032	488	52		1.1		1.1		0.94		267-286 [20]
287-306 [1]	553	559	560	365	592	573	534	84	-447		0.5		0.5		0.52		287-306 [1]
287-306 [10]	359	582	230	318	320	317	354	119	-626		0.3		0.4		0.37		287-306 [10]
287-306 [20]	1462	401	542	425	597	394	637	413	-344		0.6		0.6		0.62		287-306 [20]
307-326 [1]	424	1140	742	671	606	452	673	260	-308		0.7		0.7		0.66		307-326 [1]
307-326 [10]	587	645	881	675	1507	808	851	339	-130		0.9		0.9		0.85		307-326 [10]
307-326 [20]	447	531	964	793	1356	1176	878	357	-103		0.9		0.9		0.88		307-326 [20]
sAg 10	837	1067	415	218	906	3821	1211	1318	230		1.3		1.2		0.75		sAg 10
sAg 100	100	245	382	324	163	233	241	103	-739		0.2		0.2		0.29		sAg 100
N	61	55	85	64	110	104	80	23	-901		0.0		0.1		0.19		N
N	131	94	69	85	46	67	82	29	-899		0.0		0.1		0.20		N
3H	224	291	423	143	304	345	288	97	-692		0.2		0.3		0.32		3H
3H	[illegible]	1402	[illegible]	[illegible]	757	583	914	431	-67		0.9		0.9		0.95		3H
3H	3982	560	426	635	625	10076	2717	3857	1,737		2.9	*	2.8	*	0.06		3H
3H	317	771	423	556	1911	584	760	584	-220		0.8		0.8		0.75		3H
3H	548	506	344	299	438	549	447	106	-533		0.4		0.5		0.44		3H
3H	635	407	785	318	2793	929	978	918	-3		1.0		1.0		1.00		3H
3H	730	507	1301	581	380	854	726	327	-255		0.7		0.7		0.71		3H
	SMC															
N	37	45	72	59	59	75	58	15	-1,078		0.0		0.1		0.01	*	N
3H	1977	577	2075	165	1310	713	1136	781	0		1.0		1.0		1.00		3H
PHA - 1	63216	40102	53131	58545	47730	55305	53005	8180	51,869	*	49.1	*	46.7	*	0.00	*	PHA - 1
PHA - 5	40975	19600	29998	36920	36263	42787	34424	8507	33,288	*	31.9	*	30.3	*	0.00	*	PHA - 5
PHA - 10	42842	31538	42671	37192	36478	50008	40122	6437	38,985	*	37.2	*	35.3	*	0.00	*	PHA - 10
LPS - 1	8640	10951	10972	8442	14561	11891	10910	2260	9,773	*	10.1	*	9.6	*	0.00	*	LPS - 1
LPS - 5	37946	13960	20685	23166	33770	72250	33630	20852	32,493	*	31.1	*	29.6	*	0.00	*	LPS - 5
LPS - 10	59691	34245	54068	45079	50667	68528	52046	11845	50,910	*	48.2	*	45.8	*	0.00	*	LPS - 10
LPS - 20	48893	24555	38932	56198	60191	67977	49458	15715	48,322	*	45.8	*	43.5	*	0.00	*	LPS - 20
LPS - 40	45809	32399	30160	37495	47082	51880	40804	8741	39,668	*	37.8	*	35.9	*	0.00	*	LPS - 40

Raw data for Protein vaccinated duck V2L

V2L	Mean	SD													
Total N	72	19													
Total 3H	14570	10020							CPM-3H		S.I.		P/N	t-Test	
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1	>2
1-15 [1]	1593	21276	3084	9788	7302	38455	13583	14040	-987		0.9		0.9	0.83	1-15 [1]
1-15 [10]	23585	12090	6298	7275	3145	37800	15032	13251	462		1.0		1.0	0.92	1-15 [10]
1-15 [20]	25791	7856	30958	6412	9983	26005	17834	10899	3,264		1.2		1.2	0.46	1-15 [20]
7-14W-27 [1]	11130	1102	3762	13832	11742	28048	11603	9459	-2,967		0.8		0.8	0.50	7-14W-27 [1]
7-14W-27 [10]	22398	33477	16445	40030	35777	37263	30898	9333	16,328	*	2.1	*	2.1	0.00	* 7-14W-27 [10]
7-14W-27 [20]	36481	43107	36165	49264	53758	30902	41613	8706	27,043	*	2.9	*	2.9	0.00	* 7-14W-27 [20]
7-14R-27 [1]	3262	8708	1984	2640	10577	2576	4958	3699	-9,612		0.3		0.3	0.03	* 7-14R-27 [1]
7-14R-27 [10]	25375	8118	11213	18105	30089	22655	19259	8443	4,689		1.3		1.3	0.28	7-14R-27 [10]
7-14R-27 [20]	17328	49546	20156	17748	23451	30487	26453	12307	11,883	*	1.8		1.8	0.01	* 7-14R-27 [20]
22-41 [1]	2410	7935	10386	5459	4698	10995	6981	3378	-7,590		0.5		0.5	0.07	22-41 [1]
22-41 [10]	22296	13476	2044	4896	7874	18171	11460	7888	-3,111		0.8		0.8	0.47	22-41 [10]
22-41 [20]	10418	7371	879	11978	4898	5332	6813	4026	-7,757		0.5		0.5	0.07	22-41 [20]
37-56 [1]	2551	4059	5597	2375	2394	2556	3255	1314	-11,315		0.2		0.2	0.01	* 37-56 [1]
37-56 [10]	35647	7381	2917	5501	2273	4873	9765	12812	-4,805		0.7		0.7	0.29	37-56 [10]
37-56 [20]	1053	1569	9535	3087	4660	20458	6727	7386	-7,843		0.5		0.5	0.07	37-56 [20]
54-73 [1]	16064	3002	3165	1148	6555	4670	5767	5359	-8,803		0.4		0.4	0.04	* 54-73 [1]
54-73 [10]	10550	30377	3805	2072	4478	10127	10235	10459	-4,335		0.7		0.7	0.33	54-73 [10]
54-73 [20]	3770	7932	19865	5083	2776	18283	9618	7543	-4,952		0.7		0.7	0.25	54-73 [20]
71-90 [1]	8514	29071	9806	5366	3347	24347	13409	10657	-1,162		0.9		0.9	0.79	71-90 [1]
71-90 [10]	11041	48244	39837	49512	13215	21123	30495	17486	15,925	*	2.1		2.1	0.00	* 71-90 [10]
71-90 [20]	42351	42580	61478	36162	40305	56174	46508	9958	31,938	*	3.2	*	3.2	0.00	* 71-90 [20]
87-106 [1]	16853	5269	4769	8786	16401	11932	10668	5295	-3,902		0.7		0.7	0.36	87-106 [1]
87-106 [10]	4033	18370	16741	7247	9609	7537	10590	5706	-3,981		0.7		0.7	0.35	87-106 [10]
87-106 [20]	60559	19105	12741	11072	4630	12031	20023	20386	5,453	*	1.4		1.4	0.29	87-106 [20]
101-120 [1]	4760	9577	5099	44871	16617	2639	13927	15957	-643		1.0		1.0	0.89	101-120 [1]
101-120 [10]	8282	17287	13334	13101	9245	5943	11199	4128	-3,371		0.8		0.8	0.42	101-120 [10]
101-120 [20]	4098	2657	6775	19823	1970	2447	6295	6853	-8,275		0.4		0.4	0.06	101-120 [20]
116-130 [1]	2026	7516	10308	3277	6095	14080	7217	4483	-7,353		0.5		0.5	0.09	116-130 [1]
116-130 [10]	2235	20749	10840	4686	14531	22417	12576	8242	-1,994		0.9		0.9	0.64	116-130 [10]
116-130 [20]	2737	3298	248	1883	5277	4305	2958	1781	-11,612		0.2		0.2	0.01	* 116-130 [20]
126-140 [1]	2357	2817	1178	6883	1834	23064	6356	8429	-8,215		0.4		0.4	0.06	126-140 [1]
126-140 [10]	4115	13491	2397	24719	18151	13547	12737	8430	-1,833		0.9		0.9	0.67	126-140 [10]
126-140 [20]	2304	1750	2296	18439	5214	14333	7389	7192	-7,181		0.5		0.5	0.10	126-140 [20]
136-150 [1]	3824	5711	23738	14080	4987	9417	10293	7575	-4,277		0.7		0.7	0.32	136-150 [1]
136-150 [10]	8139	5455	2332	12701	26651	3036	9719	9116	-4,851		0.7		0.7	0.27	136-150 [10]
136-150 [20]	2864	18440	5094	6695	4806	8272	7695	5572	-6,875		0.5		0.5	0.11	136-150 [20]
146-160 [1]	34420	7377	8447	9137	2606	6177	11361	11529	-3,209		0.8		0.8	0.47	146-160 [1]
146-160 [10]	15694	6743	1523	6432	5597	7126	7186	4642	-7,384		0.5		0.5	0.08	146-160 [10]
146-160 [20]	2484	1174	3834	8709	10741	12782	6621	4772	-7,949		0.5		0.5	0.06	146-160 [20]
156-170 [1]	14455	3334	2988	36920	8162	18685	14091	12779	-479		1.0		1.0	0.92	156-170 [1]
156-170 [10]	7691	44412	7971	1604	3090	46224	18499	20932	3,929		1.3		1.3	0.45	156-170 [10]
156-170 [20]	4762	5353	2149	11202	6875	6558	6150	2993	-8,420		0.4		0.4	0.05	* 156-170 [20]
166-180 [1]	5940	1534	1787	16041	4973	7646	6320	5323	-8,250		0.4		0.4	0.06	166-180 [1]
166-180 [10]	11321	10662	8304	12028	13849	9144	10885	2001	-3,685		0.7		0.7	0.38	166-180 [10]
166-180 [20]	5177	8045	3183	11997	4896	15894	8199	4876	-6,371		0.6		0.6	0.13	166-180 [20]
176-195 [1]	19005	1935	8021	6183	26564	2350	10676	9948	-3,894		0.7		0.7	0.38	176-195 [1]
176-195 [10]	644	1817	2514	2224	6461	3546	2868	1998	-11,702		0.2		0.2	0.01	* 176-195 [10]
176-195 [20]	2190	11923	3226	556	2803	4477	4196	3999	-10,374		0.3		0.3	0.02	* 176-195 [20]
191-210 [1]	32779	2317	865	7095	8507	14406	10995	11709	-3,575		0.8		0.8	0.43	191-210 [1]
191-210 [10]	11130	8002	15305	1428	25754	6720	11390	8417	-3,180		0.8		0.8	0.46	191-210 [10]
191-210 [20]	2346	16494	40889	14210	8299	22505	17457	13403	2,887		1.2		1.2	0.53	191-210 [20]
210-229 [1]	9155	3596	1878	2370	5843	3000	4307	2747	-10,263		0.3		0.3	0.02	* 210-229 [1]
210-229 [10]	4819	11858	3423	8272	10375	33355	12017	10934	-2,553		0.8		0.8	0.57	210-229 [10]
210-229 [20]	9007	6686	14997	39912	16065	12201	16478	12013	1,908		1.1		1.1	0.67	210-229 [20]
229-248 [1]	5565	26461	1244	13831	8137	2169	9568	9441	-5,002		0.7		0.7	0.26	229-248 [1]
229-248 [10]	9672	25842	21623	26258	35862	26296	24259	8544	9,689	*	1.7		1.7	0.03	* 229-248 [10]
229-248 [20]	15224	35411	19784	20081	20723	22736	22327	6869	7,756	*	1.5		1.5	0.07	229-248 [20]
248-267 [1]	39689	14472	39411	35864	46814	43680	36655	11507	22,085	*	2.5	*	2.5	0.00	* 248-267 [1]
248-267 [10]	32424	45804	17088	32894	15138	25116	28077	11431	13,507	*	1.9		1.9	0.00	* 248-267 [10]
248-267 [20]	25804	46600	20918	33839	43641	41068	35312	10293	20,742	*	2.4	*	2.4	0.00	* 248-267 [20]
267-286 [1]	14388	39083	51078	7967	20570	15208	24716	16710	10,146	*	1.7		1.7	0.04	* 267-286 [1]
267-286 [10]	19140	1665	6410	1560	3576	2605	5826	6761	-8,744		0.4		0.4	0.04	* 267-286 [10]
267-286 [20]	4947	14549	7690	4949	4596	11856	8098	4196	-6,472		0.6		0.6	0.13	267-286 [20]
287-306 [1]	1014	13504	1594	3142	3463	1711	4071	4717	-10,499		0.3		0.3	0.02	* 287-306 [1]
287-306 [10]	2588	1054	2347	2003	19029	10022	6174	7088	-8,396		0.4		0.4	0.05	287-306 [10]
287-306 [20]	12747	1180	1001	2167	3311	2852	3876	4439	-10,694		0.3		0.3	0.01	* 287-306 [20]
307-326 [1]	9351	5668	1430	9866	3003	4628	5658	3386	-8,912		0.4		0.4	0.04	* 307-326 [1]
307-326 [10]	21800	999	2672	2372	37498	22856	14700	14974	129		1.0		1.0	0.98	307-326 [10]
307-326 [20]	2144	8005	32158	9844	11289	12294	12622	10221	-1,948		0.9		0.9	0.66	307-326 [20]
sAg 10	23198	13156	12963	13546	2041	33123	16338	10607	1,768		1.1		1.1	0.69	sAg 10
sAg 100	23720	18572	909	20522	21375	36659	20293	11485	5,723	*	1.4		1.4	0.20	sAg 100
N	81	70	82	60	76	51	70	12	-14,500		0.0		0.0	0.00	* N
N	35	80	54	107	91	77	74	26	-14,496		0.0		0.0	0.00	* N
3H	7960	763	6653	121	198	23167	6477	8869	-8,093		0.4		0.4	0.07	3H
3H	8038	28785	5301	8527	26791	13328	15128	10161	558		1.0		1.0	0.90	3H
3H	10678	17045	25109	12555	25857	10420	16944	7031	2,374		1.2		1.2	0.58	3H
3H	3752	10265	13745	12421	25215	26057	15243	8757	672		1.0		1.0	0.88	3H
3H	4758	7124	3253	14884	35467	31688	16196	14098	1,626		1.1		1.1	0.73	3H
3H	33573	19508	15228	20205	35358	13138	22835	9402	8,265	*	1.6		1.6	0.06	3H
3H	16266	10174	5846	9516	10640	2564	9168	4655	-5,402		0.6		0.6	0.20	3H
	SMC														
N	63	20	31	48	110	76	58	33	-1,798		0.0		0.0	0.00	* N
3H	2110	1607	1539	1010	1013	3856	1856	1063	0		1.0		1.0	1.00	3H
PHA - 1	35264	58957	57333	58836	62612	79604	58768	14167	56,912	*	32.7	*	31.7	0.00	* PHA - 1
PHA - 5	46638	60882	42250	50488	53265	63759	52880	8249	51,025	*	29.4	*	28.5	0.00	* PHA - 5
PHA - 10	35969	52114	48310	62184	51239	72131	53658	12359	51,802	*	29.8	*	28.9	0.00	* PHA - 10
LPS - 1	3717	18250	6783	11338	5575	10574	9373	5239	7,517	*	5.2	*	5.1	0.01	* LPS - 1
LPS - 5	12342	13488	8633	15393	25224	19610	15782	5868	13,926	*	8.7	*	8.5	0.00	* LPS - 5
LPS - 10	14482	16860	15852	11884	21987	31655	18787	7132	16,931	*	10.4	*	10.1	0.00	* LPS - 10
LPS - 20	28257	32369	19991	40980	38178	73010	38798	18340	36,942	*	21.5	*	20.9	0.00	* LPS - 20
LPS - 40	23772	22270	52463	15459	21954	39281	29200	13868	27,344	*	16.2	*	15.7	0.00	* LPS - 40

Raw data for Protein vaccinated duck V2M

V2M	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	51	18															
Total 3H	3096	2484															
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1		>2	
1-15 [1]	2644	1169	6090	1066	704	2302	2329	1991	-767		0.7		0.8		0.47		1-15 [1]
1-15 [10]	11705	8895	1060	9046	17818	8548	9512	5418	6,416	*	3.1	*	3.1	*	0.00	*	1-15 [10]
1-15 [20]	7597	2632	21655	2784	1579	23479	9954	10006	6,858	*	3.3	*	3.2	*	0.00	*	1-15 [20]
7-14W-27 [1]	1441	3527	1563	714	2893	1534	1945	1047	-1,151		0.6		0.6		0.27		7-14W-27 [1]
7-14W-27 [10]	29076	7236	30690	47636	28301	46328	31545	14736	28,449	*	10.3	*	10.2	*	0.00	*	7-14W-27 [10]
7-14W-27 [20]	9362	39737	22141	45976	73735	69908	43477	25509	40,381	*	14.3	*	14.0	*	0.00	*	7-14W-27 [20]
7-14R-27 [1]	1029	1480	790	787	1680	2267	1339	583	-1,757		0.4		0.4		0.09		7-14R-27 [1]
7-14R-27 [10]	5832	1546	18911	8464	1350	6079	7030	6448	3,934		2.3	*	2.3	*	0.01	*	7-14R-27 [10]
7-14R-27 [20]	3131	5000	3908	3954	16037	5425	6243	4869	3,147		2.0		2.0		0.01	*	7-14R-27 [20]
22-41 [1]	444	757	679	1415	1266	2544	1184	761	-1,912		0.4		0.4		0.07		22-41 [1]
22-41 [10]	1290	884	443	1347	3794	10978	3123	4021	27		1.0		1.0		0.98		22-41 [10]
22-41 [20]	1268	1366	4155	977	914	2379	1843	1249	-1,253		0.6		0.6		0.23		22-41 [20]
37-56 [1]	5662	694	435	1908	981	392	1679	2029	-1,417		0.5		0.5		0.19		37-56 [1]
37-56 [10]	1347	1565	249	5556	941	1270	1821	1886	-1,275		0.6		0.6		0.23		37-56 [10]
37-56 [20]	1190	2083	330	587	291	690	862	680	-2,234		0.3		0.3		0.03	*	37-56 [20]
54-73 [1]	1692	830	2053	550	1034	2553	1452	775	-1,644		0.5		0.5		0.12		54-73 [1]
54-73 [10]	955	3162	2199	1294	2698	3183	2249	948	-847		0.7		0.7		0.42		54-73 [10]
54-73 [20]	691	656	3180	729	372	992	1103	1036	-1,993		0.3		0.4		0.06		54-73 [20]
71-90 [1]	1109	521	466	1698	2821	1604	1370	880	-1,726		0.4		0.4		0.10		71-90 [1]
71-90 [10]	1211	1162	445	1817	663	2148	1241	653	-1,855		0.4		0.4		0.08		71-90 [10]
71-90 [20]	973	526	5019	227	11387	5746	3980	4339	884		1.3		1.3		0.46		71-90 [20]
87-106 [1]	1772	575	528	529	994	3475	1312	1163	-1,784		0.4		0.4		0.09		87-106 [1]
87-106 [10]	3883	1285	1008	435	4933	524	2011	1912	-1,085		0.6		0.6		0.31		87-106 [10]
87-106 [20]	2421	2504	1762	2993	6168	6985	3806	2197	710		1.2		1.2		0.51		87-106 [20]
101-120 [1]	16140	988	17252	3178	2127	46496	14364	17308	11,268	*	4.7	*	4.6	*	0.00	*	101-120 [1]
101-120 [10]	6063	810	1210	1105	1482	1703	2062	1984	-1,034		0.7		0.7		0.34		101-120 [10]
101-120 [20]	9839	572	723	448	768	1080	2238	3730	-858		0.7		0.7		0.46		101-120 [20]
116-130 [1]	6977	1613	1383	949	577	1691	2198	2379	-898		0.7		0.7		0.41		116-130 [1]
116-130 [10]	394	480	720	347	589	2677	868	897	-2,228		0.3		0.3		0.04	*	116-130 [10]
116-130 [20]	1354	737	1220	641	890	3721	1427	1157	-1,669		0.5		0.5		0.11		116-130 [20]
126-140 [1]	1023	685	402	1422	735	2821	1181	875	-1,915		0.4		0.4		0.07		126-140 [1]
126-140 [10]	556	1204	5286	765	1019	3242	2012	1873	-1,084		0.6		0.6		0.31		126-140 [10]
126-140 [20]	973	984	509	325	1276	1147	869	372	-2,227		0.3		0.3		0.03	*	126-140 [20]
136-150 [1]	768	1044	2177	583	2068	4963	1934	1627	-1,162		0.6		0.6		0.27		136-150 [1]
136-150 [10]	1518	2031	1292	1247	1208	750	1341	421	-1,755		0.4		0.4		0.09		136-150 [10]
136-150 [20]	898	633	875	715	545	414	680	189	-2,416		0.2		0.2		0.02	*	136-150 [20]
146-160 [1]	462	3294	346	690	2244	1054	1348	1175	-1,748		0.4		0.4		0.10		146-160 [1]
146-160 [10]	563	813	1761	1012	822	985	993	409	-2,103		0.3		0.3		0.05	*	146-160 [10]
146-160 [20]	348	635	1257	1912	432	1190	962	601	-2,134		0.3		0.3		0.04	*	146-160 [20]
156-170 [1]	357	276	822	865	664	524	585	242	-2,511		0.2		0.2		0.02	*	156-170 [1]
156-170 [10]	1609	721	1084	1310	724	1466	1152	376	-1,944		0.4		0.4		0.06		156-170 [10]
156-170 [20]	407	968	477	1355	362	1511	847	506	-2,249		0.3		0.3		0.03	*	156-170 [20]
166-180 [1]	531	1501	671	299	4875	1435	1552	1700	-1,544		0.5		0.5		0.15		166-180 [1]
166-180 [10]	3167	401	752	1171	3203	1236	1655	1223	-1,441		0.5		0.5		0.17		166-180 [10]
166-180 [20]	650	406	1339	1928	1137	1838	1216	615	-1,880		0.4		0.4		0.07		166-180 [20]
176-195 [1]	821	588	478	368	497	964	619	227	-2,477		0.2		0.2		0.02	*	176-195 [1]
176-195 [10]	516	456	420	2254	1055	978	947	696	-2,149		0.3		0.3		0.04	*	176-195 [10]
176-195 [20]	1651	367	408	920	939	402	781	501	-2,315		0.2		0.3		0.03	*	176-195 [20]
191-210 [1]	5648	512	438	523	327	1341	1465	2081	-1,631		0.5		0.5		0.13		191-210 [1]
191-210 [10]	695	738	1145	599	332	1075	764	304	-2,332		0.2		0.2		0.03	*	191-210 [10]
191-210 [20]	826	542	4996	1151	1741	1566	1804	1626	-1,292		0.6		0.6		0.22		191-210 [20]
210-229 [1]	574	2119	534	796	1421	5938	1897	2070	-1,199		0.6		0.6		0.27		210-229 [1]
210-229 [10]	782	539	479	822	1288	1740	942	484	-2,154		0.3		0.3		0.04	*	210-229 [10]
210-229 [20]	2911	1105	1805	1110	1588	10031	3092	3463	-4		1.0		1.0		1.00		210-229 [20]
229-248 [1]	2115	1232	1616	2559	1720	1696	1823	457	-1,273		0.6		0.6		0.22		229-248 [1]
229-248 [10]	5496	3067	707	6124	1578	7817	4132	2786	1,036		1.3		1.3		0.35		229-248 [10]
229-248 [20]	8001	23164	2062	4111	23126	2161	10438	10075	7,342	*	3.4	*	3.4	*	0.00	*	229-248 [20]
248-267 [1]	2138	1500	1958	2555	703	762	1603	755	-1,493		0.5		0.5		0.15		248-267 [1]
248-267 [10]	1682	993	611	765	3081	1955	1515	930	-1,581		0.5		0.5		0.13		248-267 [10]
248-267 [20]	1761	1034	478	1083	4376	603	1556	1453	-1,540		0.5		0.5		0.15		248-267 [20]
267-286 [1]	1283	578	6051	622	1110	787	1739	2131	-1,357		0.6		0.6		0.21		267-286 [1]
267-286 [10]	386	651	1225	1909	674	3594	1407	1202	-1,689		0.4		0.5		0.11		267-286 [10]
267-286 [20]	1412	1035	5016	2010	1495	7509	3080	2607	-16		1.0		1.0		0.99		267-286 [20]
287-306 [1]	1506	640	738	926	2141	1175	1188	562	-1,908		0.4		0.4		0.07		287-306 [1]
287-306 [10]	276	572	701	712	626	2502	898	802	-2,198		0.3		0.3		0.04	*	287-306 [10]
287-306 [20]	358	436	750	394	775	833	591	217	-2,505		0.2		0.2		0.02	*	287-306 [20]
307-326 [1]	1404	389	486	1193	537	607	769	421	-2,327		0.2		0.2		0.03	*	307-326 [1]
307-326 [10]	1016	1446	279	1838	589	1627	1133	613	-1,963		0.4		0.4		0.06		307-326 [10]
307-326 [20]	45668	1733	43429	44649	3840	1806	23521	23095	20,425	*	7.7	*	7.6	*	0.00	*	307-326 [20]
sAg 10	1507	1460	194	42284	314	4522	8380	16683	5,284	*	2.7	*	2.7	*	0.05	*	sAg 10
sAg 100	1841	1202	654	4171	23118	733	5287	8831	2,191		1.7		1.7		0.19		sAg 100
N	63	38	36	30	71	73	52	19	-3,044		0.0		0.0		0.00	*	N
N	36	58	51	34	42	83	51	18	-3,045		0.0		0.0		0.00	*	N
3H	1478	71	5307	134	104	548	1274	2047	-1,822		0.4		0.4		0.09		3H
3H	2128	8286	1738	2693	2412	4067	3554	2451	458		1.2		1.1		0.67		3H
3H	3095	1135	2016	4586	2459	2572	2644	1156	-452		0.9		0.9		0.66		3H
3H	1240	966	3862	1276	7896	5891	3522	2886	426		1.1		1.1		0.70		3H
3H	1153	1629	1995	7215	3682	4035	3285	2241	189		1.1		1.1		0.86		3H
3H	1092	2579	5178	4308	2507	1383	2841	1611	-255		0.9		0.9		0.81		3H
3H	1259	3754	7293	11279	2073	1656	4552	3969	1,456		1.5		1.5		0.22		3H
	SMC																
N	47	82	35	30	34	68	49	21	-1,107		0.0		0.0		0.03	*	N
3H	752	3224	547	848	456	1108	1156	1039	0		1.0		1.0		1.00		3H
PHA - 1	59603	39117	48220	41544	45001	76469	51659	14103	50,503	*	46.6	*	44.7	*	0.00	*	PHA - 1
PHA - 5	62983	52991	58343	55132	52944	41213	53934	7300	52,779	*	48.7	*	46.7	*	0.00	*	PHA - 5
PHA - 10	73003	51217	53398	58233	46706	75194	59625	11829	58,469	*	53.8	*	51.6	*	0.00	*	PHA - 10
LPS - 1	2061	1403	947	708	1139	1809	1345	517	189		1.2		1.2		0.70		LPS - 1
LPS - 5	2312	1586	1557	1515	1895	2394	1877	394	721		1.7		1.6		0.14		LPS - 5
LPS - 10	2895	1687	1819	2479	1854	1824	2093	482	937		1.8		1.8		0.07		LPS - 10
LPS - 20	1660	1766	1729	2977	2246	2536	2152	530	997		1.9		1.9		0.06		LPS - 20
LPS - 40	2723	2222	2035	2000	1976	2722	2280	354	1,124		2.0		2.0		0.03	*	LPS - 40

Raw data for Protein vaccinated duck V2N

V2N	Mean	SD														
Total N	82	28														
Total 3H	1448	1092							CPM-3H		S.I.		P/N		t-Test	
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1		>2
1-15 [1]	1381	607	856	857	563	365	772	351	-676		0.5		0.5		0.14		1-15 [1]
1-15 [10]	2066	2678	2880	883	1517	3023	2175	847	727		1.5		1.5		0.13		1-15 [10]
1-15 [20]	5089	462	1837	21308	3772	46137	13101	17869	11,653	*	9.5	*	9.0	*	0.00	*	1-15 [20]
7-14W-27 [1]	4508	650	1735	989	1657	4737	2379	1786	931		1.7		1.6		0.08		7-14W-27 [1]
7-14W-27 [10]	10187	4721	3048	11664	5965	14216	8300	4375	6,852	*	6.0	*	5.7	*	0.00	*	7-14W-27 [10]
7-14W-27 [20]	51571	31921	59049	32194	9668	36814	36870	17288	35,422	*	26.9	*	25.5	*	0.00	*	7-14W-27 [20]
7-14R-27 [1]	10628	707	535	1613	587	1895	2461	3945	1,213		1.9		1.8		0.10		7-14R-27 [1]
7-14R-27 [10]	2763	1553	4668	1280	1241	19258	5127	7045	3,679		3.7	*	3.5	*	0.00	*	7-14R-27 [10]
7-14R-27 [20]	905	2896	777	241	36283	1667	7128	14312	5,680	*	5.2	*	4.9	*	0.01	*	7-14R-27 [20]
22-41 [1]	1940	2487	738	206	2764	6907	2507	2374	1,059		1.8		1.7		0.07		22-41 [1]
22-41 [10]	2439	1593	1712	1295	1028	6791	2476	2167	1,028		1.8		1.7		0.07		22-41 [10]
22-41 [20]	1898	6302	2720	2445	1572	4280	3203	1785	1,755		2.3	*	2.2	*	0.00	*	22-41 [20]
37-56 [1]	1896	5583	602	581	1720	2934	2219	1870	771		1.6		1.5		0.15		37-56 [1]
37-56 [10]	1776	2247	390	802	1237	139	1099	813	-349		0.7		0.8		0.46		37-56 [10]
37-56 [20]	2429	4207	1953	974	870	9861	3382	3398	1,934		2.4	*	2.3	*	0.01	*	37-56 [20]
54-73 [1]	1111	1495	1660	3649	1050	1482	1741	964	293		1.2		1.2		0.54		54-73 [1]
54-73 [10]	3871	815	906	7006	999	3320	2820	2444	1,372		2.0		1.9		0.02	*	54-73 [10]
54-73 [20]	909	860	1590	2198	5033	695	1881	1644	433		1.3		1.3		0.40		54-73 [20]
71-90 [1]	582	635	385	1821	836	7789	2008	2877	560		1.4		1.4		0.36		71-90 [1]
71-90 [10]	1298	1062	656	3801	1688	786	1549	1164	101		1.1		1.1		0.84		71-90 [10]
71-90 [20]	696	1119	701	1136	1293	397	890	344	-558		0.6		0.6		0.22		71-90 [20]
87-106 [1]	1720	1256	583	3746	1971	1850	1854	1057	406		1.3		1.3		0.40		87-106 [1]
87-106 [10]	1801	639	718	4879	1092	2341	1912	1595	464		1.3		1.3		0.36		87-106 [10]
87-106 [20]	1578	673	890	1511	874	1505	1172	402	-276		0.8		0.8		0.55		87-106 [20]
101-120 [1]	1522	431	1172	1694	932	652	1067	491	-381		0.7		0.7		0.41		101-120 [1]
101-120 [10]	3181	3058	2023	1324	12801	3086	4246	4256	2,798		3.0	*	2.9	*	0.00	*	101-120 [10]
101-120 [20]	486	1594	7954	3241	38961	3570	9301	14753	7,853	*	6.7	*	6.4	*	0.00	*	101-120 [20]
116-130 [1]	968	1133	976	1030	2228	1919	1376	552	-72		0.9		1.0		0.88		116-130 [1]
116-130 [10]	911	1248	942	365	2460	1605	1255	719	-193		0.9		0.9		0.68		116-130 [10]
116-130 [20]	1172	742	3018	5085	764	3758	2423	1809	975		1.7		1.7		0.07		116-130 [20]
126-140 [1]	830	691	1103	871	1164	2386	1174	619	-274		0.8		0.8		0.55		126-140 [1]
126-140 [10]	3866	5247	2160	713	1510	1279	2463	1743	1,015		1.7		1.7		0.05		126-140 [10]
126-140 [20]	2302	936	1462	1014	1567	3541	1804	981	356		1.3		1.2		0.45		126-140 [20]
136-150 [1]	2318	2320	1926	852	1793	897	1684	662	236		1.2		1.2		0.61		136-150 [1]
136-150 [10]	4774	13183	7115	2641	2293	1989	5333	4308	3,885		3.8	*	3.7	*	0.00	*	136-150 [10]
136-150 [20]	8241	2175	1597	1285	3743	1292	3056	2702	1,608		2.2	*	2.1	*	0.01	*	136-150 [20]
146-160 [1]	1417	2992	2725	2052	2050	2263	2250	557	802		1.6		1.6		0.09		146-160 [1]
146-160 [10]	2188	958	497	2530	2924	566	1611	1064	163		1.1		1.1		0.73		146-160 [10]
146-160 [20]	3030	1574	1850	535	1707	6411	2518	2066	1,070		1.8		1.7		0.05		146-160 [20]
156-170 [1]	1904	955	849	3963	2815	3699	2364	1344	916		1.7		1.6		0.07		156-170 [1]
156-170 [10]	1652	1907	1226	1312	1048	3121	1711	757	263		1.2		1.2		0.57		156-170 [10]
156-170 [20]	1082	449	682	2626	2821	3753	1902	1347	454		1.3		1.3		0.36		156-170 [20]
166-180 [1]	1019	1861	4119	1314	4404	2120	2473	1442	1,025		1.8		1.7		0.04	*	166-180 [1]
166-180 [10]	2764	447	2024	1236	724	1331	1421	854	-27		1.0		1.0		0.95		166-180 [10]
166-180 [20]	977	1433	1319	3386	2734	2043	1982	926	534		1.4		1.4		0.26		166-180 [20]
176-195 [1]	2293	3082	9780	2197	2370	8323	4673	3438	3,225		3.4	*	3.2	*	0.00	*	176-195 [1]
176-195 [10]	9759	720	477	1675	520	1902	2509	3603	1,061		1.8		1.7		0.13		176-195 [10]
176-195 [20]	1873	706	727	1182	914	1195	1100	434	-348		0.7		0.8		0.45		176-195 [20]
191-210 [1]	7109	413	10844	489	1041	8037	4656	4565	3,208		3.3	*	3.2	*	0.00	*	191-210 [1]
191-210 [10]	1824	1142	2740	4846	1156	2803	2419	1394	971		1.7		1.7		0.05		191-210 [10]
191-210 [20]	1178	2024	834	2421	2444	530	1572	833	124		1.1		1.1		0.79		191-210 [20]
210-229 [1]	1997	655	1095	714	776	1829	1178	592	-270		0.8		0.8		0.56		210-229 [1]
210-229 [10]	3830	2425	2316	392	691	2234	1981	1263	533		1.4		1.4		0.28		210-229 [10]
210-229 [20]	9543	12476	6162	19446	5235	5752	9769	5490	8,321	*	7.1	*	6.7	*	0.00	*	210-229 [20]
229-248 [1]	2460	316	4194	3536	1881	6087	3079	1998	1,631		2.2	*	2.1	*	0.00	*	229-248 [1]
229-248 [10]	1679	636	1285	1143	2715	12014	3245	4352	1,797		2.3	*	2.2	*	0.02	*	229-248 [10]
229-248 [20]	1428	2426	2745	1722	2137	18643	4850	6774	3,402		3.5	*	3.3	*	0.00	*	229-248 [20]
248-267 [1]	545	1135	461	1129	3048	5567	1981	1991	533		1.4		1.4		0.32		248-267 [1]
248-267 [10]	471	474	516	1647	709	[redacted]	763	504	-685		0.5		0.5		0.18		248-267 [10]
248-267 [20]	7199	2390	1020	879	2306	2515	2718	2309	1,270		1.9		1.9		0.03	*	248-267 [20]
267-286 [1]	1023	3785	1167	1173	1739	2342	1872	1059	424		1.3		1.3		0.38		267-286 [1]
267-286 [10]	1114	587	1364	3424	3309	5389	2531	1829	1,083		1.8		1.7		0.04	*	267-286 [10]
267-286 [20]	534	3504	1337	1243	1363	1446	1571	1003	123		1.1		1.1		0.80		267-286 [20]
287-306 [1]	1202	3677	1585	894	1334	1179	1645	1020	197		1.1		1.1		0.68		287-306 [1]
287-306 [10]	1615	517	1295	655	945	694	954	425	-494		0.6		0.7		0.28		287-306 [10]
287-306 [20]	1112	723	792	531	390	534	680	256	-768		0.4		0.5		0.10		287-306 [20]
307-326 [1]	926	590	789	746	718	973	790	141	-658		0.5		0.5		0.15		307-326 [1]
307-326 [10]	2902	1418	2327	9646	4888	2227	3901	3047	2,453		2.8	*	2.7	*	0.00	*	307-326 [10]
307-326 [20]	3952	4479	3669	2498	2585	8862	4341	2347	2,893		3.1	*	3.0	*	0.00	*	307-326 [20]
sAg 10	855	147	269	289	826	2231	770	777	-678		0.5		0.5		0.15		sAg 10
sAg 100	1196	733	555	652	491	1307	822	344	-626		0.5		0.6		0.17		sAg 100
N	106	29	79	70	85	115	81	30	-1,367		0.0		0.1		0.00	*	N
N	107	126	76	74	65	54	84	27	-1,364		0.0		0.1		0.00	*	N
3H	1023	1132	746	741	273	643	893	532	-555		0.6		0.6		0.23		3H
3H	2784	823	3169	390	4183	817	2028	1557	580		1.4		1.4		0.25		3H
3H	1534	2566	625	2518	1661	3255	2027	936	579		1.4		1.4		0.22		3H
3H	1119	1610	1091	538	316	1520	1032	518	-416		0.7		0.7		0.37		3H
3H	1132	580	997	1232	509	893	891	293	-557		0.6		0.6		0.22		3H
3H	1517	1607	973	2360	1896	5396	2292	1588	844		1.6		1.6		0.10		3H
3H	571	620	835	496	1048	2275	974	668	-474		0.7		0.7		0.31		3H
	SMC															
N	169	47	75	97	37	16	74	55	-546		0.0		0.1		0.00	*	N
3H	388	234	965	645	864	621	620	276	0		1.0		1.0		1.00		3H
PHA - 1	61291	61293	57442	49223	58798	70877	59821	7006	59,201	*	109.4	*	96.6	*	0.00	*	PHA - 1
PHA - 5	46732	46352	31245	28834	55046	49538	42958	10506	42,338	*	78.5	*	69.3	*	0.00	*	PHA - 5
PHA - 10	50790	37503	32586	32488	33585	49208	39360	8456	38,741	*	72.0	*	63.5	*	0.00	*	PHA - 10
LPS - 1	9143	9849	13223	8598	10195	12281	10548	1815	9,929	*	19.2	*	17.0	*	0.00	*	LPS - 1
LPS - 5	34517	24897	21263	10932	21330	19912	22142	7652	21,522	*	40.4	*	35.7	*	0.00	*	LPS - 5
LPS - 10	31034	13305	22122	31895	8612	36641	23935	11192	23,315	*	43.7	*	38.6	*	0.00	*	LPS - 10
LPS - 20	36178	28648	14384	5643	19902	28366	22187	11095	21,567	*	40.5	*	35.8	*	0.00	*	LPS - 20
LPS - 40	19568	15485	20512	11473	25689	18569	18549	4803	17,930	*	33.8	*	29.9	*	0.00	*	LPS - 40

Raw data for Protein vaccinated duck V2O

V2O	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	121	106															
Total 3H	761	661															
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1		>2	
1-15 [1]	173	149	215	558	116	301	252	163	-509		0.2		0.3		0.07		1-15 [1]
1-15 [10]	255	210	2217	1564	171	111	755	905	-6		1.0		1.0		0.98		1-15 [10]
1-15 [20]	413	297	318	1737	353	938	676	573	-85		0.9		0.9		0.77		1-15 [20]
7-14W-27 [1]	2800	137	1478	1582	205	1574	1296	999	535		1.8		1.7		0.09		7-14W-27 [1]
7-14W-27 [10]	1608	317	2778	3673	451	18332	4527	6888	3,766		6.9	*	5.9	*	0.00	*	7-14W-27 [10]
7-14W-27 [20]	18941	10848	11619	62706	9212	58105	28572	24926	27,811	*	44.5	*	37.5	*	0.00	*	7-14W-27 [20]
7-14R-27 [1]	4332	6477	3666	1487	2769	2386	3520	1755	2,759		5.3	*	4.6	*	0.00	*	7-14R-27 [1]
7-14R-27 [10]	2862	1222	4537	6300	1463	692	2846	2190	2,085		4.3	*	3.7	*	0.00	*	7-14R-27 [10]
7-14R-27 [20]	2381	846	1729	954	2594	1377	1647	726	886		2.4	*	2.2	*	0.00	*	7-14R-27 [20]
22-41 [1]	1398	580	954	405	933	1403	946	410	185		1.3		1.2		0.51		22-41 [1]
22-41 [10]	923	822	2699	482	2522	1080	1421	943	660		2.0		1.9		0.04	*	22-41 [10]
22-41 [20]	448	1470	310	1664	449	2157	1083	780	322		1.5		1.4		0.28		22-41 [20]
37-56 [1]	1698	1600	1183	529	1496	2092	1433	532	672		2.1		1.9		0.02	*	37-56 [1]
37-56 [10]	2025	1158	1062	519	1477	950	1199	511	438		1.7		1.6		0.13		37-56 [10]
37-56 [20]	1084	389	643	3623	2024	1382	1524	1178	763		2.2	*	2.0		0.02	*	37-56 [20]
54-73 [1]	1013	1004	2624	502	1260	3521	1654	1161	893		2.4	*	2.2	*	0.01	*	54-73 [1]
54-73 [10]	570	601	1316	2314	6047	784	1939	2117	1,178		2.8	*	2.5	*	0.01	*	54-73 [10]
54-73 [20]	1048	1512	440	1247	348	1148	957	464	196		1.3		1.3		0.49		54-73 [20]
71-90 [1]	714	517	275	399	369	591	478	161	-284		0.6		0.6		0.31		71-90 [1]
71-90 [10]	665	621	161	822	879	388	589	272	-172		0.7		0.8		0.54		71-90 [10]
71-90 [20]	981	615	129	177	1416	2177	916	787	155		1.2		1.2		0.60		71-90 [20]
87-106 [1]	1619	626	307	844	464	2393	1042	806	281		1.4		1.4		0.35		87-106 [1]
87-106 [10]	590	636	143	520	2221	2025	1023	872	262		1.4		1.3		0.39		87-106 [10]
87-106 [20]	693	300	564	935	1098	3452	1174	1151	413		1.6		1.5		0.20		87-106 [20]
101-120 [1]	2806	1596	1401	1830	2092	4100	2304	1006	1,543		3.4	*	3.0	*	0.00	*	101-120 [1]
101-120 [10]	3630	666	747	4754	6303	29129	7538	10808	6,777	*	11.6	*	9.9	*	0.00	*	101-120 [10]
101-120 [20]	2351	5448	807	14332	1698	1690	4388	5128	3,627		6.7	*	5.8	*	0.00	*	101-120 [20]
116-130 [1]	2943	738	1692	610	1012	1430	1404	858	643		2.0		1.8		0.04	*	116-130 [1]
116-130 [10]	958	540	239	740	1001	495	662	293	-99		0.8		0.9		0.72		116-130 [10]
116-130 [20]	2059	335	754	334	522	1446	908	699	147		1.2		1.2		0.61		116-130 [20]
126-140 [1]	1285	353	1218	717	3495	3403	1745	1363	984		2.5	*	2.3	*	0.01	*	126-140 [1]
126-140 [10]	1380	844	846	421	1281	2621	1232	763	471		1.7		1.6		0.12		126-140 [10]
126-140 [20]	5392	2085	769	159	898	6307	2602	2608	1,841		3.9	*	3.4	*	0.00	*	126-140 [20]
136-150 [1]	1103	3335	1191	865	1210	3177	1814	1125	1,053		2.6	*	2.4	*	0.00	*	136-150 [1]
136-150 [10]	791	732	740	8200	3565	1706	2622	2943	1,861		3.9	*	3.4	*	0.00	*	136-150 [10]
136-150 [20]	2019	2224	951	6908	1152	3932	2864	2245	2,103		4.3	*	3.8	*	0.00	*	136-150 [20]
146-160 [1]	2878	1848	2208	455	382	2445	1703	1050	942		2.5	*	2.2	*	0.00	*	146-160 [1]
146-160 [10]	1798	931	604	613	305	663	819	519	58		1.1		1.1		0.84		146-160 [10]
146-160 [20]	833	3527	713	1337	2912	507	1638	1270	877		2.4	*	2.2	*	0.01	*	146-160 [20]
156-170 [1]	1347	1105	1021	1398	2715	2221	1635	679	874		2.4	*	2.1	*	0.00	*	156-170 [1]
156-170 [10]	545	923	1445	373	648	275	702	429	-60		0.9		0.9		0.83		156-170 [10]
156-170 [20]	3673	2511	898	1503	2315	1394	2049	997	1,288		3.0	*	2.7	*	0.00	*	156-170 [20]
166-180 [1]	2123	2481	2935	1487	1324	1275	1938	685	1,177		2.8	*	2.5	*	0.00	*	166-180 [1]
166-180 [10]	2574	1041	5821	707	437	689	1878	2078	1,117		2.7	*	2.5	*	0.01	*	166-180 [10]
166-180 [20]	3016	6645	3965	1411	3665	1975	3446	1846	2,685		5.2	*	4.5	*	0.00	*	166-180 [20]
176-195 [1]	1183	1392	1924	6529	4072	2749	2975	2035	2,214		4.5	*	3.9	*	0.00	*	176-195 [1]
176-195 [10]	2765	450	251	704	590	1449	1035	941	274		1.4		1.4		0.37		176-195 [10]
176-195 [20]	1213	412	713	871	2090	869	1028	581	267		1.4		1.4		0.35		176-195 [20]
191-210 [1]	6648	4798	474	1213	952	1426	2585	2520	1,824		3.9	*	3.4	*	0.00	*	191-210 [1]
191-210 [10]	1245	611	1000	385	1246	1159	941	362	180		1.3		1.2		0.52		191-210 [10]
191-210 [20]	179	584	983	974	823	986	755	322	-6		1.0		1.0		0.98		191-210 [20]
210-229 [1]	1903	956	1735	924	758	936	1202	486	441		1.7		1.6		0.12		210-229 [1]
210-229 [10]	2477	872	455	3283	3573	2852	2252	1292	1,491		3.3	*	3.0	*	0.00	*	210-229 [10]
210-229 [20]	1819	4206	5185	3654	1951	3734	3425	1312	2,664		5.2	*	4.5	*	0.00	*	210-229 [20]
229-248 [1]	2825	936	401	1064	1054	875	1193	836	432		1.7		1.6		0.15		229-248 [1]
229-248 [10]	1010	971	1935	1880	691	6731	2203	2276	1,442		3.3	*	2.9	*	0.00	*	229-248 [10]
229-248 [20]	1065	798	1161	1620	2302	2691	1606	749	845		2.3	*	2.1	*	0.01	*	229-248 [20]
248-267 [1]	1005	836	737	807	1137	1478	1000	276	239		1.4		1.3		0.39		248-267 [1]
248-267 [10]	1766	600	304	1626	1723	581	1100	673	339		1.5		1.4		0.25		248-267 [10]
248-267 [20]	1514	1046	468	692	1112	662	916	382	155		1.2		1.2		0.58		248-267 [20]
267-286 [1]	383	476	1745	255	1044	6080	1664	2233	903		2.4	*	2.2	*	0.04	*	267-286 [1]
267-286 [10]	1664	525	428	962	1052	124	793	549	32		1.0		1.0		0.91		267-286 [10]
267-286 [20]	887	270	1383	2565	2512	1705	1554	904	793		2.2	*	2.0		0.01	*	267-286 [20]
287-306 [1]	592	1009	8541	1990	419	615	2194	3160	1,433		3.2	*	2.9	*	0.01	*	287-306 [1]
287-306 [10]	2182	226	310	156	239	148	544	805	-218		0.7		0.7		0.47		287-306 [10]
287-306 [20]	463	501	631	548	1179	321	607	298	-154		0.8		0.8		0.58		287-306 [20]
307-326 [1]	809	250	200	631	1344	582	636	418	-125		0.8		0.8		0.66		307-326 [1]
307-326 [10]	3037	607	2517	406	333	716	1269	1187	508		1.8		1.7		0.12		307-326 [10]
307-326 [20]	5331	2023	337	1656	219	401	1661	1950	900		2.4	*	2.2	*	0.03	*	307-326 [20]
sAg 10	230	102	234	128	113	162	162	58	-600		0.1		0.2		0.03	*	sAg 10
sAg 100	198	161	134	250	166	243	192	47	-569		0.1		0.3		0.04	*	sAg 100
N	170	37	40	38	84	110	80	53	-681		-0.1		0.1		0.02	*	N
N	74	65	120	70	257	392	163	134	-598		0.1		0.2		0.03	*	N
3H	148	111	143	160	123	71	126	32	-635		0.0		0.2		0.02	*	3H
3H	841	859	2602	242	1559	2661	1461	998	700		2.1		1.9		0.03	*	3H
3H	1306	422	758	722	582	903	782	304	21		1.0		1.0		0.94		3H
3H	1085	482	897	341	1384	780	828	384	67		1.1		1.1		0.81		3H
3H	286	294	334	383	164	499	327	111	-434		0.3		0.4		0.12		3H
3H	588	429	302	242	329	364	376	121	-385		0.4		0.5		0.16		3H
3H	2252	715	1857	1085	1381	1276	1428	550	667		2.0		1.9		0.02	*	3H
	SMC																
N	35	60	56	25	58	79	52	19	-186		0.0		0.2		0.00	*	N
3H	180	125	389	217	165	353	238	108	0		1.0		1.0		1.00		3H
PHA - 1	65925	56334	67829	59985	58906	77830	64468	7863	64,230	*	346.3	*	270.7	*	0.00	*	PHA - 1
PHA - 5	34148	45237	40468	37772	49021	42975	41604	5323	41,365	*	223.4	*	174.7	*	0.00	*	PHA - 5
PHA - 10	35131	36946	32969	34798	45330	58900	40679	9924	40,441	*	218.4	*	170.8	*	0.00	*	PHA - 10
LPS - 1	692	583	826	1485	1957	1236	1130	530	892		5.8	*	4.7	*	0.00	*	LPS - 1
LPS - 5	905	1274	2729	1304	2651	2272	1856	789	1,618		9.7	*	7.8	*	0.00	*	LPS - 5
LPS - 10	3365	1265	6130	1007	3325	2570	2944	1854	2,706		15.5	*	12.4	*	0.01	*	LPS - 10
LPS - 20	3305	878	2423	3845	1744	2836	2505	1075	2,267		13.2	*	10.5	*	0.00	*	LPS - 20
LPS - 40	1505	2820	1951	1392	1992	3784	2241	908	2,003		11.8	*	9.4	*	0.00	*	LPS - 40

Raw data for Protein vaccinated duck V2P

V2P	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1		>2	
Total N	130	95															
Total 3H	2799	3059															
1-15 [1]	232	277	673	324	419	310	373	160	-2,427		0.1		0.1		0.06		1-15 [1]
1-15 [10]	387	571	13512	1708	3742	1881	3634	4986	834		1.3		1.3		0.57		1-15 [10]
1-15 [20]	715	568	721	3189	1485	990	1278	991	-1,521		0.4		0.5		0.24		1-15 [20]
7-14W-27 [1]	11968	284	10191	17981	1317	2044	7298	7168	4,498		2.7	*	2.6	*	0.01	*	7-14W-27 [1]
7-14W-27 [10]	28100	13976	14606	12963	15020	21686	17725	5952	14,926	*	6.6	*	6.3	*	0.00	*	7-14W-27 [10]
7-14W-27 [20]	54678	20862	41678	28460	58418	37977	40346	14562	37,546	*	15.1	*	14.4	*	0.00	*	7-14W-27 [20]
7-14R-27 [1]	8494	8716	3496	3540	46628	3241	12353	16983	9,553	*	4.6	*	4.4	*	0.00	*	7-14R-27 [1]
7-14R-27 [10]	726	6740	1551	11059	3895	768	4123	4109	1,324		1.5		1.5		0.35		7-14R-27 [10]
7-14R-27 [20]	43483	6239	4972	1755	11152	14529	13688	15289	10,889	*	5.1	*	4.9	*	0.00	*	7-14R-27 [20]
22-41 [1]	1242	1274	1854	2652	1155	10954	3189	3846	389		1.1		1.1		0.78		22-41 [1]
22-41 [10]	6003	3206	5327	2489	1076	781	3147	2156	348		1.1		1.1		0.79		22-41 [10]
22-41 [20]	21682	3565	5893	7710	2890	320	7010	7624	4,211		2.6	*	2.5	*	0.02	*	22-41 [20]
37-56 [1]	5267	4079	1018	2688	5783	14254	5515	4621	2,716		2.0		2.0		0.06		37-56 [1]
37-56 [10]	30218	3833	1636	893	3460	1439	6913	11477	4,114		2.5	*	2.5	*	0.05		37-56 [10]
37-56 [20]	24065	4553	30389	7503	4338	3225	12346	11785	9,546	*	4.6	*	4.4	*	0.00	*	37-56 [20]
54-73 [1]	895	3222	1271	1573	2793	3751	2251	1162	-548		0.8		0.8		0.67		54-73 [1]
54-73 [10]	20803	6183	3249	1544	3894	5083	6793	7044	3,994		2.5	*	2.4	*	0.02	*	54-73 [10]
54-73 [20]	673	17145	15875	1367	855	2426	6390	7873	3,591		2.3	*	2.3	*	0.04	*	54-73 [20]
71-90 [1]	8336	3006	3011	775	2270	1038	3073	2749	274		1.1		1.1		0.84		71-90 [1]
71-90 [10]	619	15254	1011	952	1528	3902	3878	5698	1,079		1.4		1.4		0.48		71-90 [10]
71-90 [20]	10273	2615	7025	1415	8668	764	5127	4043	2,328		1.9		1.8		0.10		71-90 [20]
87-106 [1]	4891	527	3239	2358	548	7206	3128	2599	329		1.1		1.1		0.80		87-106 [1]
87-106 [10]	896	1026	1170	814	3110	776	1299	899	-1,500		0.4		0.5		0.24		87-106 [10]
87-106 [20]	1909	5388	1168	4162	3417	1769	2969	1630	170		1.1		1.1		0.90		87-106 [20]
101-120 [1]	347	954	3110	5239	217	3024	2149	1982	-651		0.8		0.8		0.62		101-120 [1]
101-120 [10]	13176	3094	3290	9934	1246	3105	5641	4753	2,842		2.1		2.0		0.05		101-120 [10]
101-120 [20]	42509	11638	10047	15522	[illegible]	23501	20643	13284	17,844	*	7.7	*	7.4	*	0.00	*	101-120 [20]
116-130 [1]	4200	8003	1684	5516	4035	6636	5012	2215	2,213		1.8		1.8		0.10		116-130 [1]
116-130 [10]	4796	14949	9319	4354	2599	1299	6219	5072	3,420		2.3	*	2.2	*	0.02	*	116-130 [10]
116-130 [20]	3933	9980	2183	3728	13417	3464	6118	4499	3,318		2.2	*	2.2	*	0.02	*	116-130 [20]
126-140 [1]	2837	6356	2802	930	7766	936	3605	2842	805		1.3		1.3		0.55		126-140 [1]
126-140 [10]	13845	14388	6003	[illegible]	3741	2048	8005	5756	5,206	*	3.0	*	2.9	*	0.00	*	126-140 [10]
126-140 [20]	22069	7580	1026	1099	2753	2099	6104	8187	3,305		2.2	*	2.2	*	0.06		126-140 [20]
136-150 [1]	10119	1943	4597	12909	1798	5113	6080	4506	3,281		2.2	*	2.2	*	0.03	*	136-150 [1]
136-150 [10]	3163	3627	1499	2185	865	1449	2131	1075	-668		0.7		0.8		0.60		136-150 [10]
136-150 [20]	14954	2609	3811	1120	8033	6583	6185	4991	3,386		2.3	*	2.2	*	0.02	*	136-150 [20]
146-160 [1]	7036	5880	1137	2092	6033	4821	4500	2362	1,701		1.6		1.6		0.20		146-160 [1]
146-160 [10]	4087	2866	3311	3291	1138	11152	4308	3494	1,508		1.6		1.5		0.27		146-160 [10]
146-160 [20]	3175	1020	2958	1504	5377	7102	3523	2325	724		1.3		1.3		0.58		146-160 [20]
156-170 [1]	13241	1826	3608	3887	6232	6233	5838	4000	3,039		2.1	*	2.1		0.03	*	156-170 [1]
156-170 [10]	21661	3281	1746	12170	1736	1267	6977	8291	4,178		2.6	*	2.5	*	0.02	*	156-170 [10]
156-170 [20]	3756	2055	1595	1382	2533	2993	2386	896	-413		0.8		0.9		0.75		156-170 [20]
166-180 [1]	2187	4362	914	2566	3687	4056	2962	1314	163		1.1		1.1		0.90		166-180 [1]
166-180 [10]	5411	3207	2492	1833	2739	2300	2997	1268	198		1.1		1.1		0.88		166-180 [10]
166-180 [20]	2683	3559	1618	2265	2286	13340	4292	4478	1,493		1.6		1.5		0.30		166-180 [20]
176-195 [1]	4059	1744	3228	3527	1865	5979	3400	1564	601		1.2		1.2		0.64		176-195 [1]
176-195 [10]	5841	3380	2255	1517	1781	16669	5241	5817	2,441		1.9		1.9		0.11		176-195 [10]
176-195 [20]	1581	1573	746	977	869	2054	1300	514	-1,499		0.4		0.5		0.24		176-195 [20]
191-210 [1]	9355	3481	3190	[illegible]	4717	1944	4537	2868	1,738		1.7		1.6		0.23		191-210 [1]
191-210 [10]	2164	5655	10351	3359	775	4982	4548	3359	1,749		1.7		1.6		0.20		191-210 [10]
191-210 [20]	4040	1323	1368	686	698	2691	1801	1318	-998		0.6		0.6		0.44		191-210 [20]
210-229 [1]	1484	2480	1303	572	787	2027	1442	725	-1,357		0.5		0.5		0.29		210-229 [1]
210-229 [10]	1582	18585	2241	5165	4129	1554	5543	6553	2,744		2.0		2.0		0.09		210-229 [10]
210-229 [20]	10007	11536	11002	12080	3124	7292	9174	3412	6,374	*	3.4	*	3.3	*	0.00	*	210-229 [20]
229-248 [1]	7005	6142	11527	3306	2444	3919	5724	3327	2,925		2.1		2.0		0.04	*	229-248 [1]
229-248 [10]	5804	16394	4966	4881	11690	8974	8785	4584	5,986	*	3.2	*	3.1	*	0.00	*	229-248 [10]
229-248 [20]	14012	35139	3694	16282	46987	1556	19612	17957	16,813	*	7.3	*	7.0	*	0.00	*	229-248 [20]
248-267 [1]	1734	6076	1325	21439	1357	10325	7043	7901	4,244		2.6	*	2.5	*	0.02	*	248-267 [1]
248-267 [10]	6442	7760	3356	25332	1727	1783	7733	8967	4,934		2.8	*	2.8	*	0.01	*	248-267 [10]
248-267 [20]	4241	13644	8491	5419	5064	36981	12307	12571	9,508	*	4.6	*	4.4	*	0.00	*	248-267 [20]
267-286 [1]	30560	2355	2017	3072	2245	5094	7557	11325	4,758		2.8	*	2.7	*	0.03	*	267-286 [1]
267-286 [10]	37480	32780	2559	17550	5730	2156	16376	15635	13,577	*	6.1	*	5.9	*	0.00	*	267-286 [10]
267-286 [20]	9883	27589	4015	37873	16209	16593	18694	12253	15,895	*	7.0	*	6.7	*	0.00	*	267-286 [20]
287-306 [1]	11313	1161	2234	4100	1755	2222	3798	3811	998		1.4		1.4		0.47		287-306 [1]
287-306 [10]	2831	1725	1600	1026	1964	1834	1830	588	-969		0.6		0.7		0.45		287-306 [10]
287-306 [20]	1112	2502	756	1332	951	706	1227	666	-1,573		0.4		0.4		0.22		287-306 [20]
307-326 [1]	3192	1276	1789	908	1636	1088	1648	825	-1,151		0.6		0.6		0.37		307-326 [1]
307-326 [10]	2264	1432	2382	1241	3190	10203	3452	3381	653		1.2		1.2		0.63		307-326 [10]
307-326 [20]	1557	1374	3397	10782	3917	12935	5660	4950	2,861		2.1		2.0		0.05		307-326 [20]
sAg 10	134	560	295	365	472	455	380	151	-2,419		0.1		0.1		0.06		sAg 10
sAg 100	695	608	560	859	348	329	567	204	-2,233		0.2		0.2		0.08		sAg 100
N	336	64	33	72	313	118	156	134	-2,643		0.0		0.1		0.04	*	N
N	86	94	125	106	115	97	104	14	-2,695		0.0		0.0		0.04	*	N
3H	488	688	74	125	304	94	296	249	-2,504		0.1		0.1		0.05		3H
3H	1226	947	1759	2522	6938	833	2371	2322	-428		0.8		0.8		0.74		3H
3H	2421	1634	15626	1852	1777	5276	4764	5494	1,965		1.7		1.7		0.19		3H
3H	7987	2453	4429	1064	2324	1319	3263	2601	464		1.2		1.2		0.73		3H
3H	2674	1387	1777	3941	4243	1632	2609	1232	-190		0.9		0.9		0.88		3H
3H	3193	1832	1672	816	1301	8116	2822	2713	23		1.0		1.0		0.99		3H
3H	2396	2673	2326	820	2121	10482	3470	3496	671		1.3		1.2		0.62		3H
	SMC																
N	34	59	21	25	60	41	40	17	-792		0.0		0.0		0.00	*	N
3H'	616	555	470	1591	1138	620	832	440	0		1.0		1.0		1.00		3H
PHA - 1	58660	41830	50751	60203	62532	68560	57089	9449	56,258	*	72.1	*	68.6	*	0.00	*	PHA - 1
PHA - 5	63226	55758	39320	39131	50018	55043	50416	9640	49,584	*	63.6	*	60.6	*	0.00	*	PHA - 5
PHA - 10	54474	40278	47739	43398	52055	59877	49637	7269	48,805	*	62.6	*	59.7	*	0.00	*	PHA - 10
LPS - 1	3254	6653	3035	4860	6100	5667	4928	1502	4,097		6.2	*	5.9	*	0.00	*	LPS - 1
LPS - 5	8382	10776	9667	10064	7583	13880	10059	2200	9,227	*	12.7	*	12.1	*	0.00	*	LPS - 5
LPS - 10	12923	22314	24222	16725	16253	15114	17925	4383	17,094	*	22.6	*	21.6	*	0.00	*	LPS - 10
LPS - 20	21537	27498	38208	33523	31387	25173	29554	6028	28,723	*	37.3	*	35.5	*	0.00	*	LPS - 20
LPS - 40	13493	30745	27631	25451	22965	21542	23638	5958	22,806	*	29.8	*	28.4	*	0.00	*	LPS - 40

Raw data for Protein vaccinated duck V2Q

V2Q	Mean	SD														
Total N	134	65														
Total 3H	2207	3003							CPM-3H		S.I.		P/N		t-Test	
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1		>2
1-15 [1]	8056	3591	767	459	414	1030	2386	3022	180		1.1		1.1		0.89		1-15 [1]
1-15 [10]	2184	1957	526	380	960	782	1132	758	-1,075		0.5		0.5		0.39		1-15 [10]
1-15 [20]	780	1225	469	441	800	566	714	293	-1,493		0.3		0.3		0.23		1-15 [20]
7-14W-27 [1]	214	1014	874	776	358	662	650	308	-1,557		0.2		0.3		0.22		7-14W-27 [1]
7-14W-27 [10]	1349	926	926	920	758	857	956	203	-1,251		0.4		0.4		0.32		7-14W-27 [10]
7-14W-27 [20]	689	231	821	1493	556	1506	883	516	-1,324		0.4		0.4		0.29		7-14W-27 [20]
7-14R-27 [1]	438	762	635	453	649	923	643	185	-1,563		0.2		0.3		0.21		7-14R-27 [1]
7-14R-27 [10]	1356	883	645	429	827	1080	870	325	-1,337		0.4		0.4		0.29		7-14R-27 [10]
7-24R-27 [20]	2129	3063	591	1131	792	1998	1617	945	-589		0.7		0.7		0.64		7-14R-27 [20]
22-41 [1]	2054	321	555	644	796	755	854	612	-1,352		0.3		0.4		0.28		22-41 [1]
22-41 [10]	715	1749	856	526	761	973	930	428	-1,277		0.4		0.4		0.31		22-41 [10]
22-41 [20]	1567	627	355	344	1412	559	811	540	-1,396		0.3		0.4		0.27		22-41 [20]
37-56 [1]	1520	656	590	825	573	1003	861	362	-1,345		0.4		0.4		0.28		37-56 [1]
37-56 [10]	1037	586	462	736	672	1716	868	458	-1,338		0.4		0.4		0.29		37-56 [10]
37-56 [20]	943	1898	776	879	610	570	946	489	-1,261		0.4		0.4		0.31		37-56 [20]
54-73 [1]	1056	442	792	610	542	449	649	237	-1,558		0.2		0.3		0.21		54-73 [1]
54-73 [10]	1018	395	1436	383	499	1099	805	440	-1,402		0.3		0.4		0.26		54-73 [10]
54-73 [20]	704	1707	404	362	943	482	767	509	-1,440		0.3		0.3		0.25		54-73 [20]
71-90 [1]	1075	699	630	310	581	517	635	253	-1,571		0.2		0.3		0.21		71-90 [1]
71-90 [10]	1353	684	575	290	545	1212	777	415	-1,430		0.3		0.4		0.25		71-90 [10]
71-90 [20]	996	662	418	332	781	1073	710	300	-1,496		0.3		0.3		0.23		71-90 [20]
87-106 [1]	1349	1466	583	620	864	1208	1015	379	-1,192		0.4		0.5		0.34		87-106 [1]
87-106 [10]	604	442	582	1002	788	690	685	194	-1,522		0.3		0.3		0.23		87-106 [10]
87-106 [20]	367	1277	1328	1105	921	2552	1258	723	-948		0.5		0.6		0.45		87-106 [20]
101-120 [1]	584	1477	714	4900	1392	739	1634	1643	-572		0.7		0.7		0.65		101-120 [1]
101-120 [10]	807	674	392	807	492	2726	983	870	-1,224		0.4		0.4		0.33		101-120 [10]
101-120 [20]	1430	416	748	583	508	2842	1088	933	-1,119		0.5		0.5		0.37		101-120 [20]
116-130 [1]	1057	639	1091	574	702	560	771	241	-1,436		0.3		0.3		0.25		116-130 [1]
116-130 [10]	954	427	328	365	587	429	515	233	-1,692		0.2		0.2		0.18		116-130 [10]
116-130 [20]	333	220	458	497	501	337	391	113	-1,816		0.1		0.2		0.15		116-130 [20]
126-140 [1]	493	589	1313	573	584	996	758	325	-1,449		0.3		0.3		0.25		126-140 [1]
126-140 [10]	662	1373	399	519	278	586	636	386	-1,570		0.2		0.3		0.21		126-140 [10]
126-140 [20]	760	724	623	332	570	393	567	174	-1,640		0.2		0.3		0.19		126-140 [20]
136-150 [1]	1492	660	1163	709	648	811	914	342	-1,293		0.4		0.4		0.30		136-150 [1]
136-150 [10]	564	566	473	1337	456	320	619	363	-1,587		0.2		0.3		0.21		136-150 [10]
136-150 [20]	13623	1903	1639	1501	530	1055	3375	5044	1,169		1.6		1.5		0.42		136-150 [20]
146-160 [1]	1688	331	3366	1039	1071	1112	1435	1040	-772		0.6		0.7		0.54		146-160 [1]
146-160 [10]	895	1002	927	175	689	1470	860	423	-1,347		0.4		0.4		0.28		146-160 [10]
146-160 [20]	884	755	1059	775	696	1136	884	178	-1,322		0.4		0.4		0.29		146-160 [20]
156-170 [1]	623	340	1845	567	1063	793	872	534	-1,335		0.4		0.4		0.29		156-170 [1]
156-170 [10]	827	404	826	183	927	860	671	303	-1,535		0.3		0.3		0.22		156-170 [10]
156-170 [20]	1279	634	224	532	2243	1128	1007	720	-1,200		0.4		0.5		0.34		156-170 [20]
166-180 [1]	425	667	449	577	414	620	525	110	-1,681		0.2		0.2		0.18		166-180 [1]
166-180 [10]	1000	1126	919	1735	1060	2486	1388	612	-819		0.6		0.6		0.51		166-180 [10]
166-180 [20]	19513	1733	996	394	825	4346	4635	7424	2,428		2.2	*	2.1	*	0.14		166-180 [20]
176-195 [1]	2377	3214	544	1071	2929	1895	2005	1046	-202		0.9		0.9		0.87		176-195 [1]
176-195 [10]	1370	1026	679	638	785	1270	961	310	-1,245		0.4		0.4		0.32		176-195 [10]
176-195 [20]	853	1974	544	525	1146	113	859	647	-1,347		0.3		0.4		0.28		176-195 [20]
191-210 [1]	4841	1389	670	1619	1490	1401	1902	1478	-305		0.9		0.9		0.81		191-210 [1]
191-210 [10]	5558	667	719	699	723	2228	1766	1956	-441		0.8		0.8		0.73		191-210 [10]
191-210 [20]	1261	1691	481	284	646	538	817	541	-1,390		0.3		0.4		0.27		191-210 [20]
210-229 [1]	2439	1168	873	3890	1553	561	1747	1234	-459		0.8		0.8		0.72		210-229 [1]
210-229 [10]	27940	3667	12080	5883	11488	5005	11011	8992	8,804	*	5.2	*	5.0	*	0.00	*	210-229 [10]
210-229 [20]	5932	13972	15027	4539	8384	25121	12163	7624	9,956	*	5.8	*	5.5	*	0.00	*	210-229 [20]
229-248 [1]	6404	7780	1736	3010	1781	1658	3728	2688	1,522		1.7		1.7		0.25		229-248 [1]
229-248 [10]	14071	25703	4123	1759	9110	912	9280	9443	7,073	*	4.4	*	4.2	*	0.00	*	229-248 [10]
229-248 [20]	3931	4828	25122	3320	4654	7034	8148	8410	5,942	*	3.9	*	3.7	*	0.00	*	229-248 [20]
248-267 [1]	1021	20923	2581	1054	1852	1242	4779	7931	2,572		2.2	*	2.2	*	0.13		248-267 [1]
248-267 [10]	1398	812	2874	676	1842	952	1426	829	-781		0.6		0.6		0.53		248-267 [10]
248-267 [20]	592	7453	2513	1172	1011	878	2270	2626	63		1.0		1.0		0.96		248-267 [20]
267-286 [1]	2714	5497	11360	1168	1386	2543	4111	3872	1,905		1.9		1.9		0.17		267-286 [1]
267-286 [10]	1227	6467	18805	4906	12010	907	7387	6909	5,181	*	3.5	*	3.3	*	0.00	*	267-286 [10]
267-286 [20]	8553	16796	12962	17261	37476	6304	16559	11138	14,352	*	7.9	*	7.5	*	0.00	*	267-286 [20]
287-306 [1]	1617	2285	1610	1895	1684	2212	1884	302	-323		0.8		0.9		0.80		287-306 [1]
287-306 [10]	3858	7872	19560	15160	5000	12039	10582	6122	8,375	*	5.0	*	4.8	*	0.00	*	287-306 [10]
287-306 [20]	10387	1522	1107	2018	51165	13610	13302	19272	11,095	*	6.4	*	6.0	*	0.00	*	287-306 [20]
307-326 [1]	7042	18161	43859	2771	13297	1923	14509	15673	12,302	*	6.9	*	6.6	*	0.00	*	307-326 [1]
307-326 [10]	2099	6992	1501	191	2235	2263	2547	2314	340		1.2		1.2		0.79		307-326 [10]
307-326 [20]	1870	2031	1033	1318	4372	1866	2082	1185	-125		0.9		0.9		0.92		307-326 [20]
sAg 10	609	580	708	642	288	431	543	155	-1,664		0.2		0.2		0.19		sAg 10
sAg 100	1006	477	775	543	541	1004	724	240	-1,482		0.3		0.3		0.24		sAg 100
N	132	147	267	193	187	188	186	47	-2,021		0.0		0.1		0.11		N
N	102	107	77	107	37	68	83	28	-2,124		0.0		0.0		0.09		N
3H	839	442	247	217	155	450	392	250	-1,815		0.1		0.2		0.15		3H
3H	793	765	568	536	597	953	702	162	-1,505		0.3		0.3		0.23		3H
3H	745	1988	1402	994	718	7561	2235	2653	28		1.0		1.0		0.98		3H
3H	2412	5302	4183	5007	7686	14967	6593	4445	4,386		3.1	*	3.0	*	0.00	*	3H
3H	456	783	519	532	525	547	560	113	-1,646		0.2		0.3		0.19		3H
3H	712	2167	2131	829	1162	2927	1655	886	-552		0.7		0.7		0.66		3H
3H	1598	10108	1722	1208	2910	2310	3309	3384	1,103		1.5		1.5		0.41		3H
	SMC															
N	58	28	17	42	90	49	47	26	-236		0.0		0.2		0.17		N
3H	115	60	78	62	1038	346	283	385	0		1.0		1.0		1.00		3H
PHA - 1	67303	47697	63031	53841	59857	63862	59265	7260	58,982	*	25.1	*	209.3	*	0.00	*	PHA - 1
PHA - 5	111376	101106	99415	97553	127706	107399	107426	11211	107,143	*	45.3	*	379.4	*	0.00	*	PHA - 5
PHA - 10	54658	53786	40358	39204	51343	72436	51964	12063	51,681	*	22.0.1	*	183.5	*	0.00	*	PHA - 10
LPS - 1	7771	6530	1269	2279	1535	3050	3739	2743	3,456		15.7	*	13.2	*	0.01	*	LPS - 1
LPS - 5	19232	12755	13284	12861	15879	44302	19719	12298	19,436	*	83.4	*	69.6	*	0.00	*	LPS - 5
LPS - 10	34414	34126	29776	36835	41999	40517	36278	4503	35,995	*	153.6	*	128.1	*	0.00	*	LPS - 10
LPS - 20	25012	27388	32577	25042	18606	17957	24430	5507	24,147	*	103.4	*	86.3	*	0.00	*	LPS - 20
LPS - 40	43020	4336	20600	42763	24471	19260	25742	14943	25,459	*	109.0	*	90.9	*	0.00	*	LPS - 40

Raw data for Protein vaccinated duck V2S

V2S	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	70	44															
Total 3H	523	276															
	R1	R2	R3	R4	R5	R6	Mean	SD	>5000		>2.1		>2.1		>2		
1-15 [1]	380	582	248	707	270	666	476	202	-48		0.9		0.9		0.69		1-15 [1]
1-15 [10]	368	240	253	244	168	354	271	76	-252		0.4		0.5		0.03	*	1-15 [10]
1-15 [20]	242	185	716	376	188	251	326	203	-197		0.6		0.6		0.10		1-15 [20]
7-14W-27 [1]	401	260	383	550	942	217	459	264	-65		0.9		0.9		0.59		7-14W-27 [1]
7-14W-27 [10]	447	621	397	180	450	658	459	172	-65		0.9		0.9		0.58		7-14W-27 [10]
7-14W-27 [20]	201	700	341	731	550	[redacted]	505	229	-19		1.0		1.0		0.88		7-14W-27 [20]
7-14R-27 [1]	461	306	222	101	411	348	308	131	-215		0.5		0.6		0.07		7-14R-27 [1]
7-14R-27 [10]	407	383	227	589	173	141	320	171	-203		0.6		0.6		0.09		7-14R-27 [10]
7-14R-27 [20]	796	270	158	194	287	244	325	236	-199		0.6		0.6		0.10		7-14R-27 [20]
22-41 [1]	360	440	519	177	497	503	416	131	-107		0.8		0.8		0.36		22-41 [1]
22-41 [10]	214	212	352	417	217	330	290	88	-233		0.5		0.6		0.05	*	22-41 [10]
22-41 [20]	216	141	175	217	124	716	265	224	-259		0.4		0.5		0.03	*	22-41 [20]
37-56 [1]	207	279	202	190	106	229	202	57	-321		0.3		0.4		0.01	*	37-56 [1]
37-56 [10]	259	185	179	269	311	205	235	53	-289		0.4		0.4		0.01	*	37-56 [10]
37-56 [20]	456	232	224	308	147	157	254	115	-269		0.4		0.5		0.02	*	37-56 [20]
54-73 [1]	254	375	479	352	1020	686	528	282	4		1.0		1.0		0.97		54-73 [1]
54-73 [10]	240	424	377	353	179	106	280	125	-244		0.5		0.5		0.04	*	54-73 [10]
54-73 [20]	458	167	269	961	331	638	471	290	-53		0.9		0.9		0.67		54-73 [20]
71-90 [1]	326	201	210	443	327	361	311	92	-212		0.5		0.6		0.07		71-90 [1]
71-90 [10]	312	213	162	132	225	201	208	62	-316		0.3		0.4		0.01	*	71-90 [10]
71-90 [20]	715	286	572	244	255	681	459	222	-65		0.9		0.9		0.59		71-90 [20]
87-106 [1]	638	358	173	281	279	390	353	159	-170		0.6		0.7		0.15		87-106 [1]
87-106 [10]	239	238	188	330	324	688	335	182	-189		0.6		0.6		0.11		87-106 [10]
87-106 [20]	231	271	231	358	647	444	364	162	-160		0.6		0.7		0.18		87-106 [20]
101-120 [1]	317	520	249	785	408	314	432	197	-91		0.8		0.8		0.44		101-120 [1]
101-120 [10]	1281	837	552	908	482	330	732	346	208		1.5		1.4		0.10		101-120 [10]
101-120 [20]	630	465	394	566	631	216	484	161	-40		0.9		0.9		0.73		101-120 [20]
116-130 [1]	406	302	342	489	211	596	391	138	-132		0.7		0.7		0.26		116-130 [1]
116-130 [10]	607	452	1004	142	445	607	543	283	19		1.0		1.0		0.87		116-130 [10]
116-130 [20]	479	206	267	228	286	320	298	98	-226		0.5		0.6		0.06		116-130 [20]
126-140 [1]	809	404	163	182	186	892	439	332	-84		0.8		0.8		0.50		126-140 [1]
126-140 [10]	313	457	254	406	213	315	326	91	-197		0.6		0.6		0.09		126-140 [10]
126-140 [20]	150	266	266	257	362	328	272	73	-252		0.4		0.5		0.03	*	126-140 [20]
136-150 [1]	469	1034	202	362	250	303	437	307	-87		0.8		0.8		0.48		136-150 [1]
136-150 [10]	435	298	260	271	259	422	324	82	-199		0.6		0.6		0.09		136-150 [10]
136-150 [20]	1766	648	626	536	2843	425	1141	967	617		2.4	*	2.2	*	0.00	*	136-150 [20]
146-160 [1]	676	976	533	361	468	673	615	215	91		1.2		1.2		0.44		146-160 [1]
146-160 [10]	389	963	1229	376	582	518	676	345	153		1.3		1.3		0.23		146-160 [10]
146-160 [20]	1480	615	551	520	392	616	696	393	172		1.4		1.3		0.18		146-160 [20]
156-170 [1]	1094	499	480	780	520	762	689	239	166		1.4		1.3		0.17		156-170 [1]
156-170 [10]	492	598	1183	737	371	626	668	281	144		1.3		1.3		0.24		156-170 [10]
156-170 [20]	439	692	873	1019	576	1454	842	364	319		1.7		1.6		0.01	*	156-170 [20]
166-180 [1]	643	681	655	585	756	372	615	132	92		1.2		1.2		0.43		166-180 [1]
166-180 [10]	591	399	1666	652	605	564	746	459	223		1.5		1.4		0.10		166-180 [10]
166-180 [20]	1623	477	2139	535	790	578	1024	692	500		2.1	*	2.0		0.00	*	166-180 [20]
176-195 [1]	1016	676	319	1211	569	661	742	321	219		1.5		1.4		0.08		176-195 [1]
176-195 [10]	572	641	470	560	427	507	530	77	6		1.0		1.0		0.96		176-195 [10]
176-195 [20]	652	557	525	360	413	559	511	107	-12		1.0		1.0		0.91		176-195 [20]
191-210 [1]	989	1014	339	559	547	614	677	268	154		1.3		1.3		0.21		191-210 [1]
191-210 [10]	888	449	359	383	362	1189	605	351	82		1.2		1.2		0.52		191-210 [10]
191-210 [20]	789	598	299	431	341	393	475	185	-48		0.9		0.9		0.68		191-210 [20]
210-229 [1]	3540	876	1509	587	521	504	1256	1181	733		2.6	*	2.4	*	0.00	*	210-229 [1]
210-229 [10]	11937	616	523	2655	972	2351	3176	4385	2,652		6.8	*	6.1	*	0.00	*	210-229 [10]
210-229 [20]	1104	5155	890	935	749	1542	1729	1700	1,206		3.7	*	3.3	*	0.00	*	210-229 [20]
229-248 [1]	1438	1713	826	709	1410	634	1122	454	598		2.3	*	2.1	*	0.00	*	229-248 [1]
229-248 [10]	691	1250	314	399	209	644	585	376	61		1.1		1.1		0.63		229-248 [10]
229-248 [20]	568	462	291	192	177	262	325	157	-198		0.6		0.6		0.09		229-248 [20]
248-267 [1]	1130	970	499	418	296	960	712	349	189		1.4		1.4		0.14		248-267 [1]
248-267 [10]	629	465	462	424	618	522	520	86	-3		1.0		1.0		0.98		248-267 [10]
248-267 [20]	535	420	380	384	248	778	458	182	-66		0.9		0.9		0.58		248-267 [20]
267-286 [1]	1026	767	540	808	501	549	699	205	175		1.4		1.3		0.14		267-286 [1]
267-286 [10]	909	538	647	557	732	377	627	182	103		1.2		1.2		0.38		267-286 [10]
267-286 [20]	584	831	1428	647	9263	483	2206	3474	1,683		4.7	*	4.2	*	0.00	*	267-286 [20]
287-306 [1]	458	330	318	627	2059	427	703	674	180		1.4		1.3		0.24		287-306 [1]
287-306 [10]	1109	1067	810	327	405	851	762	329	238		1.5		1.5		0.06		287-306 [10]
287-306 [20]	764	704	637	265	198	662	538	242	15		1.0		1.0		0.90		287-306 [20]
307-326 [1]	1637	575	511	368	370	410	645	493	122		1.3		1.2		0.37		307-326 [1]
307-326 [10]	787	696	502	494	165	550	532	214	9		1.0		1.0		0.94		307-326 [10]
307-326 [20]	1879	874	1118	1139	361	821	1032	501	509		2.1	*	2.0		0.00	*	307-326 [20]
sAg 10	453	355	1578	347	269	342	557	503	34		1.1		1.1		0.80		sAg 10
sAg 100	599	351	447	288	381	495	427	111	-97		0.8		0.8		0.41		sAg 100
N	23	35	44	38	94	121	59	39	-464		0.0		0.1		0.00	*	N
N	109	34	88	42	48	161	80	49	-443		0.0		0.2		0.00	*	N
3H	128	169	333	704	708	338	397	254	-127		0.7		0.8		0.30		3H
3H	930	214	211	178	111	278	320	304	-203		0.6		0.6		0.10		3H
3H	399	1240	602	415	393	520	595	327	71		1.2		1.1		0.57		3H
3H	833	1242	810	469	537	536	738	290	214		1.5		1.4		0.08		3H
3H	600	377	389	318	568	380	439	116	-85		0.8		0.8		0.47		3H
3H	1218	740	449	557	351	365	613	329	90		1.2		1.2		0.47		3H
3H	541	479	567	664	467	655	562	84	39		1.1		1.1		0.74		3H
	SMC																
N	34	50	33	16	22	84	40	25	-784		0.0		0.0		0.00	*	N
3H,	487	510	722	490	1338	1397	824	430	0		1.0		1.0		1.00		3H
PHA - 1	49537	7187	13310	22068	38235	23137	25579	15756	24,755	*	32.6	*	31.0	*	0.00	*	PHA - 1
PHA - 5	51894	53170	66151	56399	59253	58271	57523	5097	56,699	*	73.3	*	69.8	*	0.00	*	PHA - 5
PHA - 10	54625	41001	47620	54304	60893	46701	50857	7095	50,033	*	64.8	*	61.7	*	0.00	*	PHA - 10
LPS - 1	1310	2867	3003	2452	2464	2912	2501	629	1,677		3.1	*	3.0	*	0.00	*	LPS - 1
LPS - 5	1871	2608	3493	3760	6193	4637	3760	1527	2,936		4.7	*	4.6	*	0.00	*	LPS - 5
LPS - 10	3715	5650	4395	3266	4569	6033	4605	1073	3,781		5.8	*	5.6	*	0.00	*	LPS - 10
LPS - 20	4532	2032	2660	2895	4911	2706	3289	1153	2,465		4.1	*	4.0	*	0.00	*	LPS - 20
LPS - 40	1530	4440	2280	3793	5275	3203	3420	1381	2,596		4.3	*	4.2	*	0.00	*	LPS - 40

Raw data for Positive control duck P72W48

P72W48	Mean	SD							CPM-3H	>5000	S.I.	>2.1	P/N	>2.1	t-Test	<0.05	
Total N	82	145															
Total 3H	1782	1130															
	R1	R2	R3	R4	R5	R6	Mean	SD									
1-15 [1]	6426	3095	3495	4592	3641	4332	4263	1194	2481		2.5	*	2.4	*	0.000	*	1-15 [1]
1-15 [10]	3945	2084	3044	3699	6115	5231	4019	1460	2237		2.3	*	2.3	*	0.001	*	1-15 [10]
1-15 [20]	4263	9476	4963	4481	4359	5420	5494	1999	3711		3.2	*	3.1	*	0.000	*	1-15 [20]
7-14W-27 [1]	3600	6037	10603	10290	7532	9832	7982	2786	6200	*	4.6	*	4.5	*	0.000	*	7-14W-27 [1]
7-14W-27 [10]	6255	12672	9683	6398	5991	9549	8424	2669	6642	*	4.9	*	4.7	*	0.000	*	7-14W-27 [10]
7-14W-27 [20]	5072	9070	7895	15649	23905	16754	13057	6991	11275	*	7.6	*	7.3	*	0.000	*	7-14W-27 [20]
7-14R-27 [1]	8927	18273	10139	12176	14863	11776	12692	3396	10910	*	7.4	*	7.1	*	0.000	*	7-14R-27 [1]
7-14R-27 [10]	18278	10405	12711	11741	7180	8621	11489	3889	9707	*	6.7	*	6.4	*	0.000	*	7-14R-27 [10]
7-14R-27 [20]	21384	18860	22072	11831	12634	8973	15959	5515	14177	*	9.3	*	9.0	*	0.000	*	7-14R-27 [20]
22-41 [1]	5162	9693	3325	4900	6814	10821	6786	2929	5003	*	3.9	*	3.8	*	0.000	*	22-41 [1]
22-41 [10]	8996	6928	4452	5019	4324	5616	5889	1793	4107		3.4	*	3.3	*	0.000	*	22-41 [10]
22-41 [20]	5587	6716	6148	6413	11243	16833	8823	4425	7041	*	5.1	*	5.0	*	0.000	*	22-41 [20]
37-56 [1]	1910	1881	403	2017	1137	1212	1426	627	-356		0.8		0.8		0.440		37-56 [1]
37-56 [10]	2595	1246	728	1626	3192	3074	2076	1022	294		1.2		1.2		0.566		37-56 [10]
37-56 [20]	830	367	1721	818	1668	965	1061	531	-721		0.6		0.6		0.120		37-56 [20]
54-73 [1]	1238	1362	1823	607	3181	477	1448	984	-334		0.8		0.8		0.511		54-73 [1]
54-73 [10]	384	641	1541	1099	354	802	803	455	-979		0.4		0.5		0.038	*	54-73 [10]
54-73 [20]	771	885	1146	493	3196	2617	1518	1111	-264		0.8		0.9		0.616		54-73 [20]
71-90 [1]	795	917	1376	1221	1608	2415	1389	584	-394		0.8		0.8		0.389		71-90 [1]
71-90 [10]	1738	445	570	989	1134	1896	1128	593	-654		0.6		0.6		0.162		71-90 [10]
71-90 [20]	1154	873	3432	1571	722	620	1395	1055	-387		0.8		0.8		0.457		71-90 [20]
87-106 [1]	423	444	571	1026	628	4436	1254	1574	-528		0.7		0.7		0.395		87-106 [1]
87-106 [10]	1234	1635	2004	858	978	1740	1408	455	-374		0.8		0.8		0.400		87-106 [10]
87-106 [20]	876	930	1348	941	1462	570	1021	329	-761		0.6		0.6		0.091		87-106 [20]
101-120 [1]	498	971	1725	1720	1889	553	1226	629	-556		0.7		0.7		0.234		101-120 [1]
101-120 [10]	1162	1863	680	1667	1246	714	1222	483	-560		0.7		0.7		0.216		101-120 [10]
101-120 [20]	2094	696	1672	1958	1433	3435	1881	907	99		1.1		1.1		0.841		101-120 [20]
116-130 [1]	2611	469	854	2740	2201	913	1631	998	-151		0.9		0.9		0.766		116-130 [1]
116-130 [10]	1173	1340	1589	1538	1476	3143	1710	718	-73		1.0		1.0		0.876		116-130 [10]
116-130 [20]	1709	3009	2645	438	809	1319	1655	1013	-128		0.9		0.9		0.802		116-130 [20]
126-140 [1]	1967	1230	2008	1429	417	1073	1354	597	-429		0.7		0.8		0.351		126-140 [1]
126-140 [10]	1054	781	824	345	1980	756	956	551	-826		0.5		0.5		0.080		126-140 [10]
126-140 [20]	560	1676	1359	1627	2851	1410	1580	741	-202		0.9		0.9		0.669		126-140 [20]
136-150 [1]	743	1100	718	304	1541	1644	1008	519	-774		0.5		0.6		0.096		136-150 [1]
136-150 [10]	1011	1107	1599	1155	604	908	1064	327	-718		0.6		0.6		0.109		136-150 [10]
136-150 [20]	1530	1868	2793	2119	786	1364	1743	688	-39		1.0		1.0		0.933		136-150 [20]
146-160 [1]	1396	665	1119	669	744	874	911	292	-871		0.5		0.5		0.055		146-160 [1]
146-160 [10]	864	760	995	1841	1122	611	1032	434	-750		0.6		0.6		0.101		146-160 [10]
146-160 [20]	1987	3070	1195	1371	587	1729	1656	843	-126		0.9		0.9		0.795		146-160 [20]
156-170 [1]	3091	2150	1707	721	417	554	1440	1063	-342		0.8		0.8		0.511		156-170 [1]
156-170 [10]	594	620	961	876	1368	687	851	292	-931		0.5		0.5		0.042	*	156-170 [10]
156-170 [20]	1637	3993	471	1148	1908	697	1642	1273	-140		0.9		0.9		0.801		156-170 [20]
166-180 [1]	882	1143	360	781	508	1985	943	580	-840		0.5		0.5		0.077		166-180 [1]
166-180 [10]	2940	480	1283	1503	3806	995	1834	1270	52		1.0		1.0		0.925		166-180 [10]
166-180 [20]	1404	1656	655	2382	551	525	1195	751	-587		0.7		0.7		0.224		166-180 [20]
176-195 [1]	657	1405	1357	1223	1562	1121	1221	315	-562		0.7		0.7		0.203		176-195 [1]
176-195 [10]	1265	5339	1961	414	1791	433	1867	1822	85		1.0		1.0		0.899		176-195 [10]
176-195 [20]	1632	1131	3028	1688	559	2552	1765	905	-17		1.0		1.0		0.972		176-195 [20]
191-210 [1]	1930	1368	3379	988	2143	2943	2125	910	343		1.2		1.2		0.491		191-210 [1]
191-210 [10]	1258	2192	1555	1260	1830	1958	1675	383	-107		0.9		0.9		0.806		191-210 [10]
191-210 [20]	1137	909	2497	1392	1630	1230	1466	560	-317		0.8		0.8		0.485		191-210 [20]
210-229 [1]	1066	590	1371	357	242	3113	1123	1065	-659		0.6		0.6		0.214		210-229 [1]
210-229 [10]	329	377	1819	1588	319	1332	961	696	-822		0.5		0.5		0.091		210-229 [10]
210-229 [20]	1799	1822	764	1042	597	540	1094	582	-688		0.6		0.6		0.141		210-229 [20]
229-248 [1]	917	667	1465	606	1292	2085	1172	561	-610		0.6		0.7		0.187		229-248 [1]
229-248 [10]	2483	983	938	1930	375	526	1206	828	-577		0.7		0.7		0.242		229-248 [10]
229-248 [20]	3559	2189	3193	4154	2325	1362	2797	1023	1015		1.6		1.6		0.061		229-248 [20]
248-267 [1]	1410	642	461	728	410	1096	791	389	-991		0.4		0.4		0.034	*	248-267 [1]
248-267 [10]	782	2211	3654	726	1183	1363	1653	1117	-129		0.9		0.9		0.806		248-267 [10]
248-267 [20]	965	509	2219	2382	1797	4795	2111	1501	329		1.2		1.2		0.585		248-267 [20]
267-286 [1]	5101	997	940	193	415	604	1388	1883	-394		0.8		0.8		0.564		267-286 [1]
267-286 [10]	211	371	865	1304	395	443	598	409	-1184		0.3		0.3		0.014	*	267-286 [10]
267-286 [20]	1044	1671	2140	452	2331	1286	1487	704	-295		0.8		0.8		0.529		267-286 [20]
287-306 [1]	2829	1708	1894	2948	1207	2335	2153	675	371		1.2		1.2		0.427		287-306 [1]
287-306 [10]	1373	2892	2305	3311	1078	337	1882	1143	100		1.1		1.1		0.850		287-306 [10]
287-306 [20]	1168	935	1505	2621	990	3554	1795	1062	13		1.0		1.0		0.980		287-306 [20]
307-326 [1]	662	241	2776	1247	924	629	1080	896	-703		0.6		0.6		0.166		307-326 [1]
307-326 [10]	498	605	4580	2073	377	836	1495	1632	-288		0.8		0.8		0.647		307-326 [10]
307-326 [20]	2322	1412	3741	2031	1040	1516	2010	963	228		1.1		1.1		0.651		307-326 [20]
sAg 10	907	1432	1781	1650	2175	823	1461	522	-321		0.8		0.8		0.475		sAg 10
sAg 100	1454	2066	857	735	1493	1684	1381	504	-401		0.8		0.8		0.372		sAg 100
N	412	267	332	289	74	23	233	152	-1549		0.1		0.1		0.002	*	N
N	43	32	55	25	31	34	36	11	-1746		0.0		0.0		0.001	*	N
N	29	23	26	19	27	28	25	4	-1757		0.0		0.0		0.001	*	N
N	47	39	39	13	28	35	33	12	-1749		0.0		0.0		0.001	*	N
3H	1074	3283	1264	1292	587	1189	1448	935	-334		0.8		0.8		0.504		3H
3H	1284	1977	1694	3820	1126	2799	2117	1023	334		1.2		1.2		0.516		3H
	SMC																
N	41	52	45	53	42	85	53	16	-1346		0.0		0.0		0.00	*	N
3H	1177	1436	1462	1584	1626	1107	1399	212	0		1.0		1.0		1.00		3H
PHA - 1	2633	4402	4099	3793	3360	2784	3512	713	2113		2.6	*	2.5	*	0.00	*	PHA - 1
PHA - 5	4473	7949	6549	6644	6542	4864	6170	1285	4772		4.5	*	4.4	*	0.00	*	PHA - 5
PHA - 10	5067	8165	7063	6233	5918	6008	6409	1072	5010	*	4.7	*	4.6	*	0.00	*	PHA - 10
LPS - 1	1513	2158	2170	1860	1567	1305	1762	358	364		1.3		1.3		0.06		LPS - 1
LPS - 5	1693	2383	2417	2218	1720	2093	2087	317	689		1.5		1.5		0.00	*	LPS - 5
LPS - 10	2091	2646	2423	2721	1402	1930	2202	498	803		1.6		1.6		0.00	*	LPS - 10
LPS - 20	2409	2488	2879	3057	2656	2022	2585	367	1186		1.9		1.8		0.00	*	LPS - 20
LPS - 40	2487	3197	3137	2834	2240	2042	2656	476	1258		1.9		1.9		0.00	*	LPS - 40
	PBMC																
N	67	42	36	54	51	38	48	12	-484		0.0		0.1		0.00	*	N
3H	848	565	506	554	417	300	531	184	0		1.0		1.0		1.00		3H
PHA - 1	1556	1862	1661	1175	1642	1115	1502	295	970		3.0	*	2.8	*	0.00	*	PHA - 1
PHA - 5	2182	2013	1294	1688	1302	1740	1703	362	1171		3.4	*	3.2	*	0.00	*	PHA - 5
PHA - 10	2721	1875	1504	1680	1681	2044	1917	435	1386		3.9	*	3.6	*	0.00	*	PHA - 10
LPS - 1	804	451	498	325	442	383	484	168	-48		0.9		0.9		0.65		LPS - 1
LPS - 5	398	254	293	195	259	263	277	67	-255		0.5		0.5		0.01	*	LPS - 5
LPS - 10	363	205	178	236	201	144	221	76	-311		0.4		0.4		0.00	*	LPS - 10
LPS - 20	426	192	15	143	152	147	179	135	-353		0.3		0.3		0.00	*	LPS - 20
LPS - 40	698	254	95	129	185	131	249	227	-283		0.4		0.5		0.04	*	LPS - 40

Raw data for Positive control duck V2R

V2R	Mean	SD							CPM-3H	S.I.	P/N	t-Test	
Total N	106	68											
Total 3H	408	358											
	R1	R2	R3	R4	R5	R6	Mean	SD	>5000	>2.1	>2.1	>2	
1-15 [1]		253	106	614	347	128	290	206	-119	0.6	0.7	0.47	1-15 [1]
1-15 [10]	216	271	581	116	107	149	240	178	-168	0.4	0.6	0.27	1-15 [10]
1-15 [20]		601	260	123	175	806	393	297	-15	0.9	1.0	0.93	1-15 [20]
7-14W-27 [1]	286	128	282	263	127	63	192	97	-217	0.3	0.5	0.15	7-14W-27 [1]
7-14W-27 [10]	589	305	315	384	338	530	410	120	2	1.0	1.0	0.99	7-14W-27 [10]
7-14W-27 [20]	322	2396	626	122	366	112	657	872	249	1.8	1.6	0.21	7-14W-27 [20]
7-14R-27 [1]	217	58	86	82	114	76	106	58	-303	0.0	0.3	0.05 *	7-14R-27 [1]
7-14R-27 [10]	196	139	344	129	110	160	180	86	-229	0.2	0.4	0.13	7-14R-27 [10]
7-14R-27 [20]	182	145	158	300	126	106	170	68	-239	0.2	0.4	0.11	7-14R-27 [20]
22-41 [1]	131	123	115	98	99	151	120	20	-289	0.0	0.3	0.06	22-41 [1]
22-41 [10]	196	148	148	653	237	198	263	194	-145	0.5	0.6	0.34	22-41 [10]
22-41 [20]	680	261	160	144	92	109	241	223	-167	0.4	0.6	0.27	22-41 [20]
37-56 [1]	177	101	85	112	93	76	107	36	-301	0.0	0.3	0.05 *	37-56 [1]
37-56 [10]	173	278	111	118	120	160	160	63	-248	0.2	0.4	0.10	37-56 [10]
37-56 [20]	253	200	181	121	147	97	167	57	-242	0.2	0.4	0.11	37-56 [20]
54-73 [1]	144	133	113	85	95	388	160	114	-249	0.2	0.4	0.10	54-73 [1]
54-73 [10]	299	40	48	106	74	117	114	96	-294	0.0	0.3	0.05	54-73 [10]
54-73 [20]	201	94	80	105	146	82	118	47	-290	0.0	0.3	0.06	54-73 [20]
71-90 [1]	412	112	147	264	304	220	243	109	-165	0.5	0.6	0.27	71-90 [1]
71-90 [10]	483	154	202	265	161	134	233	131	-175	0.4	0.6	0.24	71-90 [10]
71-90 [20]	92	292	109	110	139	107	142	75	-267	0.1	0.3	0.08	71-90 [20]
87-106 [1]	510	97	235	194	104	136	213	155	-196	0.4	0.5	0.20	87-106 [1]
87-106 [10]	128	65	58	98	116	75	90	28	-318	-0.1	0.2	0.04 *	87-106 [10]
87-106 [20]	143	85	124	317	250	114	172	91	-236	0.2	0.4	0.12	87-106 [20]
101-120 [1]	192	204	141	545	276	248	268	144	-141	0.5	0.7	0.35	101-120 [1]
101-120 [10]	125	275	129	95	230	1287	357	461	-52	0.8	0.9	0.75	101-120 [10]
101-120 [20]	208	102	96	57	177	219	143	67	-265	0.1	0.4	0.08	101-120 [20]
116-130 [1]	84	183	190	108	90	187	140	51	-268	0.1	0.3	0.08	116-130 [1]
116-130 [10]	79	172	220	97	195	220	164	62	-245	0.2	0.4	0.10	116-130 [10]
116-130 [20]	78	113	126	100	289	718	237	247	-171	0.4	0.6	0.27	116-130 [20]
126-140 [1]	124	125	140	88	81	113	112	23	-297	0.0	0.3	0.05	126-140 [1]
126-140 [10]	128	183	166	129	161	294	177	61	-232	0.2	0.4	0.12	126-140 [10]
126-140 [20]	81	110	129	112	74	130	106	24	-302	0.0	0.3	0.05 *	126-140 [20]
136-150 [1]	103	136	112	198	103	176	138	40	-270	0.1	0.3	0.07	136-150 [1]
136-150 [10]	114	139	431	194	110	145	189	122	-220	0.3	0.5	0.15	136-150 [10]
136-150 [20]	195	424	223	156	424	374	299	122	-109	0.6	0.7	0.47	136-150 [20]
146-160 [1]	570	867	376	92	2086	3538	1255	1315	846	3.8 *	3.1 *	0.00 *	146-160 [1]
146-160 [10]	245	192	272	399	578	368	342	139	-66	0.8	0.8	0.66	146-160 [10]
146-160 [20]	304	227	198	357	384	456	321	98	-87	0.7	0.8	0.56	146-160 [20]
156-170 [1]	214	373	224	303	3097	303	752	1150	344	2.1 *	1.8	0.13	156-170 [1]
156-170 [10]	301	292	975	776	569	363	546	281	138	1.5	1.3	0.37	156-170 [10]
156-170 [20]	155	229	620	561	458	319	390	186	-18	0.9	1.0	0.90	156-170 [20]
166-180 [1]	486	467	575	236	254	1863	647	611	238	1.8	1.6	0.17	166-180 [1]
166-180 [10]	113	256	211	213	375	247	236	85	-173	0.4	0.6	0.25	166-180 [10]
166-180 [20]	316	393	507	286	354	390	374	77	-34	0.9	0.9	0.82	166-180 [20]
176-195 [1]	608	221	248	495	188	291	340	168	-68	0.8	0.8	0.65	176-195 [1]
176-195 [10]	121	85	104	158	162	579	202	187	-207	0.3	0.5	0.17	176-195 [10]
176-195 [20]	179	165	188	117	540	1063	375	370	-33	0.9	0.9	0.83	176-195 [20]
191-210 [1]	289	311	210	154	216	216	233	58	-176	0.4	0.6	0.24	191-210 [1]
191-210 [10]	217	228	448	224	544	638	383	185	-25	0.9	0.9	0.87	191-210 [10]
191-210 [20]	101	299	224	166	203	390	231	102	-178	0.4	0.6	0.24	191-210 [20]
210-229 [1]	239	401	233	934	241	555	434	276	25	1.1	1.1	0.87	210-229 [1]
210-229 [10]	1885	1272	922	946	763	492	1047	483	638	3.1 *	2.6 *	0.00 *	210-229 [10]
210-229 [20]	358	348	3045	2825	3048	4556	2363	1676	1,955	7.5 *	5.8 *	0.00 *	210-229 [20]
229-248 [1]	1234	985	572	528	159	468	658	387	249	1.8	1.6	0.12	229-248 [1]
229-248 [10]	1883	305	782	853	158	265	708	643	299	2.0	1.7	0.09	229-248 [10]
229-248 [20]	474	502	485	870	407	560	550	164	141	1.5	1.3	0.35	229-248 [20]
248-267 [1]	1158	558	404	182	418	201	487	358	78	1.3	1.2	0.62	248-267 [1]
248-267 [10]	588	429	330	314	623	363	441	134	33	1.1	1.1	0.83	248-267 [10]
248-267 [20]	347	163	162	161	181	606	270	180	-138	0.5	0.7	0.36	248-267 [20]
267-286 [1]	655	321	335	1430	121	182	507	488	99	1.3	1.2	0.55	267-286 [1]
267-286 [10]	208	122	213	1048	244	241	346	347	-62	0.8	0.8	0.69	267-286 [10]
267-286 [20]	520	212	661	303	788	501	498	215	89	1.3	1.2	0.56	267-286 [20]
287-306 [1]	279	347	369	241	207	212	279	67	-129	0.6	0.7	0.39	287-306 [1]
287-306 [10]	209	468	891	680	663	621	589	230	180	1.6	1.4	0.24	287-306 [10]
287-306 [20]	322	281	351	650	397	317	386	135	-22	0.9	0.9	0.88	287-306 [20]
307-326 [1]	184	324	292	298	335	1852	548	641	139	1.5	1.3	0.43	307-326 [1]
307-326 [10]	273	909	533	490	350	851	568	260	159	1.5	1.4	0.30	307-326 [10]
307-326 [20]	238	543	336	215	300	155	298	136	-111	0.6	0.7	0.46	307-326 [20]
sAg 10	307	113	141	237	174	136	185	74	-224	0.3	0.5	0.14	sAg 10
sAg 100	173	93	169	155	245	140	163	50	-246	0.2	0.4	0.10	sAg 100
N	86	60	87	152	60	53	83	37	-325	-0.1	0.2	0.03 *	N
N	64	78	251	227	103	50	129	87	-280	0.1	0.3	0.07	N
3H	118	111	219	97	67	101	119	52	-290	0.0	0.3	0.06	3H
3H	241	102	110	87	104	129	129	57	-280	0.1	0.3	0.06	3H
3H	240	293	440	135	449	738	383	212	-26	0.9	0.9	0.86	3H
3H	263	197	197	534	742	737	445	260	37	1.1	1.1	0.81	3H
3H	105	318	289	602	397	739	408	229	0	1.0	1.0	1.00	3H
3H	137	668	434	982	482	679	564	285	155	1.5	1.4	0.32	3H
3H	587	689	294	716	2031	552	812	616	403	2.3 *	2.0	0.02 *	3H
	SMC												
N	72	119	23	31	40	20	51	38	-718	0.0	0.1	0.00 *	N
3H	592	771	649	773	578	1249	769	250	0	1.0	1.0	1.00	3H
PHA - 1	34831	387	415	61	486	817	6166	14045	5,398 *	8.5 *	8.0 *	0.37	PHA - 1
PHA - 5	17110	9263	24943	27154	29428	39750	24608	10496	23,839 *	34.2 *	32.0 *	0.00 *	PHA - 5
PHA - 10	2121	1648	1036	1159	5399	10562	3654	3747	2,886	5.0 *	4.8 *	0.09	PHA - 10
LPS - 1	290	209	357	493	317	106	295	132	-473	0.3	0.4	0.00 *	LPS - 1
LPS - 5	371	136	376	134	123	143	214	124	-555	0.2	0.3	0.00 *	LPS - 5
LPS - 10	431	193	238	225	154	146	231	105	-538	0.3	0.3	0.00 *	LPS - 10
LPS - 20	278	124	88	97	142	222	159	76	-610	0.1	0.2	0.00 *	LPS - 20
LPS - 40	376	174	115	149	109	180	184	99	-585	0.2	0.2	0.00 *	LPS - 40

Raw data for Positive control duck G531

G531	Mean	SD														
Total N	100	60														
Total 3H	191	114							CPM-3H		S.I.		P/N		t-Test	
	R1	R2	R3	R4	R5	R6	Mean	SD	>5000		>2.1		>2.1		<0.05	
1-15 [1]																1-15 [1]
1-15 [10]																1-15 [10]
1-15 [20]																1-15 [20]
7-14W-27 [1]																7-14W-27 [1]
7-14W-27 [10]																7-14W-27 [10]
7-14W-27 [20]																7-14W-27 [20]
7-14R-27 [1]																7-14R-27 [1]
7-14R-27 [10]																7-14R-27 [10]
7-14R-27 [20]																7-14R-27 [20]
22-41 [1]																22-41 [1]
22-41 [10]																22-41 [10]
22-41 [20]																22-41 [20]
37-56 [1]	30	58	79	62	161	40	72	47	-120		-0.3		0.4		0.027	* 37-56 [1]
37-56 [10]	43	32	26	29	31	24	31	7	-161		-0.8		0.2		0.004	* 37-56 [10]
37-56 [20]	118	126	176	117	82	91	118	33	-73		0.2		0.6		0.149	37-56 [20]
54-73 [1]	39	93	154	110	161		112	50	-80		0.1		0.6		0.159	54-73 [1]
54-73 [10]	44	93	129	110	124	3045	590	1203	399		5.4	*	3.1	*	0.257	54-73 [10]
54-73 [20]	93	1594	262	567	1044	1030	765	562	574		7.3	*	4.0	*	0.003	* 54-73 [20]
71-90 [1]	781	1124	314	206	191	157	462	399	270		4.0	*	2.4	*	0.040	* 71-90 [1]
71-90 [10]	216	230	2011	759	1334	60	768	770	577		7.3	*	4.0	*	0.019	* 71-90 [10]
71-90 [20]	552	224	354	423	2400	104	676	859	485		6.3	*	3.5	*	0.065	71-90 [20]
87-106 [1]	146	181	287	517	1249	558	489	409	298		4.3	*	2.6	*	0.029	* 87-106 [1]
87-106 [10]	584	273	178	778	565	287	444	233	253		3.8	*	2.3	*	0.006	* 87-106 [10]
87-106 [20]	269	117	150	76	372	149	189	110	-3		1.0		1.0		0.963	87-106 [20]
101-120 [1]	53	86	110	214	132	32	104	65	-87		0.0		0.5		0.105	101-120 [1]
101-120 [10]	57	33	35	38	40	562	127	213	-64		0.3		0.7		0.412	101-120 [10]
101-120 [20]	36	81	30	78	31	191	74	62	-117		-0.3		0.4		0.034	* 101-120 [20]
116-130 [1]	55	72	264	189	157	114	142	78	-50		0.5		0.7		0.354	116-130 [1]
116-130 [10]	162	119	322	359	63	279	217	120	26		1.3		1.1		0.661	116-130 [10]
116-130 [20]	79	112	427	614	114	106	242	224	50		1.6		1.3		0.529	116-130 [20]
126-140 [1]	573	308	260	291	245	836	419	238	227		3.5	*	2.2	*	0.013	* 126-140 [1]
126-140 [10]	256	366	220	338	210	124	252	89	61		1.7		1.3		0.271	126-140 [10]
126-140 [20]	166	146	142	245	174	78	158	54	-33		0.6		0.8		0.516	126-140 [20]
136-150 [1]	133	257	181	135	550	125	230	164	39		1.4		1.2		0.566	136-150 [1]
136-150 [10]	592	178	184	122	147	80	217	188	25		1.3		1.1		0.723	136-150 [10]
136-150 [20]	104	161	127	117	163	75	124	34	-67		0.3		0.6		0.185	136-150 [20]
146-160 [1]	226	236	379	167	268	49	221	110	29		1.3		1.2		0.613	146-160 [1]
146-160 [10]	239	1169	334	288	202	71	384	395	193		3.1	*	2.0		0.128	146-160 [10]
146-160 [20]	647	149	1446	401	369	3243	1042	1168	851		10.3	*	5.4	*	0.020	* 146-160 [20]
156-170 [1]	1227	217	166	153	258	95	353	432	161		2.8	*	1.8		0.232	156-170 [1]
156-170 [10]	238	574	248	279	563	73	329	199	138		2.5	*	1.7		0.078	156-170 [10]
156-170 [20]	93	320	167	82	103	102	144	91	-47		0.5		0.8		0.395	156-170 [20]
166-180 [1]	44	74	65	146	50	23	67	43	-125		-0.4		0.3		0.021	* 166-180 [1]
166-180 [10]	35	132	131	122	83	319	137	97	-55		0.4		0.7		0.331	166-180 [10]
166-180 [20]	40	266	107	154	77	31	113	88	-79		0.1		0.6		0.160	166-180 [20]
176-195 [1]	40	89	99	235	146	56	111	71	-81		0.1		0.6		0.136	176-195 [1]
176-195 [10]	60	84	103	209	163	79	116	58	-75		0.2		0.6		0.153	176-195 [10]
176-195 [20]	32	74	140	126	201	67	107	61	-85		0.1		0.6		0.111	176-195 [20]
191-210 [1]	197	160	180	306	369	687	316	199	125		2.4	*	1.7		0.106	191-210 [1]
191-210 [10]	84	154	231	164	400	1902	489	700	298		4.3	*	2.6	*	0.159	191-210 [10]
191-210 [20]	75	181	257	173	429	2894	668	1097	477		6.2	*	3.5	*	0.144	191-210 [20]
210-229 [1]	48	683	175	1291	804	90	515	496	324		4.5	*	2.7	*	0.042	* 210-229 [1]
210-229 [10]	1713	1414	183	221	919	120	762	692	570		7.2	*	4.0	*	0.011	* 210-229 [10]
210-229 [20]	369	275	267	150	273	98	239	98	47		1.5		1.2		0.402	210-229 [20]
229-248 [1]																229-248 [1]
229-248 [10]																229-248 [10]
229-248 [20]																229-248 [20]
248-267 [1]	741	1016	944	885	985	453	837	212	646		8.1	*	4.4	*	0.000	* 248-267 [1]
248-267 [10]	269	738	265	707	420	751	525	234	334		4.7	*	2.7	*	0.001	* 248-267 [10]
248-267 [20]	87	3775	93	291	1302		1109	1572	918		11.1	*	5.8	*	0.052	248-267 [20]
267-286 [1]	101	174	244	328	465	93	234	144	43		1.5		1.2		0.502	267-286 [1]
267-286 [10]	128	141	1378	185	209	137	363	498	172		2.9	*	1.9		0.260	267-286 [10]
267-286 [20]	161	218	190	189	181	118	176	34	-15		0.8		0.9		0.758	267-286 [20]
287-306 [1]	36	122	178	118	150	33	106	59	-85		0.1		0.6		0.109	287-306 [1]
287-306 [10]	27	105	163	125	94	50	94	49	-98		-0.1		0.5		0.065	287-306 [10]
287-306 [20]	33	56	140	99	180	34	90	60	-101		-0.1		0.5		0.061	287-306 [20]
307-326 [1]																307-326 [1]
307-326 [10]																307-326 [10]
307-326 [20]																307-326 [20]
sAg 10	70	74	45	68	35	54	58	16	-134		-0.5		0.3		0.013	* sAg 10
sAg 100	741	114	189	138	252	66	250	249	58		1.6		1.3		0.498	sAg 100
N	128	148	171	174	99	88	135	36	-57		0.4		0.7		0.258	N
N	41	47	44	28	192	43	66	62	-126		-0.4		0.3		0.024	* N
3H	133	151	208	196	175	488	225	132	34		1.4		1.2		0.581	3H
3H	40	114	289	140	247	116	158	93	-34		0.6		0.8		0.540	3H
	SMC															
N	32	36	99	88	110	46	69	34	-30		0.0		0.7		0.16	N
3H	35	106	137	121	104	91	98	35	0		1.0		1.0		1.00	3H
PHA - 1	45	218	562	317	258	111	252	181	153		6.0	*	2.5	*	0.07	PHA - 1
PHA - 5	85	361	252	181	170	110	193	101	94		4.1	*	1.9		0.06	PHA - 5
PHA - 10	70	74	256	214	72	71	126	85	27		1.9		1.3		0.49	PHA - 10
LPS - 1	39	160	175	202	179	157	152	57	53		2.7	*	1.5		0.08	LPS - 1
LPS - 5		136	149	221	302	67	175	90	76		3.5	*	1.8		0.09	LPS - 5
LPS - 10		108	154	136	148	60	121	39	22		1.7		1.2		0.35	LPS - 10
LPS - 20		73	270	153	77	51	125	90	25		1.8		1.3		0.54	LPS - 20
LPS - 40	54	35	36	37	38	56	42	10	-57		-0.9		0.4		0.00	* LPS - 40
	FBMC															
N	221	273	286	231	221	184	236	37	-193		0.0		0.6		0.00	* N
3H	391	276	462	469	465	508	429	84	0		1.0		1.0		1.00	3H
PHA - 1	809	870	1171	1005	798	927	930	141	501		3.6	*	2.2	*	0.00	* PHA - 1
PHA - 5	2779	3603	4530	5184	7115	64	3879	2385	3450		18.9	*	9.0	*	0.01	* PHA - 5
PHA - 10	6243	6705	12302	74	11293	9478	7682	4439	7254	*	38.6	*	17.9	*	0.00	* PHA - 10
LPS - 1	37	112	105	116	107	82	93	30	-335		-0.7		0.2		0.00	* LPS - 1
LPS - 5	35	175	107	111	70	96	99	47	-330		-0.7		0.2		0.00	* LPS - 5
LPS - 10	64	115	144	127	129	104	114	28	-315		-0.6		0.3		0.00	* LPS - 10
LPS - 20	56	66	130	152	109	54	94	42	-334		-0.7		0.2		0.00	* LPS - 20
LPS - 40	54	40	65	35	28	45	45	13	-384		-1.0		0.1		0.00	* LPS - 40

Raw data for Positive control duck G58

G58	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	68	43															
Total 3H	217	162								>5000		>2.1		>2.1		<0.05	
	R1	R2	R3	R4	R5	R6	Mean	SD									
1-15 [1]																	1-15 [1]
1-15 [10]																	1-15 [10]
1-15 [20]																	1-15 [20]
7-14W-27 [1]																	7-14W-27 [1]
7-14W-27 [10]																	7-14W-27 [10]
7-14W-27 [20]																	7-14W-27 [20]
7-14R-27 [1]																	7-14R-27 [1]
7-14R-27 [10]																	7-14R-27 [10]
7-14R-27 [20]																	7-14R-27 [20]
22-41 [1]																	22-41 [1]
22-41 [10]																	22-41 [10]
22-41 [20]																	22-41 [20]
37-56 [1]	38	377	160	243	132	37	165	130	-52		0.6		0.8		0.507		37-56 [1]
37-56 [10]	50	89	79	84	53	41	66	20	-151		0.0		0.3		0.040	*	37-56 [10]
37-56 [20]	43	48	145	234	541	50	177	194	-40		0.7		0.8		0.651		37-56 [20]
54-73 [1]	117	385	266	501	465	136	312	165	95		1.6		1.4		0.261		54-73 [1]
54-73 [10]	276	358	380	429	717	61	370	214	154		2.0		1.7		0.107		54-73 [10]
54-73 [20]	52	375	562	223	582	54	308	237	91		1.6		1.4		0.348		54-73 [20]
71-90 [1]	682	313	338	147	223	58	293	217	77		1.5		1.4		0.409		71-90 [1]
71-90 [10]	87	417	379	178	192	61	219	148	2		1.0		1.0		0.976		71-90 [10]
71-90 [20]	64	165	306	266	110	66	163	103	-54		0.6		0.8		0.474		71-90 [20]
87-106 [1]	69	294	457	615	500	391	387	189	171		2.2	*	1.8		0.063		87-106 [1]
87-106 [10]	250	2271	634	611	539	1210	919	732	703		5.7	*	4.2	*	0.005	*	87-106 [10]
87-106 [20]	186	1070	1072	4949	2536	245	1676	1814	1460		10.8	*	7.7	*	0.011	*	87-106 [20]
101-120 [1]	44	333	512	446	879	114	388	302	171		2.2	*	1.8		0.132		101-120 [1]
101-120 [10]	66	125	119	133	122	56	103	33	-113		0.2		0.5		0.116		101-120 [10]
101-120 [20]	47	62	498	740	728	62	356	339	140		1.9		1.6		0.247		101-120 [20]
116-130 [1]	316	287	221	430	276	56	264	123	48		1.3		1.2		0.536		116-130 [1]
116-130 [10]	186	367	410	299	399	90	292	129	75		1.5		1.3		0.340		116-130 [10]
116-130 [20]	308	201	278	552	563	135	339	179	123		1.8		1.6		0.163		116-130 [20]
126-140 [1]	37	203	254	315	225	33	178	117	-39		0.7		0.8		0.612		126-140 [1]
126-140 [10]	48	188	171	244	226	52	155	85	-62		0.6		0.7		0.398		126-140 [10]
126-140 [20]	49	163	209	324	265	73	181	107	-36		0.8		0.8		0.632		126-140 [20]
136-150 [1]	54	702	257	291	607	71	331	270	114		1.8		1.5		0.276		136-150 [1]
136-150 [10]	46	267	180	399	300	66	209	138	-7		1.0		1.0		0.927		136-150 [10]
136-150 [20]	30	202	278	175	226	49	160	100	-56		0.6		0.7		0.450		136-150 [20]
146-160 [1]	39	424	239	223	574	146	274	194	58		1.4		1.3		0.515		146-160 [1]
146-160 [10]	177	313	497	505	757	132	397	235	180		2.2	*	1.8		0.074		146-160 [10]
146-160 [20]	31	545	542	516	481	56	362	248	145		2.0		1.7		0.152		146-160 [20]
156-170 [1]	51	170	208	418	297	43	198	145	-19		0.9		0.9		0.814		156-170 [1]
156-170 [10]	41	197	264	253	342	30	188	127	-29		0.8		0.9		0.711		156-170 [10]
156-170 [20]	32	189	303	248	490	64	221	168	4		1.0		1.0		0.958		156-170 [20]
166-180 [1]	64	52	47	43	50	63	53	9	-163		-0.1		0.2		0.028	*	166-180 [1]
166-180 [10]	46	295	878	359	104	34	286	319	69		1.5		1.3		0.543		166-180 [10]
166-180 [20]	72	445	362	200	182	54	219	156	3		1.0		1.0		0.975		166-180 [20]
176-195 [1]	46	207	171	402	246	16	181	141	-35		0.8		0.8		0.658		176-195 [1]
176-195 [10]	124	230	136	220	381	339	238	104	22		1.1		1.1		0.772		176-195 [10]
176-195 [20]	70	313	166	211	404	90	209	130	-8		0.9		1.0		0.921		176-195 [20]
191-210 [1]	218	217	257	161	681	39	262	219	46		1.3		1.2		0.623		191-210 [1]
191-210 [10]	287	165	309	306	779	45	315	250	99		1.7		1.5		0.323		191-210 [10]
191-210 [20]	1103	245	317	256	885	58	477	415	261		2.8	*	2.2	*	0.070		191-210 [20]
210-229 [1]	94	112	126	348	279	108	178	108	-39		0.7		0.8		0.604		210-229 [1]
210-229 [10]	70	126	150	190	223	217	163	59	-54		0.6		0.8		0.449		210-229 [10]
210-229 [20]	55	570	264	274	320		296	184	80		1.5		1.4		0.387		210-229 [20]
229-248 [1]																	229-248 [1]
229-248 [10]																	229-248 [10]
229-248 [20]																	229-248 [20]
248-267 [1]	492	367	911	715	1172	904	760	297	544		4.7	*	3.5	*	0.000	*	248-267 [1]
248-267 [10]	885	895	350	734			716	255	499		4.4	*	3.3	*	0.000	*	248-267 [10]
248-267 [20]	138	401	18	190	463	346	259	171	43		1.3		1.2		0.612		248-267 [20]
267-286 [1]	38	220	112	273	218	52	152	98	-65		0.6		0.7		0.388		267-286 [1]
267-286 [10]	42	154	120	247	194	60	136	79	-80		0.5		0.6		0.273		267-286 [10]
267-286 [20]	42	139	163	228	151	33	126	75	-91		0.4		0.6		0.218		267-286 [20]
287-306 [1]	42	99	135	237	242	66	137	85	-80		0.5		0.6		0.280		287-306 [1]
287-306 [10]	38	140	131	371	331	102	185	134	-31		0.8		0.9		0.690		287-306 [10]
287-306 [20]	31	162	109	288	148	77	136	88	-81		0.5		0.6		0.277		287-306 [20]
307-326 [1]																	307-326 [1]
307-326 [10]																	307-326 [10]
307-326 [20]																	307-326 [20]
sAg 10	40	71	54	57	48	39	51	12	-165		-0.1		0.2		0.026	*	sAg 10
sAg 100	288	364	376	307	299	44	279	121	63		1.4		1.3		0.416		sAg 100
N	32	27	35	37	32	32	32	4	-184		-0.2		0.1		0.015	*	N
N	118	97	145	118	88	57	104	30	-113		0.2		0.5		0.116		N
3H	28	337	440	344	105	42	216	178	-1		1.0		1.0		0.994		3H
3H	87	201	483	148	327	56	217	162	1		1.0		1.0		0.994		3H
	SMC																
N	27		54	56	52	41	46	12	-221		0.0		0.2		0.04	*	N
3H	27		327	496	384	102	267	196	0		1.0		1.0		1.00		3H
PHA - 1	25		21	26	24	36	26	6	-241		-0.1		0.1		0.03	*	PHA - 1
PHA - 5	49		957	762	700	158	525	398	258		2.2	*	2.0		0.23		PHA - 5
PHA - 10	78	688	1271	942	848	169	666	462	399		2.8	*	2.5	*	0.11		PHA - 10
LPS - 1	39	158	214	168	186	85	142	66	-126		0.4		0.5		0.17		LPS - 1
LPS - 5	55	149	245	139	141	62	132	69	-135		0.4		0.5		0.15		LPS - 5
LPS - 10	62	157	154	163	103	43	113	52	-154		0.3		0.4		0.10		LPS - 10
LPS - 20	41	73	153	99	62	62	81	40	-186		0.2		0.3		0.05	*	LPS - 20
LPS - 40	26	27	25	26	27	84	36	23	-232		0.0		0.1		0.02	*	LPS - 40
	PBMC																
N		705	801	766	919	543	747	138	-205		0.0		0.8		0.24		N
3H	1597	906	734	820	1006	653	952	339	0		1.0		1.0		1.00		3H
PHA - 1	3564	3304	4386	5046	3079	2994	3729	817	2776		14.5	*	3.9	*	0.00	*	PHA - 1
PHA - 5	10689	16526	16934	17436	10951	8488	13504	3898	12552	*	62.1	*	14.2	*	0.00	*	PHA - 5
PHA - 10	21681	26993	21861	20782	20262	10877	20409	5256	19457	*	95.7	*	21.4	*	0.00	*	PHA - 10
LPS - 1	435	846	1018	914	639	215	678	308	-275		-0.3		0.7		0.17		LPS - 1
LPS - 5	440	781	812	645	647	379	617	176	-335		-0.6		0.6		0.06		LPS - 5
LPS - 10	842	895	601	659	451	284	622	231	-331		-0.6		0.7		0.08		LPS - 10
LPS - 20	390	675	502	577	435	492	512	102	-441		-1.1		0.5		0.01	*	LPS - 20
LPS - 40	462	432	508	402	309	290	401	86	-552		-1.7		0.4		0.00	*	LPS - 40

Raw data for Positive control duck P631

P631	Mean	SD							CPM-3H	S.I.	P/N	t-Test	
Total N	294	111											
Total 3H	382	347							>5000	>2.1	>2.1	<0.05	
	R1	R2	R3	R4	R5	R6	Mean	SD					
1-15 [1]													1-15 [1]
1-15 [10]													1-15 [10]
1-15 [20]													1-15 [20]
7-14W-27 [1]													7-14W-27 [1]
7-14W-27 [10]													7-14W-27 [10]
7-14W-27 [20]													7-14W-27 [20]
7-14R-27 [1]													7-14R-27 [1]
7-14R-27 [10]													7-14R-27 [10]
7-14R-27 [20]													7-14R-27 [20]
22-41 [1]													22-41 [1]
22-41 [10]													22-41 [10]
22-41 [20]													22-41 [20]
37-56 [1]	295	272	233	317	360	240	286	48	-96	-0.1	0.7	0.515	37-56 [1]
37-56 [10]	234	213	187	238	281	341	249	55	-134	-0.5	0.7	0.370	37-56 [10]
37-56 [20]	436			308	448	448	410	68	28	1.3	1.1	0.879	37-56 [20]
54-73 [1]	534	294		374	320	414	387	94	5	1.1	1.0	0.976	54-73 [1]
54-73 [10]	295	791	263	393	206	406	392	210	10	1.1	1.0	0.950	54-73 [10]
54-73 [20]	376	347	363	362	388	405	373	21	-9	0.9	1.0	0.951	54-73 [20]
71-90 [1]	419	249	595	311	308	179	344	146	-39	0.6	0.9	0.799	71-90 [1]
71-90 [10]	210	370	427	260	320	278	311	79	-71	0.2	0.8	0.631	71-90 [10]
71-90 [20]	308	242	246	220	188	229	239	40	-144	-0.6	0.6	0.335	71-90 [20]
87-106 [1]	509	335	593	298	303	475	419	124	36	1.4	1.1	0.810	87-106 [1]
87-106 [10]	1251	369	362	205	523	523	539	369	157	2.8 *	1.4	0.390	87-106 [10]
87-106 [20]	569	344	395	383	271	471	406	103	23	1.3	1.1	0.876	87-106 [20]
101-120 [1]	575	540	248	290	624	476	459	155	77	1.9	1.2	0.617	101-120 [1]
101-120 [10]	485	275	427	469	327	298	380	91	-2	1.0	1.0	0.989	101-120 [10]
101-120 [20]	243	521	352	494	325	346	380	107	-2	1.0	1.0	0.989	101-120 [20]
116-130 [1]	462		372	360	382	100	335	137	-47	0.5	0.9	0.776	116-130 [1]
116-130 [10]		382	174	194	364	270	277	95	-106	-0.2	0.7	0.521	116-130 [10]
116-130 [20]	456	430	386	202	448	294	369	101	-13	0.9	1.0	0.931	116-130 [20]
126-140 [1]	352	13	406	248	256	341	269	139	-113	-0.3	0.7	0.460	126-140 [1]
126-140 [10]	259	222	623	329	289	303	337	145	-45	0.5	0.9	0.768	126-140 [10]
126-140 [20]	291	200	343	231	385	258	284	69	-98	-0.1	0.7	0.510	126-140 [20]
136-150 [1]	216	206	218	182	284	356	244	65	-139	-0.6	0.6	0.354	136-150 [1]
136-150 [10]	116	284	246	234	366		249	91	-133	-0.5	0.7	0.419	136-150 [10]
136-150 [20]	314	348	222	444	276	382	331	79	-51	0.4	0.9	0.729	136-150 [20]
146-160 [1]	297	350	271	379	373	458	354	66	-28	0.7	0.9	0.850	146-160 [1]
146-160 [10]	311	405	319	409	394	427	377	50	-5	0.9	1.0	0.973	146-160 [10]
146-160 [20]	374	630	420	460	491	480	475	87	93	2.1	1.2	0.533	146-160 [20]
156-170 [1]	656	318	168	352	216	278	331	173	-51	0.4	0.9	0.741	156-170 [1]
156-170 [10]	452	480	94	338	212	472	341	159	-41	0.5	0.9	0.789	156-170 [10]
156-170 [20]		240		466	166	22	224	185	-159	-0.8	0.6	0.404	156-170 [20]
166-180 [1]	206	49	235	411	351	256	251	126	-131	-0.5	0.7	0.389	166-180 [1]
166-180 [10]	430	313	251	331	262	251	306	69	-76	0.1	0.8	0.609	166-180 [10]
166-180 [20]	441	323	269	303	399	319	342	65	-40	0.5	0.9	0.786	166-180 [20]
176-195 [1]	431	272	290	300	409	325	338	66	-45	0.5	0.9	0.762	176-195 [1]
176-195 [10]	336	304	259	328	340	239	301	42	-82	0.1	0.8	0.580	176-195 [10]
176-195 [20]	373	272	244	410	283	269	308	66	-74	0.2	0.8	0.618	176-195 [20]
191-210 [1]	384	381	344	384	284		355	43	-27	0.7	0.9	0.867	191-210 [1]
191-210 [10]	612	381	420	674	2273		872	793	490	6.6 *	2.3 *	0.089	191-210 [10]
191-210 [20]	1269	374	429	376	245		539	414	156	2.8 *	1.4	0.435	191-210 [20]
210-229 [1]	208	309	327	657	253	582	389	185	7	1.1	1.0	0.964	210-229 [1]
210-229 [10]	179	320	299	75	142	692	284	220	-98	-0.1	0.7	0.541	210-229 [10]
210-229 [20]	494	247	63	214	194	586	299	198	-83	0.1	0.8	0.598	210-229 [20]
229-248 [1]													229-248 [1]
229-248 [10]													229-248 [10]
229-248 [20]													229-248 [20]
248-267 [1]	176	796	448	1175	231	278	517	393	135	2.5 *	1.4	0.468	248-267 [1]
248-267 [10]	617	526	636	1084	882	1125	811	256	429	5.9 *	2.1 *	0.017 *	248-267 [10]
248-267 [20]	87	351	2279	78	84	61	490	883	108	2.2 *	1.3	0.711	248-267 [20]
267-286 [1]	309	250	252	292	336	266	284	34	-98	-0.1	0.7	0.506	267-286 [1]
267-286 [10]	436	531	247	318	219	240	332	126	-50	0.4	0.9	0.738	267-286 [10]
267-286 [20]	304	99	157	154	776	349	306	249	-76	0.1	0.8	0.641	267-286 [20]
287-306 [1]	494	346	250	362	416		374	90	-9	0.9	1.0	0.957	287-306 [1]
287-306 [10]	396	310	400	388	294	206	332	77	-50	0.4	0.9	0.736	287-306 [10]
287-306 [20]	106	270	228	464	318	350	289	121	-93	-0.1	0.8	0.539	287-306 [20]
307-326 [1]													307-326 [1]
307-326 [10]													307-326 [10]
307-326 [20]													307-326 [20]
sAg 10	136	274	248				219	73	-163	-0.9	0.6	0.445	sAg 10
sAg 100	324	192	104	152	290	224	214	83	-168	-0.9	0.6	0.266	sAg 100
N	442	312	370	218	180	157	280	114	-103	-0.2	0.7	0.496	N
N	196	374	482	287	224		312	117	-70	0.2	0.8	0.671	N
3H	256	1469	186	243	274	199	438	506	56	1.6	1.1	0.787	3H
3H	356	338	357	268	365	277	327	43	-56	0.4	0.9	0.706	3H
	SMC												
N	32		38	56	63	65	51	15	-12	0.0	0.8	0.25	N
3H	68	60	45	80	41	86	63	18	0	1.0	1.0	1.00	3H
PHA - 1	33	65	73	68	76	100	69	21	6	1.5	1.1	0.62	PHA - 1
PHA - 5		376	331	686	277	475	429	161	366	30.4 *	6.8 *	0.00 *	PHA - 5
PHA - 10		145	143	480	334	269	274	141	211	18.0 *	4.3 *	0.01 *	PHA - 10
LPS - 1	63	45	28	49	117	356	110	124	46	4.7 *	1.7	0.39	LPS - 1
LPS - 5	24	96	27	33	41	134	59	45	-4	0.7	0.9	0.85	LPS - 5
LPS - 10	27	40	33	66	46	74	48	19	-16	-0.3	0.8	0.17	LPS - 10
LPS - 20	31	42	29	33	45	53	39	9	-24	-1.0	0.6	0.01 *	LPS - 20
LPS - 40	27	28	24	43		52	35	12	-28	-1.3	0.6	0.01 *	LPS - 40
	FBMC												
N	95	65	88	68	106	64	81	18	-44	0.0	0.6	0.00 *	N
3H	92	131	143	139	124	124	125	18	0	1.0	1.0	1.00	3H
PHA - 1	167	309	111	42	320	79	171	118	46	2.0	1.4	0.37	PHA - 1
PHA - 5	181	337	173	114	107	146	176	84	51	2.2 *	1.4	0.18	PHA - 5
PHA - 10	165	116	194	95	134	9	119	64	-6	0.9	0.9	0.82	PHA - 10
LPS - 1	86	91	176	31	22	20	71	60	-54	-0.2	0.6	0.06	LPS - 1
LPS - 5	118	86	142	107	31	34	86	45	-39	0.1	0.7	0.08	LPS - 5
LPS - 10	81	69	29	24	26	24	42	26	-83	-0.9	0.3	0.00 *	LPS - 10
LPS - 20	27	130	55	40	26	31	52	40	-74	-0.7	0.4	0.00 *	LPS - 20
LPS - 40	86	91	45	37	41	31	55	26	-70	-0.6	0.4	0.00 *	LPS - 40

Raw data for Positive control duck G631

G631	Mean	SD												
Total N	381	344												
Total 3H	597	240							CPM-3H	S.I.	P/N	t-Test		
	R1	R2	R3	R4	R5	R6	Mean	SD	>5000	>2.1	>2.1	<0.05		
1-15 [1]														1-15 [1]
1-15 [10]														1-15 [10]
1-15 [20]														1-15 [20]
7-14W-27 [1]														7-14W-27 [1]
7-14W-27 [10]														7-14W-27 [10]
7-14W-27 [20]														7-14W-27 [20]
7-14R-27 [1]														7-14R-27 [1]
7-14R-27 [10]														7-14R-27 [10]
7-14R-27 [20]														7-14R-27 [20]
22-41 [1]														22-41 [1]
22-41 [10]														22-41 [10]
22-41 [20]														22-41 [20]
37-56 [1]	71	483	318	679	606	147	384	247	-213	0.0	0.6	0.098		37-56 [1]
37-56 [10]	102	277	808	283	572	112	359	278	-238	-0.1	0.6	0.078		37-56 [10]
37-56 [20]		188			124		156	45	-441	-1.0	0.3	0.027	*	37-56 [20]
54-73 [1]	43	38	29	43	41	32	38	6	-559	-1.6	0.1	0.000	*	54-73 [1]
54-73 [10]	753	506	304	501	300	98	410	226	-186	0.1	0.7	0.133		54-73 [10]
54-73 [20]	493	703	539	236	128	70	361	253	-235	-0.1	0.6	0.072		54-73 [20]
71-90 [1]	307	583	502	287	526	1455	610	431	13	1.1	1.0	0.934		71-90 [1]
71-90 [10]	341	306	895	883	2135	2203	1127	846	530	3.5 *	1.9	0.055		71-90 [10]
71-90 [20]	382	550	754	832	1154	1384	843	373	246	2.1 *	1.4	0.107		71-90 [20]
87-106 [1]	1208	617	245	201	406	635	552	369	-45	0.8	0.9	0.757		87-106 [1]
87-106 [10]	1108	456	344	1638	913	935	899	468	302	2.4 *	1.5	0.085		87-106 [10]
87-106 [20]	1433	810	822	577	722	969	889	296	292	2.4 *	1.5	0.038	*	87-106 [20]
101-120 [1]	357	668	968	1057	315	780	691	307	94	1.4	1.2	0.484		101-120 [1]
101-120 [10]	77	408	906	1238	1677	294	767	616	170	1.8	1.3	0.405		101-120 [10]
101-120 [20]	43	40	62	50	43	51	48	8	-549	-1.5	0.1	0.000	*	101-120 [20]
116-130 [1]		300	334	198	442	80	271	138	-326	-0.5	0.5	0.013	*	116-130 [1]
116-130 [10]		230	266	1262	430	74	452	470	-144	0.3	0.8	0.407		116-130 [10]
116-130 [20]		518	984	1084	654		810	268	213	2.0	1.4	0.155		116-130 [20]
126-140 [1]	774	439	478	480	839	1381	732	360	135	1.6	1.2	0.354		126-140 [1]
126-140 [10]	1137	668	383	459	1769	14	738	626	142	1.7	1.2	0.492		126-140 [10]
126-140 [20]	547	1289	518	217	584	1284	740	443	143	1.7	1.2	0.381		126-140 [20]
136-150 [1]	1179	911	1050	853	1644	2157	1299	506	702	4.3 *	2.2 *	0.001	*	136-150 [1]
136-150 [10]	1175	2271	2305	922	805	2560	1673	788	1076	6.0 *	2.8 *	0.000	*	136-150 [10]
136-150 [20]	507	765	1373	1222	1587	1791	1207	490	611	3.8 *	2.0	0.002	*	136-150 [20]
146-160 [1]	272	630	503	959	520	97	497	298	-100	0.5	0.8	0.451		146-160 [1]
146-160 [10]	197	1748	1727	2740	1202	351	1328	957	731	4.4 *	2.2 *	0.021	*	146-160 [10]
146-160 [20]	48	796	886	2094	1324	971	1020	672	423	3.0 *	1.7	0.064		146-160 [20]
156-170 [1]	207		976	454	1152	515	661	391	64	1.3	1.1	0.682		156-170 [1]
156-170 [10]	201	1094	530	675	1691	41	705	608	108	1.5	1.2	0.589		156-170 [10]
156-170 [20]	487	848	489	805	120	2442	865	816	268	2.2 *	1.4	0.297		156-170 [20]
166-180 [1]	65	43	51	50	42	68	53	11	-544	-1.5	0.1	0.000	*	166-180 [1]
166-180 [10]	53	994	961	557	1059	81	617	461	20	1.1	1.0	0.901		166-180 [10]
166-180 [20]	380	557	1154	704	370	1842	834	571	237	2.1 *	1.4	0.225		166-180 [20]
176-195 [1]	84	541	261	260	961	1255	560	458	-36	0.8	0.9	0.825		176-195 [1]
176-195 [10]	385	709	826	1359	1334	1328	990	410	393	2.8 *	1.7	0.020	*	176-195 [10]
176-195 [20]	70	1053	452	645	1279	228	621	470	24	1.1	1.0	0.885		176-195 [20]
191-210 [1]	1436	775	170	719	1654	1217	995	545	398	2.8 *	1.7	0.044	*	191-210 [1]
191-210 [10]	485	554	848	484	1047	1233	775	319	178	1.8	1.3	0.200		191-210 [10]
191-210 [20]	648	956	983	298	1456	2207	1091	669	494	3.3 *	1.8	0.033	*	191-210 [20]
210-229 [1]	145	771	1038	1050	498	69	595	430	-2	1.0	1.0	0.992		210-229 [1]
210-229 [10]	677	1656	945	701	523	113	769	514	172	1.8	1.3	0.338		210-229 [10]
210-229 [20]	1093	1194	331	640	315	412	664	390	67	1.3	1.1	0.654		210-229 [20]
229-248 [1]														229-248 [1]
229-248 [10]														229-248 [10]
229-248 [20]														229-248 [20]
248-267 [1]	67	65	226	169	137	935	267	333	-330	-0.5	0.4	0.028	*	248-267 [1]
248-267 [10]	118	118	177	886			325	375	-272	-0.3	0.5	0.108		248-267 [10]
248-267 [20]	98	51	70	1374	1085	41	453	609	-144	0.3	0.8	0.476		248-267 [20]
267-286 [1]	135	1130	434	308	265	1345	603	505	6	1.0	1.0	0.973		267-286 [1]
267-286 [10]	297	695	539	492	445	2093	760	666	163	1.8	1.3	0.450		267-286 [10]
267-286 [20]	72	536	1268	1467	978	759	847	507	250	2.2 *	1.4	0.168		267-286 [20]
287-306 [1]		450	454	982	498	262	529	269	-68	0.7	0.9	0.616		287-306 [1]
287-306 [10]		494	1460	510	892	204	712	484	115	1.5	1.2	0.514		287-306 [10]
287-306 [20]		964	794	734	832	108	686	334	90	1.4	1.2	0.540		287-306 [20]
307-326 [1]														307-326 [1]
307-326 [10]														307-326 [10]
307-326 [20]														307-326 [20]
sAg 10	105	208	520	2155	1091	175	709	797	112	1.5	1.2	0.652		sAg 10
sAg 100	607	1244	1420	1250	709	150	897	489	300	2.4 *	1.5	0.095		sAg 100
N	54	65	585	265	520	43	255	245	-342	-0.6	0.4	0.012	*	N
N	219	280	1143	532	798	75	508	403	-89	0.6	0.9	0.562		N
3H	309	685	543	480	349	358	454	144	-143	0.3	0.8	0.202		3H
3H	693	621	1192	639	780	512	740	238	143	1.7	1.2	0.250		3H
	SMC													
N	60	196	264	217	230	139	184	73	-140	0.0	0.6	0.05		N
3H	106	427	447	314	420	228	324	135	0	1.0	1.0	1.00		3H
PHA - 1	52	274	269	333	260	220	235	97	-89	0.4	0.7	0.22		PHA - 1
PHA - 5	83	800	764	918	563	405	589	308	265	2.9 *	1.8	0.08		PHA - 5
PHA - 10	185	1126	852	1003	1007	381	759	384	435	4.1 *	2.3 *	0.03	*	PHA - 10
LPS - 1	43	339	346	222	296	171	236	117	-88	0.4	0.7	0.26		LPS - 1
LPS - 5	61	376	400	330	349	150	278	138	-46	0.7	0.9	0.57		LPS - 5
LPS - 10	91	430	459	456	367	127	322	168	-2	1.0	1.0	0.98		LPS - 10
LPS - 20	70	317	379	264	334	110	246	127	-78	0.4	0.8	0.33		LPS - 20
LPS - 40	61	76	134	114	73	87	91	28	-233	-0.7	0.3	0.00	*	LPS - 40

Raw data for Positive control duck G72

G72	Mean	SD											
Total N	55	35											
Total 3H	247	422							CPM-3H	S.I.	P/N	t-Test	
	R1	R2	R3	R4	R5	R6	Mean	SD	>5000	>2.1	>2.1	<0.05	
1-15 [1]													1-15 [1]
1-15 [10]													1-15 [10]
1-15 [20]													1-15 [20]
7-14W-27 [1]													7-14W-27 [1]
7-14W-27 [10]													7-14W-27 [10]
7-14W-27 [20]													7-14W-27 [20]
7-14R-27 [1]													7-14R-27 [1]
7-14R-27 [10]													7-14R-27 [10]
7-14R-27 [20]													7-14R-27 [20]
22-41 [1]													22-41 [1]
22-41 [10]													22-41 [10]
22-41 [20]													22-41 [20]
37-56 [1]	56	45	53	80	83	42	60	18	-187	0.0	0.2	0.302	37-56 [1]
37-56 [10]	72	41	35	36	47	47	46	14	-201	0.0	0.2	0.269	37-56 [10]
37-56 [20]	33	33	35	34	33	35	34	1	-213	-0.1	0.1	0.242	37-56 [20]
54-73 [1]	48	68	238	50	300		141	119	-106	0.4	0.6	0.596	54-73 [1]
54-73 [10]	60	86	84	386	122		148	135	-99	0.5	0.6	0.621	54-73 [10]
54-73 [20]	74	78	136	298	132		144	91	-103	0.5	0.6	0.603	54-73 [20]
71-90 [1]	46	150	165	115	259	278	169	88	-78	0.6	0.7	0.665	71-90 [1]
71-90 [10]	41	167	109	121	250	138	138	69	-109	0.4	0.6	0.544	71-90 [10]
71-90 [20]	39	44	83	69	206	96	89	61	-157	0.2	0.4	0.384	71-90 [20]
87-106 [1]	46	69	90	82	240	63	98	71	-148	0.2	0.4	0.412	87-106 [1]
87-106 [10]	73	58	83	83	116	55	78	22	-169	0.1	0.3	0.349	87-106 [10]
87-106 [20]	43	114	75	75	180	41	88	52	-159	0.2	0.4	0.379	87-106 [20]
101-120 [1]	37	31	141	121	68	32	72	48	-175	0.1	0.3	0.333	101-120 [1]
101-120 [10]	92	56	56	51	52	59	61	15	-186	0.0	0.2	0.304	101-120 [10]
101-120 [20]	40	34		54	40		42	8	-205	-0.1	0.2	0.359	101-120 [20]
116-130 [1]	47	161	352	77	87	42	128	118	-119	0.4	0.5	0.514	116-130 [1]
116-130 [10]	41	114	121	104	334	50	127	107	-119	0.4	0.5	0.511	116-130 [10]
116-130 [20]	42	70	71	285	113	86	111	88	-135	0.3	0.5	0.455	116-130 [20]
126-140 [1]	45	65	141	92	111	29	80	42	-166	0.1	0.3	0.357	126-140 [1]
126-140 [10]	49	58	69	240	145	31	99	80	-148	0.2	0.4	0.414	126-140 [10]
126-140 [20]	59	132	90	102	85	29	83	35	-164	0.1	0.3	0.364	126-140 [20]
136-150 [1]	58	47	245	137	136	434	176	145	-70	0.6	0.7	0.701	136-150 [1]
136-150 [10]	153	96	116	104	215	231	152	58	-94	0.5	0.6	0.599	136-150 [10]
136-150 [20]	197	73	6352	156	197	262	1206	2522	959	6.0 *	4.9 *	0.205	136-150 [20]
146-160 [1]	48	58	142	120	402		154	144	-93	0.5	0.6	0.644	146-160 [1]
146-160 [10]			658	1310	334		767	497	521	3.7 *	3.1 *	0.086	146-160 [10]
146-160 [20]	48	1644	424	560	1940		923	822	677	4.5 *	3.7 *	0.038 *	146-160 [20]
156-170 [1]	63	109	184	72	104	198	122	57	-125	0.3	0.5	0.487	156-170 [1]
156-170 [10]	91	118	116	135	128	98	114	17	-132	0.3	0.5	0.461	156-170 [10]
156-170 [20]	152	131	170	147	477	248	221	132	-26	0.9	0.9	0.886	156-170 [20]
166-180 [1]	46	114	85	185	61	41	88	55	-158	0.2	0.4	0.381	166-180 [1]
166-180 [10]	47	96	1434	209	105	35	321	549	74	1.4	1.3	0.754	166-180 [10]
166-180 [20]	57	99	126	165	113	49	101	44	-145	0.2	0.4	0.420	166-180 [20]
176-195 [1]	57	168	76	87	167	40	99	55	-148	0.2	0.4	0.412	176-195 [1]
176-195 [10]	188	127	529	94	196	53	198	171	-49	0.7	0.8	0.791	176-195 [10]
176-195 [20]	119	107	74	82	92	72	91	19	-156	0.2	0.4	0.387	176-195 [20]
191-210 [1]	52	125	133	189	211	49	126	67	-120	0.4	0.5	0.504	191-210 [1]
191-210 [10]	43	195	121	432	168	145	184	132	-63	0.7	0.7	0.730	191-210 [10]
191-210 [20]	35	113	102	440	306	76	179	159	-68	0.6	0.7	0.712	191-210 [20]
210-229 [1]	45	37	47	48	62	66	51	11	-196	0.0	0.2	0.280	210-229 [1]
210-229 [10]	36	176	94	234		39	116	87	-131	0.3	0.5	0.509	210-229 [10]
210-229 [20]	54	71	90	87	69	43	69	18	-178	0.1	0.3	0.325	210-229 [20]
229-248 [1]													229-248 [1]
229-248 [10]													229-248 [10]
229-248 [20]													229-248 [20]
248-267 [1]													248-267 [1]
248-267 [10]													248-267 [10]
248-267 [20]													248-267 [20]
267-286 [1]	36	50	67	75	102	25	59	28	-187	0.0	0.2	0.300	267-286 [1]
267-286 [10]	41	74	107	154	186	68	105	55	-142	0.3	0.4	0.432	267-286 [10]
267-286 [20]	30	85	77	108	65	30	66	31	-181	0.1	0.3	0.317	267-286 [20]
287-306 [1]	48	68	79	69	84	36	64	18	-183	0.0	0.3	0.312	287-306 [1]
287-306 [10]	41	85	208	74	236	46	115	85	-132	0.3	0.5	0.466	287-306 [10]
287-306 [20]	43	73	56	65	251	40	88	81	-159	0.2	0.4	0.381	287-306 [20]
307-326 [1]													307-326 [1]
307-326 [10]													307-326 [10]
307-326 [20]													307-326 [20]
sAg 10	41	108	93	99	922	95	226	342	-20	0.9	0.9	0.920	sAg 10
sAg 100	48	209	93	66	162	133	118	61	-128	0.3	0.5	0.476	sAg 100
N	125	31	30	21	35	27	45	40	-202	-0.1	0.2	0.266	N
N	45	55	58	62	53	123	66	28	-181	0.1	0.3	0.317	N
3H	95	166	267	136	1568	60	382	585	135	1.7	1.5	0.580	3H
3H	38	65	222	170	130	45	112	75	-135	0.3	0.5	0.454	3H
	SMC												
N	61	87	95	116	79	58	82	22	-40	0.0	0.7	0.04 *	N
3H	57	133	120	156	137	132	123	34	0	1.0	1.0	1.00	3H
PHA - 1	60	259	231	325	246	121	207	98	84	3.1 *	1.7	0.07	PHA - 1
PHA - 5	56	1694	1809	1668	1096	486	1135	726	1012	26.2 *	9.3 *	0.01 *	PHA - 5
PHA - 10	112	704	1155	1493	1601	745	968	559	846	22.0 *	7.9 *	0.00 *	PHA - 10
LPS - 1	35	161	176	174	123	106	129	54	6	1.2	1.1	0.81	LPS - 1
LPS - 5	56	224	218	182	221	181	180	64	57	2.4 *	1.5	0.08	LPS - 5
LPS - 10	156	248	233	289	213	122	210	62	87	3.2 *	1.7	0.01 *	LPS - 10
LPS - 20	92	193	178	210	192	107	162	50	39	2.0	1.3	0.14	LPS - 20
LPS - 40	49	101	149	160	82	62	101	46	-22	0.4	0.8	0.36	LPS - 40

Raw data for Positive control duck G89

G89	Mean	SD							CPM-3H	S.I.	P/N	t-Test	
Total N	146	164											
Total 3H	160	125											
	R1	R2	R3	R4	R5	R6	Mean	SD	>5000	>2.1	>2.1	<0.05	
1-15 [1]													1-15 [1]
1-15 [10]													1-15 [10]
1-15 [20]													1-15 [20]
7-14W-27 [1]													7-14W-27 [1]
7-14W-27 [10]													7-14W-27 [10]
7-14W-27 [20]													7-14W-27 [20]
7-14R-27 [1]													7-14R-27 [1]
7-14R-27 [10]													7-14R-27 [10]
7-14R-27 [20]													7-14R-27 [20]
22-41 [1]													22-41 [1]
22-41 [10]													22-41 [10]
22-41 [20]													22-41 [20]
37-56 [1]	70	73	62	80	95	47	71	16	-89	-5.3	0.4	0.107	37-56 [1]
37-56 [10]	110	44	44	250	33	50	89	84	-71	-4.1	0.6	0.231	37-56 [10]
37-56 [20]	232	413	125	132	156	150	201	111	41	3.9 *	1.3	0.510	37-56 [20]
54-73 [1]	37	70	66	58	83	43	59	17	-101	-6.2	0.4	0.072	54-73 [1]
54-73 [10]	42	60	60	60	404	39	111	144	-49	-2.5	0.7	0.470	54-73 [10]
54-73 [20]	39	56	101	70	44	37	58	24	-102	-6.3	0.4	0.069	54-73 [20]
71-90 [1]	111	922	79	65	224	87	248	335	88	7.2 *	1.5	0.442	71-90 [1]
71-90 [10]	176	100	103	135	323	74	152	91	-8	0.4	0.9	0.890	71-90 [10]
71-90 [20]	77	155	427	162	130	58	168	133	8	1.6	1.1	0.900	71-90 [20]
87-106 [1]	53	61	67	78	1218	324	300	462	140	11.0 *	1.9	0.349	87-106 [1]
87-106 [10]	36	66	74	49	293	61	96	97	-64	-3.5	0.6	0.297	87-106 [10]
87-106 [20]	42	43	62	85	160	87	79	44	-81	-4.7	0.5	0.152	87-106 [20]
101-120 [1]	49	62	85	141	278	71	114	86	-46	-2.3	0.7	0.439	101-120 [1]
101-120 [10]	59	37	89	61	85	1066	233	409	73	6.2 *	1.5	0.586	101-120 [10]
101-120 [20]	24	25	54	33	34	33	34	11	-126	-8.0	0.2	0.028 *	101-120 [20]
116-130 [1]	42	113	189	72		90	101	56	-59	-3.2	0.6	0.336	116-130 [1]
116-130 [10]	54	75	79	78	396	125	134	130	-26	-0.8	0.8	0.695	116-130 [10]
116-130 [20]	141	100	108	64	498	135	174	161	14	2.0	1.1	0.842	116-130 [20]
126-140 [1]	252	322	58	86	110		166	115	6	1.4	1.0	0.934	126-140 [1]
126-140 [10]	85	101	87	78	70	312	122	94	-38	-1.7	0.8	0.528	126-140 [10]
126-140 [20]	99	73	76	109	415	69	140	135	-20	-0.4	0.9	0.764	126-140 [20]
136-150 [1]	80	64	81	242	129	1299	316	486	156	12.1 *	2.0	0.321	136-150 [1]
136-150 [10]	72	52	177	164	81	940	247	343	87	7.2 *	1.5	0.451	136-150 [10]
136-150 [20]	1095	142	66	51	79	210	274	407	114	9.1 *	1.7	0.396	136-150 [20]
146-160 [1]	51	54	75	73	105	580	156	208	-4	0.7	1.0	0.962	146-160 [1]
146-160 [10]	73	787	1496	1087	496	199	690	544	530	38.7 *	4.3 *	0.006 *	146-160 [10]
146-160 [20]	51	358	472	383	181	1302	458	441	298	22.2 *	2.9 *	0.049 *	146-160 [20]
156-170 [1]	137	78	175	187	124	138	140	39	-20	-0.4	0.9	0.706	156-170 [1]
156-170 [10]	96	76	82	517	137	123	172	171	12	1.8	1.1	0.871	156-170 [10]
156-170 [20]	70	118	111	288	139	562	214	186	54	4.9 *	1.3	0.480	156-170 [20]
166-180 [1]	38	40	43	28	36	29	36	6	-124	-7.8	0.2	0.030 *	166-180 [1]
166-180 [10]	83	45	51	47	60	28	52	18	-108	-6.7	0.3	0.056	166-180 [10]
166-180 [20]	53	142	67	49	77	38	71	37	-89	-5.3	0.4	0.112	166-180 [20]
176-195 [1]	87	91	128	49	97	46	83	31	-77	-4.5	0.5	0.163	176-195 [1]
176-195 [10]	74	85	61	79	61	100	76	15	-84	-4.9	0.5	0.128	176-195 [10]
176-195 [20]	55	108	48	64	79	102	76	25	-84	-5.0	0.5	0.128	176-195 [20]
191-210 [1]	60	109	125	83	319	93	131	95	-29	-1.0	0.8	0.632	191-210 [1]
191-210 [10]	714	350	167	2010	416	127	630	707	470	34.5 *	3.9 *	0.044 *	191-210 [10]
191-210 [20]	565	501	216	815	872	623	599	236	439	32.2 *	3.7 *	0.000 *	191-210 [20]
210-229 [1]	28	33	37	53	33	39	37	8	-123	-7.8	0.2	0.031 *	210-229 [1]
210-229 [10]	33	59	89	90	117	679	178	247	18	2.3 *	1.1	0.845	210-229 [10]
210-229 [20]	54	69		62	99	1594	375	681	215	16.3 *	2.3 *	0.310	210-229 [20]
229-248 [1]													229-248 [1]
229-248 [10]													229-248 [10]
229-248 [20]													229-248 [20]
248-267 [1]													248-267 [1]
248-267 [10]													248-267 [10]
248-267 [20]													248-267 [20]
267-286 [1]	67	63	88	63	99	54	72	17	-88	-5.3	0.5	0.112	267-286 [1]
267-286 [10]	78	61	57	81	109	42	71	23	-89	-5.3	0.4	0.109	267-286 [10]
267-286 [20]	56	105	61	78	76	40	69	22	-91	-5.5	0.4	0.102	267-286 [20]
287-306 [1]	44	49	77	71	127	55	70	31	-90	-5.4	0.4	0.109	287-306 [1]
287-306 [10]	44	65	84	176	141	194	117	62	-43	-2.0	0.7	0.449	287-306 [10]
287-306 [20]	44	124	81	52	393	54	124	135	-36	-1.5	0.8	0.593	287-306 [20]
307-326 [1]													307-326 [1]
307-326 [10]													307-326 [10]
307-326 [20]													307-326 [20]
sAg 10	59	86	145	111	79	103	97	30	-63	-3.5	0.6	0.248	sAg 10
sAg 100	50	95	148	646	135	64	190	227	29	3.1 *	1.2	0.731	sAg 100
N	64	57	158	88	48	46	77	42	-83	-4.9	0.5	0.137	N
N	66	331	570	133		47	229	221	69	5.9 *	1.4	0.431	N
3H	56	83	86	314	80	43	110	101	-50	-2.6	0.7	0.415	3H
3H	67		116	405	274	238	220	134	60	5.3 *	1.4	0.398	3H
	SMC												
N	129	211	313	263	403	346	277	99	-60	0.0	0.8	0.38	N
3H	129	393	356	306	516	326	338	126	0	1.0	1.0	1.00	3H
PHA - 1	91	252	326	243	472	338	287	127	-51	0.2	0.8	0.50	PHA - 1
PHA - 5	91	2557	2639	2666	2312	1843	2018	992	1680	29.0 *	6.0 *	0.00 *	PHA - 5
PHA - 10	708	3905	4980	4136	3503	2239	3245	1533	2907	49.4 *	9.6 *	0.00 *	PHA - 10
LPS - 1	107	280	318	514	266	261	291	131	-47	0.2	0.9	0.54	LPS - 1
LPS -.5	67	323	298	385	371	371	303	120	-35	0.4	0.9	0.64	LPS - 5
LPS - 10	82	256	227	380	335	224	251	104	-87	-0.4	0.7	0.22	LPS - 10
LPS - 20	70	212	199	233	251	254	203	68	-135	-1.2	0.6	0.04 *	LPS - 20
LPS - 40	52	124	127	188	204	119	136	55	-202	-2.4	0.4	0.00 *	LPS - 40

Raw data for Positive control duck W105

W105	Mean	SD
Total N	48	26
Total 3H	142	96

	R1	R2	R3	R4	R5	R6	Mean	SD	CPM-3H >5000	S.I. >2.1	P/N >2.1	t-Test <0.05	
1-15 [1]	48	64	55	108	126	33	72	37	-70	0.3	0.5	0.110	1-15 [1]
1-15 [10]	79	85	82	94	100	69	85	11	-57	0.4	0.6	0.172	1-15 [10]
1-15 [20]	158	204	126	71	111	87	126	49	-16	0.8	0.9	0.711	1-15 [20]
7-14W-27 [1]	194	60	82	38	142	83	100	58	-42	0.5	0.7	0.341	7-14W-27 [1]
7-14W-27 [10]	94	112	135	109	135	173	126	28	-16	0.8	0.9	0.704	7-14W-27 [10]
7-14W-27 [20]	130	91	97	109	68	73	95	23	-47	0.5	0.7	0.258	7-14W-27 [20]
7-14R-27 [1]	49	167	362	63	36	44	120	128	-22	0.8	0.8	0.688	7-14R-27 [1]
7-14R-27 [10]	50	69	74	75	69	34	62	16	-80	0.1	0.4	0.063	7-14R-27 [10]
7-14R-27 [20]	77	118	68	96	54	75	81	23	-61	0.4	0.6	0.153	7-14R-27 [20]
22-41 [1]	77	111	59	49	86	44	71	25	-71	0.2	0.5	0.099	22-41 [1]
22-41 [10]	90	79	63	106	94	70	84	16	-58	0.4	0.6	0.165	22-41 [10]
22-41 [20]	87	117	65	75	118	41	84	30	-58	0.4	0.6	0.173	22-41 [20]
37-56 [1]	87	108	98	121	176	49	107	42	-36	0.6	0.7	0.405	37-56 [1]
37-56 [10]	72	77	145	133	223	100	125	56	-17	0.8	0.9	0.696	37-56 [10]
37-56 [20]	38	56	114	141	132	133	102	44	-40	0.6	0.7	0.356	37-56 [20]
54-73 [1]	119	113	116	86	339	111	147	95	5	1.1	1.0	0.914	54-73 [1]
54-73 [10]	44	182	92	158	79	80	106	53	-36	0.6	0.7	0.407	54-73 [10]
54-73 [20]	134	166	104	132	172	75	131	37	-12	0.9	0.9	0.782	54-73 [20]
71-90 [1]	56	142	139	219	103	162	137	55	-5	0.9	1.0	0.904	71-90 [1]
71-90 [10]	85	88	90	123	114	74	96	19	-46	0.5	0.7	0.266	71-90 [10]
71-90 [20]	33	67	115	117	89	57	80	33	-62	0.3	0.6	0.148	71-90 [20]
87-106 [1]	92	153	102	95	125	125	115	23	-27	0.7	0.8	0.518	87-106 [1]
87-106 [10]	82	156	90	142	104	136	118	30	-24	0.7	0.8	0.569	87-106 [10]
87-106 [20]	139	132	105	164	165	191	149	30	7	1.1	1.1	0.861	87-106 [20]
101-120 [1]	65	74	143	114	160	130	114	38	-28	0.7	0.8	0.512	101-120 [1]
101-120 [10]	29	43	123	46	117	96	76	41	-66	0.3	0.5	0.130	101-120 [10]
101-120 [20]	55	132	68	101	193	86	106	50	-36	0.6	0.7	0.405	101-120 [20]
116-130 [1]	65	112	22	36	476	23	122	177	-20	0.8	0.9	0.760	116-130 [1]
116-130 [10]	73	65	49	100	40	30	60	25	-83	0.1	0.4	0.059	116-130 [10]
116-130 [20]	117	75	54	121	101	90	93	26	-49	0.5	0.7	0.244	116-130 [20]
126-140 [1]	98	100	112	77	64	135	98	25	-44	0.5	0.7	0.290	126-140 [1]
126-140 [10]	103	85	54	69	107	98	86	21	-56	0.4	0.6	0.184	126-140 [10]
126-140 [20]	85	93	91	89	309	165	139	89	-3	1.0	1.0	0.943	126-140 [20]
136-150 [1]	55	56	45	55	111	73	66	24	-76	0.2	0.5	0.078	136-150 [1]
136-150 [10]	63	54	26	270	111	68	99	88	-43	0.5	0.7	0.369	136-150 [10]
136-150 [20]	42	39	39	42	14	41	36	11	-106	-0.1	0.3	0.018 *	136-150 [20]
146-160 [1]	62	94	58	48	45	57	61	18	-81	0.1	0.4	0.060	146-160 [1]
146-160 [10]	60	86	86	81	75	65	76	11	-67	0.3	0.5	0.116	146-160 [10]
146-160 [20]	132	74	62	53	54	63	73	30	-69	0.3	0.5	0.110	146-160 [20]
156-170 [1]	90	71	52	67	46	46	62	17	-80	0.1	0.4	0.064	156-170 [1]
156-170 [10]	62	206	61	56	74	31	82	63	-60	0.4	0.6	0.185	156-170 [10]
156-170 [20]	90	46	65	49	28	33	52	23	-90	0.0	0.4	0.040 *	156-170 [20]
166-180 [1]	65	76	75	108	92	30	74	26	-68	0.3	0.5	0.115	166-180 [1]
166-180 [10]	189	285	75	174	72	21	136	97	-6	0.9	1.0	0.901	166-180 [10]
166-180 [20]	145	556	445	177	159	111	266	187	123	2.3 *	1.9	0.079	166-180 [20]
176-195 [1]	104	172	452	92	128	107	176	138	34	1.4	1.2	0.552	176-195 [1]
176-195 [10]	135	221	94	67	123	46	114	62	-28	0.7	0.8	0.533	176-195 [10]
176-195 [20]	89	107	142	92	122	235	130	56	-13	0.9	0.9	0.773	176-195 [20]
191-210 [1]	67	71	254	87	54	30	94	81	-48	0.5	0.7	0.309	191-210 [1]
191-210 [10]	112	65	64	51	59	20	62	30	-80	0.1	0.4	0.067	191-210 [10]
191-210 [20]	55	74	53	167	103	114	94	43	-48	0.5	0.7	0.270	191-210 [20]
210-229 [1]	58	126	249	65	82	66	108	73	-34	0.6	0.8	0.455	210-229 [1]
210-229 [10]	79	128	164	305	103	82	144	85	1	1.0	1.0	0.976	210-229 [10]
210-229 [20]	111	190	345	125	109	330	202	109	60	1.6	1.4	0.254	210-229 [20]
229-248 [1]	102	166	186	142	189	106	149	38	6	1.1	1.0	0.879	229-248 [1]
229-248 [10]	87	83	190	211	137	106	136	54	-6	0.9	1.0	0.882	229-248 [10]
229-248 [20]	39	72	154	99	63	42	78	43	-64	0.3	0.6	0.145	229-248 [20]
248-267 [1]	92	46	32	687	92	85	172	253	30	1.3	1.2	0.715	248-267 [1]
248-267 [10]	85	61	61	144	291	202	141	92	-1	1.0	1.0	0.977	248-267 [10]
248-267 [20]	38	106	97	69	72	315	116	100	-26	0.7	0.8	0.603	248-267 [20]
267-286 [1]	95	129	69	80	100	215	115	53	-27	0.7	0.8	0.529	267-286 [1]
267-286 [10]	113	108	75	98	67	86	91	18	-51	0.5	0.6	0.224	267-286 [10]
267-286 [20]	101	125	84	124	81	119	106	20	-36	0.6	0.7	0.380	267-286 [20]
287-306 [1]	173	93	85	87	49	139	104	44	-38	0.6	0.7	0.380	287-306 [1]
287-306 [10]	213	99	82	99	57	56	101	58	-41	0.6	0.7	0.355	287-306 [10]
287-306 [20]	101	336	82	100	102	59	130	102	-12	0.9	0.9	0.809	287-306 [20]
307-326 [1]	226	270	133	201	143	56	172	76	29	1.3	1.2	0.525	307-326 [1]
307-326 [10]	198	218	222	187	214	78	186	55	44	1.5	1.3	0.318	307-326 [10]
307-326 [20]	160	531	438	298	258	283	328	134	186	3.0 *	2.3 *	0.004 *	307-326 [20]
sAg 10	60	59	54	45	55	16	48	17	-94	0.0	0.3	0.033 *	sAg 10
sAg 100	86	59	57	24	15	48	48	26	-94	0.0	0.3	0.034 *	sAg 100
N	69	60	13	16	60	55	46	24	-97	0.0	0.3	0.030 *	N
N	33	121	14	16	24	42	42	40	-100	-0.1	0.3	0.028 *	N
N	43	38	57	48	79	45	52	15	-90	0.0	0.4	0.039 *	N
N	84	59	18	35	53	77	54	25	-88	0.1	0.4	0.046 *	N
3H	218	133	73	93	115	58	115	57	-27	0.7	0.8	0.538	3H
3H	87	236	385	143	42	122	169	124	27	1.3	1.2	0.616	3H
	EMC												
N	95	73	27	27	64	120	68	37	36	0.0	2.1 *	0.05	N
3H	42	17	33	16	26	56	32	15	0	1.0	1.0	1.00	3H
PHA - 1	226	117	192	168	289	124	186	65	154	-3.3	5.9 *	0.00 *	PHA - 1
PHA - 5	294	228	173	373	246	211	254	71	223	-5.2	8.0 *	0.00 *	PHA - 5
PHA - 10	84	136	103	152	125	160	127	29	95	-1.6	4.0 *	0.00 *	PHA - 10
LPS - 1	154	474	267	585	771	263	419	233	387	-9.8	13.2 *	0.00 *	LPS - 1
LPS - 5	67	126	115	698	141	291	240	237	208	-4.8	7.6 *	0.06	LPS - 5
LPS - 10	29	150	142	88	146	171	121	53	89	-1.5	3.8 *	0.00 *	LPS - 10
LPS - 20	85	95	106	132	170	69	110	36	78	-1.2	3.5 *	0.00 *	LPS - 20
LPS - 40	87	54	73	75	279	60	105	86	73	-1.0	3.3 *	0.07	LPS - 40
	PBMC												
N	28	16	30	84	62	74	49	28	-4	0.0	0.9	0.77	N
3H	33	46	66	70	45	57	53	14	0	1.0	1.0	1.00	3H
PHA - 1	190	337	325	329	507	377	344	102	291	77.0 *	6.5 *	0.00 *	PHA - 1
PHA - 5	598	897	797	1111	1150	801	892	209	840	220.0 *	16.9 *	0.00 *	PHA - 5
PHA - 10	1044	1584	1141	1640	1707	1565	1447	281	1394	364.7 *	27.4 *	0.00 *	PHA - 10
LPS - 1	544	1246	1100	1753	2100	1328	1345	538	1292	338.1 *	25.5 *	0.00 *	LPS - 1
LPS - 5	862	1576	1440	1767	2038	1923	1601	423	1548	404.9 *	30.3 *	0.00 *	LPS - 5
LPS - 10	512	489	980	2102	2177	1034	1216	751	1163	304.3 *	23.0 *	0.00 *	LPS - 10
LPS - 20	520	1519	1226	1376	1840	914	1233	465	1180	308.7 *	23.3 *	0.00 *	LPS - 20
LPS - 40	89	450	929	823	500	128	487	346	434	114.1 *	9.2 *	0.01 *	LPS - 40

Raw data for Positive control duck W106

W106	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	66	51															
Total 3H	173	93								>5000		>2.1		>2.1		<0.05	
	R1	R2	R3	R4	R5	R6	Mean	SD									
1-15 [1]	92	100	185	221	125	167	148	51	-24		0.8		0.9		0.576		1-15 [1]
1-15 [10]	183	297	234	284	228	104	222	71	49		1.5		1.3		0.303		1-15 [10]
1-15 [20]	119	355	243	542	244	102	268	163	95		1.9		1.5		0.192		1-15 [20]
7-14W-27 [1]	51	120	208	125	164	197	144	58	-28		0.7		0.8		0.524		7-14W-27 [1]
7-14W-27 [10]	35	117	278	185	119	72	134	87	-38		0.6		0.8		0.448		7-14W-27 [10]
7-14W-27 [20]	69	57	129	117	62	33	78	37	-95		0.1		0.5		0.037	*	7-14W-27 [20]
7-14R-27 [1]	238	138	97	74	51	49	108	72	-65		0.4		0.6		0.183		7-14R-27 [1]
7-14R-27 [10]	63	77	150	56	66	30	74	41	-99		0.1		0.4		0.032	*	7-14R-27 [10]
7-14R-27 [20]	60	252	278	245	336	50	204	119	31		1.3		1.2		0.595		7-14R-27 [20]
22-41 [1]	162	130	127	154	148	82	134	29	-39		0.6		0.8		0.347		22-41 [1]
22-41 [10]	131	349	252	257	330	113	239	98	66		1.6		1.4		0.224		22-41 [10]
22-41 [20]	144	302	359	439	361	176	297	115	124		2.2	*	1.7		0.045	*	22-41 [20]
37-56 [1]	162	232	178	159	114	83	155	52	-18		0.8		0.9		0.679		37-56 [1]
37-56 [10]	65	89	107	221	80	35	100	64	-73		0.3		0.6		0.126		37-56 [10]
37-56 [20]	124	300	434	342	170	41	235	148	63		1.6		1.4		0.350		37-56 [20]
54-73 [1]	123	318	284	284	135	41	198	113	25		1.2		1.1		0.658		54-73 [1]
54-73 [10]	120	321	301	204	190	95	205	92	33		1.3		1.2		0.527		54-73 [10]
54-73 [20]	86	323	516	222	183	194	254	149	81		1.8		1.5		0.232		54-73 [20]
71-90 [1]	114	214	148	128	62	38	117	63	-55		0.5		0.7		0.235		71-90 [1]
71-90 [10]	116	267	94	101	75	35	115	80	-58		0.5		0.7		0.244		71-90 [10]
71-90 [20]	56	74	82	82	132	262	115	76	-58		0.5		0.7		0.238		71-90 [20]
87-106 [1]	86	147	148	172	144	99	133	33	-40		0.6		0.8		0.338		87-106 [1]
87-106 [10]	47	112	187	204	168	122	140	58	-33		0.7		0.8		0.466		87-106 [10]
87-106 [20]	59	158	201	159	143	242	160	61	-12		0.9		0.9		0.784		87-106 [20]
101-120 [1]	92	230	235	98	89	82	138	74	-35		0.7		0.8		0.463		101-120 [1]
101-120 [10]	54	108	213	116	141	37	112	63	-61		0.4		0.6		0.192		101-120 [10]
101-120 [20]	40	73	98	86	64	97	76	22	-96		0.1		0.4		0.030	*	101-120 [20]
116-130 [1]	92	218	45	81	83	65	97	61	-75		0.3		0.6		0.112		116-130 [1]
116-130 [10]	41	87	35	48	29	23	44	23	-129		-0.2		0.3		0.006	*	116-130 [10]
116-130 [20]	62	170	156	209	80	46	121	67	-52		0.5		0.7		0.267		116-130 [20]
126-140 [1]	111	306	129	75	73	53	125	93	-48		0.5		0.7		0.357		126-140 [1]
126-140 [10]	78	117	133	166	82	56	105	41	-67		0.4		0.6		0.126		126-140 [10]
126-140 [20]	90	122	211	156	83	107	128	48	-44		0.6		0.7		0.309		126-140 [20]
136-150 [1]	98	87	89	179	119	47	103	44	-69		0.3		0.6		0.118		136-150 [1]
136-150 [10]	78	45	47	46	62	48	54	13	-118		-0.1		0.3		0.010	*	136-150 [10]
136-150 [20]	88	51	56	63	29	64	59	19	-114		-0.1		0.3		0.013	*	136-150 [20]
146-160 [1]	46	63	125	144	64	89	89	39	-84		0.2		0.5		0.061		146-160 [1]
146-160 [10]	56	121	133	170	106	76	110	41	-62		0.4		0.6		0.154		146-160 [10]
146-160 [20]	53	161	109	122	73	60	96	42	-76		0.3		0.6		0.088		146-160 [20]
156-170 [1]	93	91	99	109	37	63	82	27	-91		0.1		0.5		0.041	*	156-170 [1]
156-170 [10]	37	82	61	86	47	562	146	205	-27		0.7		0.8		0.747		156-170 [10]
156-170 [20]	58	29	87	61	71	56	60	19	-112		-0.1		0.3		0.014	*	156-170 [20]
166-180 [1]	92	62	82	102	173	111	104	38	-69		0.4		0.6		0.115		166-180 [1]
166-180 [10]	34	40	52	64	45	30	44	12	-128		-0.2		0.3		0.006	*	166-180 [10]
166-180 [20]	201	123	126	115	78	58	117	49	-56		0.5		0.7		0.209		166-180 [20]
176-195 [1]	61	159	141	135	132	79	118	39	-55		0.5		0.7		0.203		176-195 [1]
176-195 [10]	66	121	199	143	169	56	126	57	-47		0.6		0.7		0.298		176-195 [10]
176-195 [20]	51	213	225	151	154	275	178	78	6		1.1		1.0		0.908		176-195 [20]
191-210 [1]	98	93	100	74	115	79	93	15	-79		0.3		0.5		0.063		191-210 [1]
191-210 [10]	82	67	59	59	104	52	71	19	-102		0.0		0.4		0.022	*	191-210 [10]
191-210 [20]	55	83	58	68	45	86	66	16	-107		0.0		0.4		0.017	*	191-210 [20]
210-229 [1]	26	117	149	98	137	90	103	44	-70		0.3		0.6		0.117		210-229 [1]
210-229 [10]	83	407	1103	267	281	84	371	380	198		2.9	*	2.1	*	0.176		210-229 [10]
210-229 [20]	134	808	694	611	309	110	444	300	272		3.6	*	2.6	*	0.031	*	210-229 [20]
229-248 [1]	93	280	82	98	114	80	125	77	-48		0.5		0.7		0.325		229-248 [1]
229-248 [10]	34	83	261	147	139	57	120	82	-52		0.5		0.7		0.294		229-248 [10]
229-248 [20]	62	96	124	92	65	114	92	25	-80		0.2		0.5		0.063		229-248 [20]
248-267 [1]	41	30	73	58	27	101	55	28	-118		-0.1		0.3		0.012	*	248-267 [1]
248-267 [10]	38	31	68	55	32	65	48	17	-124		-0.2		0.3		0.008	*	248-267 [10]
248-267 [20]	83	170	151	145	98	194	140	42	-32		0.7		0.8		0.445		248-267 [20]
267-286 [1]	56	117	153	178	112	39	109	54	-63		0.4		0.6		0.163		267-286 [1]
267-286 [10]	92	129	209	131	64	142	128	49	-45		0.6		0.7		0.307		267-286 [10]
267-286 [20]	77	135	181	162	83	80	120	46	-53		0.5		0.7		0.227		267-286 [20]
287-306 [1]	47	46	43	21	80	32	45	20	-128		-0.2		0.3		0.007	*	287-306 [1]
287-306 [10]	35	55	76	161	62	30	70	48	-103		0.0		0.4		0.030	*	287-306 [10]
287-306 [20]	132	174	236	191	183	137	176	38	3		1.0		1.0		0.945		287-306 [20]
307-326 [1]	127	186	213	216	98	94	156	56	-17		0.8		0.9		0.701		307-326 [1]
307-326 [10]	201	459	253	324	285	172	282	103	110		2.0		1.6		0.058		307-326 [10]
307-326 [20]	244	558	666	311	359	149	381	195	209		3.0	*	2.2	*	0.020	*	307-326 [20]
sAg 10	48	88	68	66	82	116	78	23	-95		0.1		0.5		0.033	*	sAg 10
sAg 100	86	73	38	50	105	148	83	40	-89		0.2		0.5		0.049	*	sAg 100
N	98	156	60	41	176	225	126	72	-47		0.6		0.7		0.328		N
N	27	40	24	40	75	44	42	18	-131		-0.2		0.2		0.006	*	N
N	47	36	36	67	53	24	44	15	-129		-0.2		0.3		0.006	*	N
N	21	50	70	58	67	55	54	18	-119		-0.1		0.3		0.010	*	N
3H	140	163	373	139	137	56	168	107	-5		1.0		1.0		0.933		3H
3H	224					149	187	53	14		1.1		1.1		0.849		3H
	SMC																
N	100	32	29	43	90	263	93	89	7		0.0		1.1		0.90		N
3H	38	46	27	33	118	255	86	89	0		1.0		1.0		1.00		3H
PHA - 1	457	514	1416	1229	584	2167	1061	674	975		-145.3		12.3	*	0.01	*	PHA - 1
PHA - 5	3962	1551	1434	1882	2795	6670	3049	2011	2963		-443.4		35.4	*	0.00	*	PHA - 5
PHA - 10	2283	2034	1998	1554	1576	4240	2281	1000	2195		-328.2		26.5	*	0.00	*	PHA - 10
LPS - 1	1129	986	884	546	475	1734	959	456	873		-129.9		11.1	*	0.00	*	LPS - 1
LPS - 5	633	568	614	606	953	777	692	147	606		-89.9		8.0	*	0.00	*	LPS - 5
LPS - 10	532	555	737	488	388	592	549	116	463		-68.4		6.4	*	0.00	*	LPS - 10
LPS - 20	212	508	342	318	361	241	330	105	244		-35.6		3.8	*	0.00	*	LPS - 20
LPS - 40	101	183	145	199	290	119	173	68	87		-12.0		2.0		0.09		LPS - 40
	PBMC																
N	22	35	27	152	167	107	85	66	-239		0.0		0.3		0.00	*	N
3H	383	422	239	441	249	211	324	102	0		1.0		1.0		1.00		3H
PHA - 1	1924	1262	619	680	899	4459	1641	1462	1316		6.5	*	5.1	*	0.05		PHA - 1
PHA - 5	3576	986	636	588	677	6124	2098	2283	1774		8.4	*	6.5	*	0.09		PHA - 5
PHA - 10	2708	1590	1389	1482	1836	2681	1948	598	1624		7.8	*	6.0	*	0.00	*	PHA - 10
LPS - 1	1811	1577	1375	1483	2283	1562	1682	328	1358		6.7	*	5.2	*	0.00	*	LPS - 1
LPS - 5	1708	2172	1420	1486	1358	1170	1552	350	1228		6.1	*	4.8	*	0.00	*	LPS - 5
LPS - 10	1011	1521	1396	1259	1809	606	1267	419	943		4.9	*	3.9	*	0.00	*	LPS - 10
LPS - 20	177	1097	1461	996	1121	181	839	535	515		3.2	*	2.6	*	0.04	*	LPS - 20
LPS - 40	116	113	295	81	170	148	154	76	-170		0.3		0.5		0.01	*	LPS - 40

Raw data for Positive control duck W107

W107	Mean	SD							CPM-3H	S.I.	P/N	t-Test	
Total N	86	59											
Total 3H	517	527							>5000	>2.1	>2.1	<0.05	
	R1	R2	R3	R4	R5	R6	Mean	SD					
1-15 [1]	82	54	90	409	61	263	160	145	-357	0.2	0.3	0.115	1-15 [1]
1-15 [10]	144	115	223	116	385	303	214	111	-303	0.3	0.4	0.178	1-15 [10]
1-15 [20]	136	160	150	115	122	410	182	113	-335	0.2	0.4	0.137	1-15 [20]
7-14W-27 [1]	125	373	204	133	151	220	201	93	-316	0.3	0.4	0.159	7-14W-27 [1]
7-14W-27 [10]	343	363	168	128	107	162	212	112	-305	0.3	0.4	0.174	7-14W-27 [10]
7-14W-27 [20]	539	444	161	206	396	760	418	221	-99	0.8	0.8	0.658	7-14W-27 [20]
7-14R-27 [1]	113	287	3491	779	153	63	814	1337	297	1.7	1.6	0.386	7-14R-27 [1]
7-14R-27 [10]	120	113	47	327	59	252	153	112	-364	0.2	0.3	0.108	7-14R-27 [10]
7-14R-27 [20]	719	196	193	261	189	128	281	219	-236	0.5	0.5	0.297	7-14R-27 [20]
22-41 [1]	324	172	588	95	101	373	276	191	-242	0.4	0.5	0.284	22-41 [1]
22-41 [10]	294	197	157	1080	209	1102	507	455	-11	1.0	1.0	0.965	22-41 [10]
22-41 [20]	449	151	114	94	93	812	286	292	-232	0.5	0.6	0.312	22-41 [20]
37-56 [1]	37	80	188	88	1187	87	278	448	-239	0.4	0.5	0.316	37-56 [1]
37-56 [10]	149	131	55	83	119	546	181	182	-337	0.2	0.3	0.139	37-56 [10]
37-56 [20]	225	97	113	94	81	122	122	52	-395	0.1	0.2	0.081	37-56 [20]
54-73 [1]	479	162	255	100	48	408	242	172	-275	0.4	0.5	0.222	54-73 [1]
54-73 [10]	207	144	238	214	68	496	228	145	-289	0.3	0.4	0.199	54-73 [10]
54-73 [20]	104	192	154	140	115	202	151	40	-366	0.2	0.3	0.104	54-73 [20]
71-90 [1]	213	99	78	222	85	387	181	120	-336	0.2	0.3	0.136	71-90 [1]
71-90 [10]	51	2049	2759	737	706	61	1061	1105	544	2.3 *	2.1	0.085	71-90 [10]
71-90 [20]	36	1078	595	687	2765	284	908	977	391	1.9	1.8	0.186	71-90 [20]
87-106 [1]	750	115	305	109	319	296	316	233	-201	0.5	0.6	0.373	87-106 [1]
87-106 [10]	284	180	320	143	617	604	358	206	-159	0.6	0.7	0.479	87-106 [10]
87-106 [20]	168	114	169	64	145	291	159	76	-359	0.2	0.3	0.112	87-106 [20]
101-120 [1]	294	109	82	121	239	71	153	92	-364	0.2	0.3	0.107	101-120 [1]
101-120 [10]	202	485	149	347	602	294	347	172	-171	0.6	0.7	0.446	101-120 [10]
101-120 [20]	1766	461	168	216	230	303	524	617	7	1.0	1.0	0.978	101-120 [20]
116-130 [1]	69	224	198	930	158	69	275	327	-242	0.4	0.5	0.294	116-130 [1]
116-130 [10]	80	52	122	280	70	784	231	283	-286	0.3	0.4	0.214	116-130 [10]
116-130 [20]	93	182	222	83	345	815	290	274	-227	0.5	0.6	0.320	116-130 [20]
126-140 [1]	154	111	208	70	55	352	158	110	-359	0.2	0.3	0.113	126-140 [1]
126-140 [10]	295	242	54	186	145	56	163	98	-354	0.2	0.3	0.117	126-140 [10]
126-140 [20]	199	78	338	106	55	528	217	184	-300	0.3	0.4	0.185	126-140 [20]
136-150 [1]	585	80	151	103	132	254	218	190	-300	0.3	0.4	0.186	136-150 [1]
136-150 [10]	135	1909	1226	100	713	92	696	747	179	1.4	1.3	0.499	136-150 [10]
136-150 [20]	19	397	236	203	257	40	192	142	-325	0.2	0.4	0.150	136-150 [20]
146-160 [1]	971	99	79	415	329	152	341	336	-176	0.6	0.7	0.445	146-160 [1]
146-160 [10]	324	250	131	505	77	312	267	153	-251	0.4	0.5	0.264	146-160 [10]
146-160 [20]	702	425	261	61	110	212	295	236	-222	0.5	0.6	0.327	146-160 [20]
156-170 [1]	775	362	281	64	190	296	328	242	-189	0.6	0.6	0.403	156-170 [1]
156-170 [10]	506	562	131	126	72	74	245	226	-272	0.4	0.5	0.231	156-170 [10]
156-170 [20]	5517	199	157	83	88	57	1017	2205	500	2.2 *	2.0	0.305	156-170 [20]
166-180 [1]	52	1109	154	1137	392	91	489	505	-28	0.9	0.9	0.908	166-180 [1]
166-180 [10]	435	200	85	279	385	649	339	197	-178	0.6	0.7	0.427	166-180 [10]
166-180 [20]	139	84	181	245	123	599	229	190	-289	0.3	0.4	0.202	166-180 [20]
176-195 [1]	1869	271	72	72	215	131	438	705	-79	0.8	0.8	0.762	176-195 [1]
176-195 [10]	311	103	165	84	103	99	144	86	-373	0.1	0.3	0.099	176-195 [10]
176-195 [20]	220	77	92	89	108	152	123	54	-394	0.1	0.2	0.082	176-195 [20]
191-210 [1]	579	247	591	365	122	421	388	184	-130	0.7	0.7	0.562	191-210 [1]
191-210 [10]	134	1581	63	1189	168	124	543	665	26	1.1	1.1	0.918	191-210 [10]
191-210 [20]	95	1143	450	1136	924	34	630	506	113	1.3	1.2	0.639	191-210 [20]
210-229 [1]	170	76	161	92	79	209	131	56	-386	0.1	0.3	0.088	210-229 [1]
210-229 [10]	301	150	114	154	85	82	148	81	-369	0.1	0.3	0.102	210-229 [10]
210-229 [20]	3222	319	889	309	454	1675	1145	1142	628	2.5 *	2.2 *	0.052	210-229 [20]
229-248 [1]	179	135	57	70	64	284	132	89	-386	0.1	0.3	0.089	229-248 [1]
229-248 [10]	1170	220	122	206	120	177	336	411	-181	0.6	0.6	0.441	229-248 [10]
229-248 [20]	255	241	84	81	57	76	132	90	-385	0.1	0.3	0.089	229-248 [20]
248-267 [1]	101	995	325	469	3153	1914	1160	1172	643	2.5 *	2.2 *	0.050	248-267 [1]
248-267 [10]	1668	964	130	199	149	335	574	620	57	1.1	1.1	0.820	248-267 [10]
248-267 [20]	421	231	120	413	75	114	229	155	-288	0.3	0.4	0.201	248-267 [20]
267-286 [1]	908	819	322	382	249	87	461	328	-56	0.9	0.9	0.807	267-286 [1]
267-286 [10]	676	80	500	226	167	456	351	229	-166	0.6	0.7	0.461	267-286 [10]
267-286 [20]	330	312	339	152	113	817	344	251	-173	0.6	0.7	0.444	267-286 [20]
287-306 [1]	166	1740	584	911	125	272	633	618	116	1.3	1.2	0.644	287-306 [1]
287-306 [10]	794	132	182	168	219	1023	420	387	-97	0.8	0.8	0.676	287-306 [10]
287-306 [20]	165	194	191	155	147	1132	331	393	-186	0.6	0.6	0.426	287-306 [20]
307-326 [1]	660	396	458	180	239	562	416	184	-101	0.8	0.8	0.650	307-326 [1]
307-326 [10]	302	87	339	82	335	151	216	123	-301	0.3	0.4	0.180	307-326 [10]
307-326 [20]	327	182	551	98	157	302	270	163	-248	0.4	0.5	0.270	307-326 [20]
sAg 10	881	271	164	96	154	97	277	303	-240	0.4	0.5	0.297	sAg 10
sAg 100	76	2312	881	259	454	27	668	863	151	1.4	1.3	0.586	sAg 100
N	47	67	144	176	86	100	103	48	-414	0.0	0.2	0.068	N
N	20	200	41	77	35	37	68	67	-449	0.0	0.1	0.049 *	N
3H	339	311	134	181	150	378	249	106	-268	0.4	0.5	0.231	3H
3H	100	2171	872	882	39	100	694	823	177	1.4	1.3	0.517	3H
3H	988	222	532	328	108	1744	654	617	137	1.3	1.3	0.586	3H
3H	401	702	731	393	525	77	472	241	-46	0.9	0.9	0.840	3H
	SMC												
N	242	113	84	58	102	160	127	66	15	0.0	1.1	0.68	N
3H	99	179	75	54	85	178	112	54	0	1.0	1.0	1.00	3H
PHA - 1	335	196	122	91	240	392	229	118	118	-6.9	2.1	0.05	PHA - 1
PHA - 5	2928	3260	4777	1861	4359	4839	3671	1188	3559	-238.9	32.9 *	0.00 *	PHA - 5
PHA - 10	1816	2469	1551	1804	2013	1967	1937	307	1825	-122.0	17.3 *	0.00 *	PHA - 10
LPS - 1	179	283	432	490	416	280	347	118	235	-14.8	3.1 *	0.00 *	LPS - 1
LPS - 5	422	408	281	227	268	389	333	83	221	-13.9	3.0 *	0.00 *	LPS - 5
LPS - 10	203	209	218	157	154	152	182	31	71	-3.8	1.6	0.02 *	LPS - 10
LPS - 20	49	109	77	58	88	40	70	26	-42	3.8 *	0.6	0.12	LPS - 20
LPS - 40	66	114	107	41	79	211	103	59	-9	1.6	0.9	0.80	LPS - 40
	PBMC												
N	159	97	159	102	98	70	114	37	-199	0.0	0.4	0.00 *	N
3H	349	337	331	327	271	265	313	36	0	1.0	1.0	1.00	3H
PHA - 1	208	236	220	155	121	117	176	52	-137	0.3	0.6	0.00 *	PHA - 1
PHA - 5	992	1701	1193	1495	1661	1504	1424	277	1111	6.6 *	4.5 *	0.00 *	PHA - 5
PHA - 10	2375	2246	1239	1328	2380	2097	1944	523	1631	9.2 *	6.2 *	0.00 *	PHA - 10
LPS - 1	470	496	492	362	462	469	459	49	145	1.7	1.5	0.00 *	LPS - 1
LPS - 5	575	663	545	579	513	351	538	104	224	2.1 *	1.7	0.00 *	LPS - 5
LPS - 10	409	221	260	209	281	320	283	74	-30	0.8	0.9	0.39	LPS - 10
LPS - 20	194	116	224	223	147	81	164	59	-149	0.3	0.5	0.00 *	LPS - 20
LPS - 40	68	224	197	181	154	72	149	66	-164	0.2	0.5	0.00 *	LPS - 40

Raw data for Positive control duck W111

W111	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	144	112								>5000		>2.1		>2.1		<0.05	
Total 3H	423	1066															
	R1	R2	R3	R4	R5	R6	Mean	SD									
1-15 [1]	281	127	150	161	298	668	281	203	-142		0.5		0.7		0.751		1-15 [1]
1-15 [10]	412	247	317	1800	348	1349	746	660	323		2.2	*	1.8		0.488		1-15 [10]
1-15 [20]	238	2574	238	633	1416	1971	1178	969	755		3.7	*	2.8	*	0.126		1-15 [20]
7-14W-27 [1]	105	210	74	144	158	396	181	115	-242		0.1		0.4		0.588		7-14W-27 [1]
7-14W-27 [10]	558	754	294	1449	391	2643	1015	897	592		3.1	*	2.4	*	0.222		7-14W-27 [10]
7-14W-27 [20]	4341	476	1118	514	2775	11982	3534	4402	3111		12.2	*	8.4	*	0.003	*	7-14W-27 [20]
7-14R-27 [1]	492	86	1154	258	45	368	401	406	-22		0.9		0.9		0.960		7-14R-27 [1]
7-14R-27 [10]	176	218	139	40	108	670	225	226	-198		0.3		0.5		0.659		7-14R-27 [10]
7-14R-27 [20]	355	169	196	232	612	222	298	167	-125		0.6		0.7		0.779		7-14R-27 [20]
22-41 [1]	185	232	182	1126	492	232	408	370	-15		0.9		1.0		0.974		22-41 [1]
22-41 [10]	74	182	162	64	91	340	152	104	-271		0.0		0.4		0.545		22-41 [10]
22-41 [20]	256	283	472	475	4140	552	1030	1528	607		3.2	*	2.4	*	0.262		22-41 [20]
37-56 [1]	152	338	227	93	153	7245	1368	2880	945		4.4	*	3.2	*	0.194		37-56 [1]
37-56 [10]	196	267	95	212	710	228	285	216	-138		0.5		0.7		0.757		37-56 [10]
37-56 [20]	560	503	294	194	179	717	408	219	-15		0.9		1.0		0.973		37-56 [20]
54-73 [1]	153	92	131	63	640	213	215	214	-208		0.3		0.5		0.643		54-73 [1]
54-73 [10]	336	242	200	252	120	251	234	71	-189		0.3		0.6		0.671		54-73 [10]
54-73 [20]	131	450	673	63	597	353	378	245	-45		0.8		0.9		0.920		54-73 [20]
71-90 [1]	597	545	197	287	712	458	466	194	43		1.2		1.1		0.923		71-90 [1]
71-90 [10]	925	1272	158	123	451	119	508	486	85		1.3		1.2		0.852		71-90 [10]
71-90 [20]	108	78	370	53	347	66	170	147	-253		0.1		0.4		0.572		71-90 [20]
87-106 [1]	530	306	188	259	179	75	256	155	-167		0.4		0.6		0.709		87-106 [1]
87-106 [10]	110	405	146	139	228	92	187	117	-236		0.2		0.4		0.597		87-106 [10]
87-106 [20]	119	75	259	198	340	130	187	99	-236		0.2		0.4		0.597		87-106 [20]
101-120 [1]	1853	77	255	735	628	402	658	633	235		1.8		1.6		0.611		101-120 [1]
101-120 [10]	341	761	138	270	141	98	292	248	-131		0.5		0.7		0.769		101-120 [10]
101-120 [20]	281	193	52	426	973	495	403	321	-20		0.9		1.0		0.965		101-120 [20]
116-130 [1]	938	90	126	1403	217	435	535	528	112		1.4		1.3		0.807		116-130 [1]
116-130 [10]	142	99	221	157	71	889	263	311	-160		0.4		0.6		0.722		116-130 [10]
116-130 [20]	369	65	134	157	596	48	228	214	-195		0.3		0.5		0.664		116-130 [20]
126-140 [1]	150	129	166	132	369	593	257	188	-166		0.4		0.6		0.710		126-140 [1]
126-140 [10]	105	256	63	145	235	971	296	339	-127		0.5		0.7		0.778		126-140 [10]
126-140 [20]	468	107	393	237	407	146	293	150	-130		0.5		0.7		0.771		126-140 [20]
136-150 [1]	347	216	139	67	289	143	200	104	-223		0.2		0.5		0.618		136-150 [1]
136-150 [10]	2084	111	598	135	544	234	618	747	195		1.7		1.5		0.678		136-150 [10]
136-150 [20]	91	71	281	149	137	117	141	74	-282		0.0		0.3		0.528		136-150 [20]
146-160 [1]	642	94	35	95	121	429	236	243	-187		0.3		0.6		0.677		146-160 [1]
146-160 [10]	555	387	115	180	46	432	286	201	-137		0.5		0.7		0.759		146-160 [10]
146-160 [20]	1509	61	146	187	35	344	380	564	-43		0.8		0.9		0.926		146-160 [20]
156-170 [1]	65	218	942	117	93	86	254	342	-169		0.4		0.6		0.707		156-170 [1]
156-170 [10]	284	46	114	185	178	345	192	109	-231		0.2		0.5		0.605		156-170 [10]
156-170 [20]	41	229	749	117	172	271	263	252	-160		0.4		0.6		0.722		156-170 [20]
166-180 [1]	166	418	93	137	207	18	173	136	-250		0.1		0.4		0.576		166-180 [1]
166-180 [10]	279	262	235	102	81	234	199	85	-224		0.2		0.5		0.616		166-180 [10]
166-180 [20]	111	545	513	959	711	233	512	310	89		1.3		1.2		0.843		166-180 [20]
176-195 [1]	1451	143	295	76	72	80	353	545	-70		0.7		0.8		0.878		176-195 [1]
176-195 [10]	235	527	414	69	175	41	244	193	-179		0.4		0.6		0.688		176-195 [10]
176-195 [20]	51	46	226	150	173	140	131	71	-292		0.0		0.3		0.514		176-195 [20]
191-210 [1]	597	153	1314	140	904	1093	700	489	277		2.0		1.7		0.544		191-210 [1]
191-210 [10]	42	4357	257	275	148	2557	1273	1787	850		4.1	*	3.0	*	0.140		191-210 [10]
191-210 [20]	155	75	164	172	168	422	193	118	-230		0.2		0.5		0.606		191-210 [20]
210-229 [1]	110	81	2196	71	219	340	503	836	80		1.3		1.2		0.866		210-229 [1]
210-229 [10]	992	517	143	313	434	188	431	309	8		1.0		1.0		0.985		210-229 [10]
210-229 [20]	642	1576	13298	1564	325	305	2952	5101	2529		10.1	*	7.0	*	0.026	*	210-229 [20]
229-248 [1]	426	623	38	143	99	69	233	237	-190		0.3		0.6		0.672		229-248 [1]
229-248 [10]	68	361	232	2514	3230	544	1158	1356	735		3.6	*	2.7	*	0.163		229-248 [10]
229-248 [20]	639	976	190	640	512	681	606	256	183		1.7		1.4		0.683		229-248 [20]
248-267 [1]	381	164	444	137	465	406	333	144	-90		0.7		0.8		0.840		248-267 [1]
248-267 [10]	79	1502	893	245	232	102	509	571	86		1.3		1.2		0.852		248-267 [10]
248-267 [20]	549	808	143	52	448	44	341	311	-82		0.7		0.8		0.855		248-267 [20]
267-286 [1]	538	118	203	29	94	1135	353	423	-70		0.7		0.8		0.877		267-286 [1]
267-286 [10]	78	447	116	137	171	265	202	136	-221		0.2		0.5		0.621		267-286 [10]
267-286 [20]	355	378	111	159	408	301	285	123	-138		0.5		0.7		0.758		267-286 [20]
287-306 [1]	249	202	267	174	670	1997	593	711	170		1.6		1.4		0.715		287-306 [1]
287-306 [10]	497	156	394	205	392	394	340	131	-83		0.7		0.8		0.852		287-306 [10]
287-306 [20]	331	181	35	326	280	124	213	120	-210		0.2		0.5		0.638		287-306 [20]
307-326 [1]	252	396	52	89	82	1217	348	445	-75		0.7		0.8		0.869		307-326 [1]
307-326 [10]	468	322	1729	332	259	81	532	600	109		1.4		1.3		0.813		307-326 [10]
307-326 [20]	326	76	269	190	127	484	245	148	-178		0.4		0.6		0.691		307-326 [20]
sAg 10	291	214	205	252	211	849	337	253	-86		0.7		0.8		0.848		sAg 10
sAg 100	179	2566	485	293	300	97	653	946	230		1.8		1.5		0.633		sAg 100
N	116	45	85	62	62	26	66	31	-357		-0.3		0.2		0.425		N
N	221	330	295	67	310	114	223	110	-200		0.3		0.5		0.654		N
3H	168	529	72	232	380	183	261	166	-162		0.4		0.6		0.716		3H
3H	692	174	85	169	267	5337	1121	2077	698		3.5	*	2.6	*	0.251		3H
3H	706	97	113	127	141	488	279	256	-144		0.5		0.7		0.748		3H
3H	60	21	37	22	19	32	32	15	-391		-0.4		0.1		0.383		3H
	SMC																
N	28	28	21	16	34	244	62	89	-591		0.0		0.1		0.00	*	N
3H	552	555	711	648	701	750	653	84	0		1.0		1.0		1.00		3H
PHA - 1	3065	5200	3512	3731	4840	6205	4426	1192	3773		7.4	*	6.8	*	0.00	*	PHA - 1
PHA - 5	8438	9533	5715	8787	9290	9510	8546	1452	7893	*	14.4	*	13.1	*	0.00	*	PHA - 5
PHA - 10	5367	6193	5701	4239	6070	4920	5415	741	4762		9.1	*	8.3	*	0.00	*	PHA - 10
LPS - 1	1774	2444	2435	1985	2504	4453	2599	954	1946		4.3	*	4.0	*	0.00	*	LPS - 1
LPS - 5	1125	1969	1382	1895	1442	2065	1646	381	994		2.7	*	2.5	*	0.00	*	LPS - 5
LPS - 10	1140	861	1434	1065	1182	1028	1118	190	466		1.8		1.7		0.00	*	LPS - 10
LPS - 20	875	820	1057	973	970	945	940	83	287		1.5		1.4		0.00	*	LPS - 20
LPS - 40	224	269	288	349	270	206	268	50	-385		0.3		0.4		0.00	*	LPS - 40
	PBMC																
N	73	110	78	68	31	23	64	32	-199		0.0		0.2		0.00	*	N
3H	286	262	258	330	190	252	263	46	0		1.0		1.0		1.00		3H
PHA - 1	596	427	190	79	119	217	271	200	8		1.0		1.0		0.92		PHA - 1
PHA - 5	9255	6168	6833	5064	3250	7658	6371	2082	6108	*	31.7	*	24.2	*	0.00	*	PHA - 5
PHA - 10	5410	3357	9341	7126	7399	7445	6680	2051	6417	*	33.2	*	25.4	*	0.00	*	PHA - 10
LPS - 1	387	225	239	219	327	318	286	68	23		1.1		1.1		0.51		LPS - 1
LPS - 5	366	184	636	276	215	209	314	171	51		1.3		1.2		0.49		LPS - 5
LPS - 10	223	256	147	227	175	211	207	39	-57		0.7		0.8		0.04	*	LPS - 10
LPS - 20	332	426	507	218	280	260	337	110	74		1.4		1.3		0.16		LPS - 20
LPS - 40	236	208	343	723	180	52	290	232	27		1.1		1.1		0.78		LPS - 40

Raw data for DNAvacc1 duck B67

B67	Mean	SD													DNA vaccinated
Total N	86	113													
Total 3H	33283	20998							CPM-3H		S.I.		P/N	t-Test	
	R1	R2	R3	R4	R5	R6	Mean	SD	>5000		>2.1		>2.1	<0.05	
1-15 [1]	20944	36234	8486	2717	71507	530	23403	27049	-9,880		0.7		0.7	0.34	1-15 [1]
1-15 [10]	3318	21194	10957	3012	53041	842	15394	19907	-17,889		0.5		0.5	0.07	1-15 [10]
1-15 [20]	15636	29794	17095	9086	31165	2664	17573	11246	-15,710		0.5		0.5	0.09	1-15 [20]
7-14W-27 [1]	3918	13411	694	5211	3930	323	4581	4743	-28,702		0.1		0.1	0.00	* 7-14W-27 [1]
7-14W-27 [10]	167	503	46330	1602	194	228	8171	18702	-25,112		0.2		0.2	0.01	* 7-14W-27 [10]
7-14W-27 [20]	191	686	195	636	459	13392	2593	5295	-30,690		0.1		0.1	0.00	* 7-14W-27 [20]
71-90 [1]	10836	7859	64551	33096	1968	67616	30988	29174	-2,295		0.9		0.9	0.83	71-90 [1]
71-90 [10]	711	12364	21752	44746	54871	25313	26626	20132	-6,657		0.8		0.8	0.49	71-90 [10]
71-90 [20]	6749	10727	45595	43459	10462	75822	32136	27533	-1,147		1.0		1.0	0.91	71-90 [20]
101-120 [1]	13484	8170	1126	3266	231	37273	10592	13977	-22,691		0.3		0.3	0.02	* 101-120 [1]
101-120 [10]	9101	54603	31404	9247	330	1014	17617	21331	-15,666		0.5		0.5	0.11	101-120 [10]
101-120 [20]	5583	3259	6833	7783	669	12117	6041	3934	-27,242		0.2		0.2	0.00	* 101-120 [20]
229-248 [1]	33056	28439	1897	39400	463	768	17337	18192	-15,946		0.5		0.5	0.10	229-248 [1]
229-248 [10]	11087	54449	5725	825	12790	9076	15659	19472	-17,624		0.5		0.5	0.07	229-248 [10]
229-248 [20]	413	9488	332	43254	6211	6833	11089	16179	-22,194		0.3		0.3	0.02	* 229-248 [20]
267-286 [1]	501	15718	445	69285	14998	106530	34580	43467	1,297		1.0		1.0	0.92	267-286 [1]
267-286 [10]	13314	16644	34327	399	8693	89407	27131	32513	-6,152		0.8		0.8	0.57	267-286 [10]
267-286 [20]	571	3346	533	473	358	1217	1083	1149	-32,200		0.0		0.0	0.00	* 267-286 [20]
307-326 [1]	36952	2774	447	5433	747	47844	15700	21041	-17,583		0.5		0.5	0.08	307-326 [1]
307-326 [10]	11039	19349	311	31983	7064	704	11742	12186	-21,541		0.4		0.4	0.02	* 307-326 [10]
307-326 [20]	2150	20197	3185	26225	22911	29662	17388	11841	-15,895		0.5		0.5	0.09	307-326 [20]
sAg 10	16121	11036	1849	18530	4898	15521	11326	6689	-21,957		0.3		0.3	0.02	* sAg 10
sAg 100	1372	33596	46821	11876	3128	7667	17410	18513	-15,873		0.5		0.5	0.10	sAg 100
N	34	30	75	36	376	54	101	136	-33,182		0.0		0.0	0.00	* N
N	76	60	157	24	26	14	60	53	-33,223		0.0		0.0	0.00	* N
N	35	35	45	390	26	58	98	143	-33,185		0.0		0.0	0.00	* N
3H	55504	29180	5584	23039	44580	33407	31882	17313	-1,401		1.0		1.0	0.88	3H
3H	33566	8564	1727	42768	47920	49642	30698	20681	-2,585		0.9		0.9	0.79	3H
3H	32361	60099	38142	30329	75938	8720	40932	23766	7,649	*	1.2		1.2	0.44	3H
3H	20071	32817	6180	65016	52083	1553	29620	25283	-3,663		0.9		0.9	0.72	3H
	BMC														
N	53	59	105	39	49	71	63	23	-152		0.0		0.3	0.00	* N
3H	126	154	166	273	298	272	215	74	0		1.0		1.0	1.00	3H
PHA - 1	2713	9346	17355	10096	16830	9799	11023	5441	10,808	*	72.0	*	51.3	* 0.00	* PHA - 1
PHA - 5	27464	38985	42873	39957	66823	41229	42889	12941	42,674	*	281.4	*	199.6	* 0.00	* PHA - 5
PHA - 10	20921	49003	48658	44420	53404	50966	44562	11955	44,347	*	292.4	*	207.4	* 0.00	* PHA - 10
LPS - 1	3974	9387	5986	10010	9839	8653	7975	2451	7,760	*	52.0	*	37.1	* 0.00	* LPS - 1
LPS - 5	8921	6927	6921	6705	5390	7277	7024	1136	6,809	*	45.7	*	32.7	* 0.00	* LPS - 5
LPS - 10	7896	6724	5236	8014	6464	4270	6434	1472	6,219	*	41.9	*	29.9	* 0.00	* LPS - 10
LPS - 20	2135	5022	5135	4148	4435	3866	4124	1091	3,909		26.7	*	19.2	* 0.00	* LPS - 20
LPS - 40	2266	2627	2219	2508	3337	2339	2549	415	2,335		16.3	*	11.9	* 0.00	* LPS - 40
	PBMC														
N	37	54	99	66	67	52	63	21	-15		0.0		0.8	0.18	N
3H	67	68	102	75	75	75	77	13	0		1.0		1.0	1.00	3H
PHA - 1	3020	1453	844	1334	4477	3892	2503	1504	2,426		168.3	*	32.5	* 0.00	* PHA - 1
PHA - 5	12797	17646	13178	14794	28332	39527	21046	10726	20,969	*	1447.1	*	273.3	* 0.00	* PHA - 5
PHA - 10	27737	14831	11347	4042	24406	29554	18653	10158	18,576	*	1282.1	*	242.2	* 0.00	* PHA - 10
LPS - 1	4128	2715	3283	2619	4097	6264	3851	1348	3,774		261.3	*	50.0	* 0.00	* LPS - 1
LPS - 5	3385	2619	3196	2347	4150	4666	3394	886	3,317		229.7	*	44.1	* 0.00	* LPS - 5
LPS - 10	3195	3031	2376	2520	3891	4809	3304	913	3,227		223.5	*	42.9	* 0.00	* LPS - 10
LPS - 20	2770	2308	3501	2505	3555	4209	3141	732	3,064		212.3	*	40.8	* 0.00	* LPS - 20
LPS - 40	1836	2096	3117	2652	3318	1822	2474	653	2,397		166.3	*	32.1	* 0.00	* LPS - 40
	preFBMC														
N	119	61	1690				623	924	378		0.0		2.5	* 0.52	N
3H	309	324	102				245	124	0		1.0		1.0	1.00	3H
RW1 - 20	193	244	286				241	47	-4		1.0		1.0	0.96	RW1 - 20
RW2 - 20	312	266	179				252	68	7		1.0		1.0	0.93	RW2 - 20
C - 20	122	255	31				136	113	-109		1.3		0.6	0.32	C - 20
E - 20	104	127	147				126	22	-119		1.3		0.5	0.18	E - 20
O - 20	124	142	133				133	9	-112		1.3		0.5	0.19	O - 20
Q - 20	101	103	83				96	11	-149		1.4		0.4	0.11	Q - 20
S - 20	211	336	71				206	133	-39		1.1		0.8	0.73	S - 20
sAg - 10	150	7213	905				2756	3878	2,511		-5.6		11.2	* 0.33	sAg - 10

Raw data for DNAvacc1 duck B68

B68	Mean	SD							CPM-3H		S.I.		P/N		t-Test		DNA vaccinated
Total N	107	58															
Total 3H	7772	7590															
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1		<0.05	
1-15 [1]	1876	1208	3763	552	4410	437	2041	1679	-5,731		0.3		0.3		0.08		1-15 [1]
1-15 [10]	26488	14021	55689	1639	6936	8601	18896	19910	11,124	*	2.5	*	2.4	*	0.03	*	1-15 [10]
1-15 [20]	38929	71213	75763	24517	42222	90266	57152	25537	49,380	*	7.4	*	7.4	*	0.00	*	1-15 [20]
7-14W-27 [1]	3627	12753	7941	14463	3395	13131	9218	4942	1,446		1.2		1.2		0.66		7-14W-27 [1]
7-14W-27 [10]	48849	91901	85745	63985	54707	26339	61921	24330	54,149	*	8.1	*	8.0	*	0.00	*	7-14W-27 [10]
7-14W-27 [20]	20366	47813	30104	16638	56704	54086	37619	17514	29,847	*	4.9	*	4.8	*	0.00	*	7-14W-27 [20]
71-90 [1]	2735	3604	3028	32455	1959	3078	7810	12086	38		1.0		1.0		0.99		71-90 [1]
71-90 [10]	2690	6137	4323	4856	1088	8344	4573	2550	-3,199		0.6		0.6		0.32		71-90 [10]
71-90 [20]	3244	35722	13866	1267	22461	2805	13228	13733	5,456	*	1.7		1.7		0.19		71-90 [20]
101-120 [1]	2066	3713	5315	6678	2265	5196	4206	1839	-3,566		0.5		0.5		0.27		101-120 [1]
101-120 [10]	8842	2235	165	1450	4673	558	2987	3284	-4,785		0.4		0.4		0.15		101-120 [10]
101-120 [20]	2699	2300	5842	5747	6601	2808	4333	1926	-3,439		0.6		0.6		0.29		101-120 [20]
229-248 [1]	5089	3346	9787	457	11936	8874	6582	4345	-1,190		0.8		0.8		0.72		229-248 [1]
229-248 [10]	8234	2579	21265	2157	7908	2428	7429	7330	-343		1.0		1.0		0.92		229-248 [10]
229-248 [20]	1545	15314	2939	3027	5218	10007	6342	5306	-1,430		0.8		0.8		0.67		229-248 [20]
267-286 [1]	2425	2191	14268	18720	11529	5771	9151	6760	1,379		1.2		1.2		0.69		267-286 [1]
267-286 [10]	3826	8218	16194	4769	52365	33504	19813	19373	12,041	*	2.6	*	2.5	*	0.02	*	267-286 [10]
267-286 [20]	49949	21657	32771	39452	45245	66507	42597	15347	34,825	*	5.5	*	5.5	*	0.00	*	267-286 [20]
307-326 [1]	201	6912	3296	4309	22185	34152	11843	13374	4,071		1.5		1.5		0.33		307-326 [1]
307-326 [10]	656	4223	1039	4080	1289	3085	2395	1596	-5,377		0.3		0.3		0.10		307-326 [10]
307-326 [20]	11955	4797	4746	17887	12091	26646	13020	8336	5,248	*	1.7		1.7		0.15		307-326 [20]
sAg 10	1022	5430	3608	1513	784	1478	2306	1829	-5,466		0.3		0.3		0.09		sAg 10
sAg 100	5971	14033	1883	3248	2540	1913	4931	4709	-2,841		0.6		0.6		0.39		sAg 100
N	51	86	85	117	60	30	72	31	-7,700		0.0		0.0		0.02	*	N
N	109	233	125	215	99	125	151	58	-7,621		0.0		0.0		0.02	*	N
N	47	55	195	105	58	127	98	57	-7,674		0.0		0.0		0.02	*	N
3H	5185	4408	2477	2269	5630	656	3438	1940	-4,334		0.4		0.4		0.18		3H
3H	3500	2767	3690	26872	4024	13040	8982	9569	1,210		1.2		1.2		0.74		3H
3H	2861	3497	13316	3931	3653	3736	5166	4009	-2,606		0.7		0.7		0.43		3H
3H	11551	12495	28290	3639	19074	5964	13502	9040	5,730	*	1.7		1.7		0.12		3H
	SMC																
N	54	42	26	27	137	93	63	44	-642		0.0		0.1		0.00	*	N
3H	637	371	708	936	632	949	706	217	0		1.0		1.0		1.00		3H
PHA - 1	10991	12385	19116	18955	24185	25559	18532	5942	17,826	*	28.8	*	26.3	*	0.00	*	PHA - 1
PHA - 5	30609	42621	48443	50677	38861	45990	42867	7324	42,161	*	66.6	*	60.8	*	0.00	*	PHA - 5
PHA - 10	19567	36666	42179	42763	46292	52706	40029	11332	39,323	*	62.2	*	56.7	*	0.00	*	PHA - 10
LPS - 1	1130	909	1269	1176	1141	1176	1134	120	428		1.7		1.6		0.00	*	LPS - 1
LPS - 5	645	1279	1461	1247	1777	7055	2244	2386	1,539		3.4	*	3.2	*	0.15		LPS - 5
LPS - 10	1101	1630	1492	1824	1775	1838	1610	282	905		2.4	*	2.3	*	0.00	*	LPS - 10
LPS - 20	1158	1498	1720	1764	1706	1661	1585	228	879		2.4	*	2.2	*	0.00	*	LPS - 20
LPS - 40	1073	1589	1312	1564	1084	1499	1354	234	648		2.0		1.9		0.00	*	LPS - 40
	PBMC																
N	31	24	82	110	74	34	59	35	-57		0.0		0.5		0.03	*	N
3H	76	86	153	104	95	180	116	41	0		1.0		1.0		1.00		3H
PHA - 1	962	644	828	1141	819	1060	909	181	793		15.0	*	7.9	*	0.00	*	PHA - 1
PHA - 5	1301	1624	951	1415	1888	2993	1695	709	1,580		29.0	*	14.7	*	0.00	*	PHA - 5
PHA - 10	2170	1109	1062	1171	2213	5098	2137	1544	2,022		36.8	*	18.5	*	0.01	*	PHA - 10
LPS - 1	1238	783	210	242	536	917	654	402	539		10.5	*	5.7	*	0.01	*	LPS - 1
LPS - 5	677	217	101	131	174	776	346	299	230		5.1	*	3.0	*	0.09		LPS - 5
LPS - 10	1123	259	147	152	148	875	451	434	335		6.9	*	3.9	*	0.09		LPS - 10
LPS - 20	1873	963	399	394	534	1124	881	573	766		14.5	*	7.6	*	0.01	*	LPS - 20
LPS - 40	1360	1263	1162	1083	1019	1218	1184	124	1,069		19.9	*	10.2	*	0.00	*	LPS - 40
	prePBMC																
N	61	74	55				63	10	-1		0.0		1.0		0.91		N
3H	72	52	69				64	11	0		1.0		1.0		1.00		3H
1-15 [20]	130	85	161				125	38	61		62.0	*	1.9		0.06		1-15 [20]
7-14W-27 [20]	205	963	253				474	424	409		410.3	*	7.4	*	0.17		7-14W-27 [20]
71-90 [20]	101	54	88				81	24	17		17.7	*	1.3		0.34		71-90 [20]
101-120 [20]	64	81	102				82	19	18		19.0	*	1.3		0.23		101-120 [20]
229-248 [20]	98	40	66				68	29	4		4.7	*	1.1		0.85		229-248 [20]
267-286 [20]	133	74	108				105	30	41		41.7	*	1.6		0.09		267-286 [20]
307-326 [20]	254	99	62				138	102	74		75.0	*	2.2	*	0.28		307-326 [20]
sAg - 10	7148	134	248				2510	4017	2,446		2446.7	*	39.0	*	0.35		sAg - 10

Raw data for DNAvacc1 duck G57

G57	Mean	SD												DNA vaccinated
Total N	48	14												
Total 3H	46990	25812						CPM-3H		S.I.		P/N		t-Test		
	R1	R2	R3	R4	R5	R6	Mean	SD	>5000		>2.1		>2.1	<0.05		
1-15 [1]	36900	38872	24741	40829	5341	35151	30306	13456	-16,685		0.6		0.6	0.14		1-15 [1]
1-15 [10]	99422	45841	29644	64074	84624	13377	56164	32816	9,173	*	1.2		1.2	0.47		1-15 [10]
1-15 [20]	85369	57137	77232	70486	32565	63630	64403	18484	17,413	*	1.4		1.4	0.13		1-15 [20]
7-14W-27 [1]	108163	57674	34965	24472	34305	30355	48322	31416	1,332		1.0		1.0	0.91		7-14W-27 [1]
7-14W-27 [10]	82543	75739	62928	78824	87246	97659	80823	11642	33,833	*	1.7		1.7	0.00	*	7-14W-27 [10]
7-14W-27 [20]	32023	2838	5474	83748	65774	6615	32745	34680	-14,245		0.7		0.7	0.27		7-14W-27 [20]
71-90 [1]	4992	889	28569	39537	11658	885	14422	16086	-32,569		0.3		0.3	0.01	*	71-90 [1]
71-90 [10]	10797	55892	8953	19062	18836	34340	24647	17739	-22,344		0.5		0.5	0.06		71-90 [10]
71-90 [20]	734	2085	38124	6420	9205	29621	14365	15643	-32,625		0.3		0.3	0.01	*	71-90 [20]
101-120 [1]	3200	17681	51972	82072	51861	1231	34670	32370	-12,321		0.7		0.7	0.33		101-120 [1]
101-120 [10]	51849	14486	38203	3685	2290	19629	21690	19692	-25,300		0.5		0.5	0.03	*	101-120 [10]
101-120 [20]	34637	5399	15258	75749	6500	64018	33594	30229	-13,397		0.7		0.7	0.28		101-120 [20]
229-248 [1]	5675	23686	29679	28972	70997	61284	36716	24583	-10,275		0.8		0.8	0.39		229-248 [1]
229-248 [10]	751	8385	47156	46327	41459	70738	35803	26353	-11,188		0.8		0.8	0.35		229-248 [10]
229-248 [20]	22138	40847	30189	26376	58511	46968	37505	13824	-9,485		0.8		0.8	0.40		229-248 [20]
267-286 [1]	36286	20902	1585	40367	7696	4377	18536	16749	-28,455		0.4		0.4	0.02	*	267-286 [1]
267-286 [10]	22339	23493	22802	70960	62591	79373	46926	26875	-64		1.0		1.0	1.00		267-286 [10]
267-286 [20]	87908	48763	23747	36338	59253	89089	57516	26792	10,526	*	1.2		1.2	0.38		267-286 [20]
307-326 [1]	22532	17053	938	6117	15778	1154	10595	9094	-36,395		0.2		0.2	0.00	*	307-326 [1]
307-326 [10]	33347	3025	31314	22341	56769	5745	25424	19892	-21,567		0.5		0.5	0.07		307-326 [10]
307-326 [20]	17137	46967	31505	3974	26209	46102	28649	16707	-18,341		0.6		0.6	0.11		307-326 [20]
sAg 10	55520	6265	51233	10873	65867	45948	39284	24718	-7,706		0.8		0.8	0.52		sAg 10
sAg 100	102352	78615	1940	3173	39292	88902	52379	43946	5,389	*	1.1		1.1	0.70		sAg 100
N	50	23	54	55	26	49	43	14	-46,947		0.0		0.0	0.00	*	N
N	44	53	56	46	56	63	53	7	-46,937		0.0		0.0	0.00	*	N
N	21	55	58	31	56	65	48	17	-46,943		0.0		0.0	0.00	*	N
3H	82992	43046	57668	66233	59021	91244	66701	17709	19,710	*	1.4		1.4	0.09		3H
3H	58101	8057	5036	69129	12634	55135	34682	29077	-12,308		0.7		0.7	0.32		3H
3H	37756	50336	32831	86454	57349	71305	56005	20324	9,015	*	1.2		1.2	0.43		3H
3H	8575	1524	41991	34295	47681	49374	30573	20582	-16,417		0.7		0.7	0.16		3H
	SMC															
N	48	39	60	67	52	132	66	34	-787		0.0		0.1	0.00	*	N
3H	739	374	844	426	932	1806	854	518	0		1.0		1.0	1.00		3H
PHA - 1	74877	70005	64200	60198	66557	78176	69002	6727	68,149	*	87.6	*	80.8	0.00	*	PHA - 1
PHA - 5	87392	78185	74167	71272	78098	107882	82833	13424	81,979	*	105.1	*	97.1	0.00	*	PHA - 5
PHA - 10	74646	54857	59578	62217	76678	89966	69657	13135	68,804	*	88.4	*	81.6	0.00	*	PHA - 10
LPS - 1	5097	7393	4490	5347	4987	5807	5520	1014	4,667		6.9	*	6.5	0.00	*	LPS - 1
LPS - 5	7884	9060	8557	10878	12733	13669	10464	2360	9,610	*	13.2	*	12.3	0.00	*	LPS - 5
LPS - 10	7996	15322	13563	15147	13022	22129	14530	4575	13,676	*	18.4	*	17.0	0.00	*	LPS - 10
LPS - 20	23549	16525	16568	16727	21516	19420	19051	2980	18,197	*	24.1	*	22.3	0.00	*	LPS - 20
LPS - 40	11810	7417	13224	14082	13181	7848	11260	2906	10,407	*	14.2	*	13.2	0.00	*	LPS - 40
	PBMC															
N	148	84	93	36	82	89	89	36	-16		0.0		0.8	0.37		N
3H	108	121	71	125	92	109	104	20	0		1.0		1.0	1.00		3H
PHA - 1	1322	1369	1415	1745	1998	2257	1684	383	1,580		101.9	*	16.1	0.00	*	PHA - 1
PHA - 5	4679	4675	6242	14463	22336	22488	12481	8508	12,376	*	791.0	*	119.6	0.01	*	PHA - 5
PHA - 10	9719	7196	7616	26433	36372	28161	19250	12615	19,145	*	1223.0	*	184.5	0.00	*	PHA - 10
LPS - 1	1247	932	1114	1354	2168	1719	1422	451	1,318		85.1	*	13.6	0.00	*	LPS - 1
LPS - 5	1195	977	606	1022	932	1847	1097	415	992		64.3	*	10.5	0.00	*	LPS - 5
LPS - 10	790	581	769	1353	1316	1513	1054	386	949		61.6	*	10.1	0.00	*	LPS - 10
LPS - 20	604	527	437	650	935	909	677	203	573		37.6	*	6.5	0.00	*	LPS - 20
LPS - 40	552	617	505	602	840	853	662	149	557		36.6	*	6.3	0.00	*	LPS - 40
	prePBMC															
N	62	52	60				58	5	-100		0.0		0.4	0.01	*	N
3H	184	113	178				158	39	0		1.0		1.0	1.00		3H
1-15 [20]	143	138	112				131	17	-27		0.7		0.8	0.33		1-15 [20]
7-14W-27 [20]	320	281	776				459	275	301		4.0	*	2.9	0.13		7-14W-27 [20]
71-90 [20]	147	144	140				144	4	-15		0.9		0.9	0.56		71-90 [20]
101-120 [20]	131	89	107				109	21	-49		0.5		0.7	0.13		101-120 [20]
229-248 [20]	329	127	263				240	103	81		1.8		1.5	0.27		229-248 [20]
267-286 [20]	333	320	448				367	70	209		3.1	*	2.3	0.01	*	267-286 [20]
307-326 [20]	153	121	129				134	17	-24		0.8		0.8	0.39		307-326 [20]
sAg - 10	182	138	345				222	109	63		1.6		1.4	0.40		sAg - 10

Raw data for DNAvacc1 duck G97

G97	Mean	SD												DNA vaccinated
Total N	53	33												
Total 3H	22571	24364							CPM-3H		S.I.	P/N	t-Test	
	R1	R2	R3	R4	R5	R6	Mean	SD	>5000		>2.1	>2.1	<0.05	
1-15 [1]	15773	9984	8349	5201	5107	4581	8166	4294	-14,405		0.4	0.4	0.17	1-15 [1]
1-15 [10]	9351	27809	15668	288	600	6709	10071	10423	-12,500		0.4	0.4	0.23	1-15 [10]
1-15 [20]	13397	33088	33866	2482	1397	892	14187	15636	-8,384		0.6	0.6	0.43	1-15 [20]
7-14W-27 [1]	6663	23476	3877	2911	29915	24522	15227	12034	-7,344		0.7	0.7	0.48	7-14W-27 [1]
7-14W-27 [10]	27210	63744	42037	56111	17204	55687	43666	18310	21,094	*	1.9	1.9	0.06	7-14W-27 [10]
7-14W-27 [20]	67373	74702	47482	29054	20888	28018	44586	22414	22,015	*	2.0	2.0	0.05	7-14W-27 [20]
71-90 [1]	69	2894	9395	27513	23570	117	10593	12132	-11,978		0.5	0.5	0.26	71-90 [1]
71-90 [10]	4948	43220	10584	28103	53907	4198	24160	21050	1,589		1.1	1.1	0.88	71-90 [10]
71-90 [20]	94973	37017	29487	10858	4093	16826	32209	33024	9,638	*	1.4	1.4	0.43	71-90 [20]
101-120 [1]	25280	4045	32619	18164	4691	235	14172	13163	-8,399		0.6	0.6	0.43	101-120 [1]
101-120 [10]	68765	4968	1552	17887	24241	807	19703	25825	-2,868		0.9	0.9	0.80	101-120 [10]
101-120 [20]	101	7710	7932	30456	27799	19505	15584	12215	-6,987		0.7	0.7	0.51	101-120 [20]
229-248 [1]	156	12907	2546	4728	64991	92	14237	25311	-8,335		0.6	0.6	0.46	229-248 [1]
229-248 [10]	212	107	1836	48257	168	104	8447	19514	-14,124		0.4	0.4	0.20	229-248 [10]
229-248 [20]	37312	43781	18649	1322	1609	6680	18226	18508	-4,346		0.8	0.8	0.69	229-248 [20]
267-286 [1]	466	40962	3077	200	22578	58838	21020	24528	-1,551		0.9	0.9	0.89	267-286 [1]
267-286 [10]	150	2128	10150	2467	641	6050	3598	3821	-18,974		0.2	0.2	0.07	267-286 [10]
267-286 [20]	9233	87994	30708	284	3329	2976	22421	33986	-151		1.0	1.0	0.99	267-286 [20]
307-326 [1]	27385	9529	3733	8640	6041	31493	14470	11843	-8,101		0.6	0.6	0.44	307-326 [1]
307-326 [10]	92	410	243	21703	6702	21854	8501	10585	-14,071		0.4	0.4	0.18	307-326 [10]
307-326 [20]	73	8090	3794	15604	399	73	4672	6205	-17,899		0.2	0.2	0.09	307-326 [20]
sAg 10	66687	15201	8523	26525	15237	974	22191	23379	-380		1.0	1.0	0.97	sAg 10
sAg 100	9050	10031	17681	6305	5734	26316	12520	7998	-10,052		0.6	0.6	0.33	sAg 100
N	23	63	31	23	89	158	65	53	-22,507		0.0	0.0	0.03	* N
N	31	59	45	53	21	29	40	15	-22,532		0.0	0.0	0.03	* N
N	38	59	44	43	55	92	55	20	-22,516		0.0	0.0	0.03	* N
3H	53053	15845	29656	22190	18503	8445	24615	15593	2,044		1.1	1.1	0.85	3H
3H	96	13040	1630	4036	47445	634	11147	18411	-11,424		0.5	0.5	0.29	3H
3H	138	49583	35358	13458	2285	87555	31396	33605	8,825	*	1.4	1.4	0.47	3H
3H	672	102	60894	51987	24832	273	23127	27636	555		1.0	1.0	0.96	3H
	SMC													
N	40	32	50	72	84	58	56	20	-363		0.0	0.1	0.00	* N
3H	328	269	321	389	538	667	419	153	0		1.0	1.0	1.00	3H
PHA - 1	38951	33931	31773	38924	33870	54021	38578	8109	38,160	*	106.2	* 92.1	* 0.00	* PHA - 1
PHA - 5	55025	39560	27756	36696	42458	57176	43112	11223	42,693	*	118.7	* 103.0	* 0.00	* PHA - 5
PHA - 10	38536	22103	21319	25919	37102	51723	32784	11858	32,365	*	90.2	* 78.3	* 0.00	* PHA - 10
LPS - 1	798	371	875	599	738	675	676	177	257		1.7	1.6	0.02	* LPS - 1
LPS - 5	527	428	674	534	520	787	578	129	160		1.4	1.4	0.08	LPS - 5
LPS - 10	635	410	428	430	638	776	553	152	134		1.4	1.3	0.16	LPS - 10
LPS - 20	661	391	302	357	367	546	437	137	19		1.1	1.0	0.83	LPS - 20
LPS - 40	314	368	244	299	559	434	370	113	-49		0.9	0.9	0.54	LPS - 40
	PBMC													
N	41	61	47	49	49	60	51	8	-60		0.0	0.5	0.00	* N
3H	104	139	81	110	81	152	111	29	0		1.0	1.0	1.00	3H
PHA - 1	453	625	509	378	1033	5160	1360	1876	1,249		21.8	* 12.2	* 0.13	PHA - 1
PHA - 5	6023	4806	1698	5194	5175	23613	7752	7913	7,640	*	128.3	* 69.7	* 0.04	* PHA - 5
PHA - 10	5818	6574	3822	4754	5401	14728	6850	3972	6,738	*	113.3	* 61.6	* 0.00	* PHA - 10
LPS - 1	646	1105	1739	1881	1282	442	1183	574	1,071		18.9	* 10.6	* 0.00	* LPS - 1
LPS - 5	536	837	1116	957	1183	389	836	317	725		13.1	* 7.5	* 0.00	* LPS - 5
LPS - 10	341	976	737	855	926	463	716	259	605		11.1	* 6.4	* 0.00	* LPS - 10
LPS - 20	189	847	861	747	692	406	624	269	513		9.5	* 5.6	* 0.00	* LPS - 20
LPS - 40	167	408	571	441	458	204	375	157	264		5.4	* 3.4	* 0.00	* LPS - 40
	prePBMC													
N	17	21	20				19	2	-129		0.0	0.1	0.00	* N
3H	123	131	192				149	38	0		1.0	1.0	1.00	3H
1-15 [20]	146	255	322				241	89	92		1.7	1.6	0.17	1-15 [20]
7-14W-27 [20]	187	246	422				285	122	136		2.1	1.9	0.14	7-14W-27 [20]
71-90 [20]	142	137	171				150	18	1		1.0	1.0	0.96	71-90 [20]
101-120 [20]	270	218	525				338	164	189		2.5	* 2.3	* 0.12	101-120 [20]
229-248 [20]	232	210	256				233	23	84		1.6	1.6	0.03	* 229-248 [20]
267-286 [20]	62		250				156	133	7		1.1	1.0	0.93	267-286 [20]
307-326 [20]	310	185	271				255	64	107		1.8	1.7	0.07	307-326 [20]
sAg - 10	94	58691	125				19637	33822	19,488	*	151.7	* 132.1	* 0.37	sAg - 10

Raw data for DNAvacc1 duck G98

G98	Mean	SD														DNA vaccinated
Total N	57	35														
Total 3H	320	424							CPM-3H		S.I.		P/N		t-Test	
	R1	R2	R3	R4	R5	R6	Mean	SD	>5000		>2.1		>2.1		<0.05	
1-15 [1]	176	142	162	163	221	927	299	309	-21		0.9		0.9		0.91	1-15 [1]
1-15 [10]	337	207	413	295	2022	289	594	703	274		2.0		1.9		0.23	1-15 [10]
1-15 [20]	63	129	107	86	125	260	128	69	-191		0.3		0.4		0.29	1-15 [20]
7-14W-27 [1]	936	135	189	151	579	123	352	334	33		1.1		1.1		0.86	7-14W-27 [1]
7-14W-27 [10]	134	208	151	106	194	74	145	51	-175		0.3		0.5		0.33	7-14W-27 [10]
7-14W-27 [20]	177	155	153	150	75	134	141	35	-179		0.3		0.4		0.32	7-14W-27 [20]
71-90 [1]	105	178	99	119	251	133	148	58	-172		0.3		0.5		0.34	71-90 [1]
71-90 [10]	126	158	240	112	192	195	171	48	-149		0.4		0.5		0.40	71-90 [10]
71-90 [20]	86	125	105	101	155	144	119	27	-200		0.2		0.4		0.26	71-90 [20]
101-120 [1]	145	225	176	161	111	94	152	47	-168		0.4		0.5		0.35	101-120 [1]
101-120 [10]	122	93	268	95	171	157	151	66	-169		0.4		0.5		0.35	101-120 [10]
101-120 [20]	149	97	496	140	101	466	242	187	-78		0.7		0.8		0.67	101-120 [20]
229-248 [1]	106	96	133	126	420	161	174	123	-146		0.4		0.5		0.42	229-248 [1]
229-248 [10]	154	186	360	196	220	207	221	72	-99		0.6		0.7		0.58	229-248 [10]
229-248 [20]	147	191	154	165	129	200	164	27	-155		0.4		0.5		0.38	229-248 [20]
267-286 [1]	89	142	588	179	224	334	259	181	-60		0.8		0.8		0.74	267-286 [1]
267-286 [10]	156	119	129	623	360	2694	680	1005	361		2.4	*	2.1	*	0.18	267-286 [10]
267-286 [20]	153	81	397	188	1332	542	449	465	129		1.5		1.4		0.52	267-286 [20]
307-326 [1]	107	106	130	147	159	249	150	53	-170		0.4		0.5		0.34	307-326 [1]
307-326 [10]	127	81	87	171	182	121	128	42	-191		0.3		0.4		0.29	307-326 [10]
307-326 [20]	125	115	308	702	137	322	285	225	-35		0.9		0.9		0.85	307-326 [20]
sAg 10	133	148	89	2740	612	252	662	1035	343		2.3	*	2.1		0.21	sAg 10
sAg 100	734	159	5042	2006	506	2573	1837	1825	1,517		6.8	*	5.7	*	0.00	* sAg 100
N	97	107	81	16	14	34	58	42	-261		0.0		0.2		0.15	N
N	87	28	37	32	21	57	44	24	-276		-0.1		0.1		0.13	N
N	11	46	57	102	93	107	69	38	-250		0.0		0.2		0.17	N
3H	291	66	134	75	569	254	232	189	-88		0.7		0.7		0.63	3H
3H	104	271	100	49	138	195	143	79	-177		0.3		0.4		0.32	3H
3H	65	183	141	2002	671	69	522	760	202		1.8		1.6		0.38	3H
3H	429	244	281	185	1016	136	382	326	62		1.2		1.2		0.74	3H
	SMC															
N	62	81	87	101	61	54	74	18	-180		0.0		0.3		0.00	* N
3H	364	189	231	315	149	276	254	80	0		1.0		1.0		1.00	3H
PHA - 1	5662	17592	16306	13642	9079	15279	12927	4622	12,673	*	71.5	*	50.9	*	0.00	* PHA - 1
PHA - 5	45791	39762	38744	49986	54956	59007	48041	8147	47,787	*	267.0	*	189.1	*	0.00	* PHA - 5
PHA - 10	33662	48977	41083	46385	61599	45669	46229	9258	45,975	*	256.9	*	182.0	*	0.00	* PHA - 10
LPS - 1	2034	4412	3041	4075	3840	2941	3391	881	3,137		18.5	*	13.3	*	0.00	* LPS - 1
LPS - 5	2345	6317	4695	6934	4700	2919	4652	1806	4,398		25.5	*	18.3	*	0.00	* LPS - 5
LPS - 10	3502	3771	2365	7007	12425	2325	5233	3916	4,979		28.7	*	20.6	*	0.01	* LPS - 10
LPS - 20	2580	4666	10981	3445	5739	2587	5000	3179	4,746		27.4	*	19.7	*	0.00	* LPS - 20
LPS - 40	2838	3434	2337	1366	1303	1500	2130	884	1,876		11.4	*	8.4	*	0.00	* LPS - 40
	PBMC															
N	60	101	77	36	22	46	57	29	-30		0.0		0.7		0.05	N
3H	72	89	111	83	96	69	87	16	0		1.0		1.0		1.00	3H
PHA - 1	1013	3220	2241	2441	1152	1684	1959	839	1,872		64.1	*	22.6	*	0.00	* PHA - 1
PHA - 5	6405	17137	20432	24816	19345	14164	17050	6303	16,963	*	572.8	*	196.7	*	0.00	* PHA - 5
PHA - 10	31945	31056	26562	40491	35846	56036	36989	10447	36,903	*	1244.9	*	426.8	*	0.00	* PHA - 10
LPS - 1	1493	1324	1276	972	1869	1805	1457	340	1,370		47.2	*	16.8	*	0.00	* LPS - 1
LPS - 5	1167	1141	938	513	1045	2104	1151	524	1,065		36.9	*	13.3	*	0.00	* LPS - 5
LPS - 10	1090	1125	1103	647	683	921	928	217	842		29.4	*	10.7	*	0.00	* LPS - 10
LPS - 20	1073	871	605	347	852	773	754	250	667		23.5	*	8.7	*	0.00	* LPS - 20
LPS - 40	448	224	414	358	496	343	381	95	294		10.9	*	4.4	*	0.00	* LPS - 40
	prePBMC															
N	23	5	82				37	40	-47		0.0		0.4		0.23	N
3H	92	38	122				84	43	0		1.0		1.0		1.00	3H
1-15 [20]	91	64	278				144	117	60		2.3	*	1.7		0.45	1-15 [20]
7-14W-27 [20]	120	159	241				173	62	89		2.9	*	2.1		0.11	7-14W-27 [20]
71-90 [20]	77	35	109				74	37	-10		0.8		0.9		0.77	71-90 [20]
101-120 [20]	84	61	84				76	13	-8		0.8		0.9		0.78	101-120 [20]
229-248 [20]	94	82	172				116	49	32		1.7		1.4		0.44	229-248 [20]
267-286 [20]	55	57	109				74	31	-10		0.8		0.9		0.75	267-286 [20]
307-326 [20]	59	2186	121				789	1211	705		15.9	*	9.4	*	0.37	307-326 [20]
sAg - 10	130	10919	203				3751	6208	3,667		78.5	*	44.7	*	0.36	sAg - 10

Raw data for DNAvacc1 duck W39

W39	Mean	SD															DNA vaccinated
Total N	46	44															
Total 3H	71093	20089							CPM-3H		S.I.		P/N		t-Test		
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1		<0.05	
1-15 [1]	73424	58848	57323	81021	63507	17095	58536	22230	-12,556		0.8		0.8		0.19		1-15 [1]
1-15 [10]	42761	66733	62360	57113	81815	55593	61063	13000	-10,030		0.9		0.9		0.26		1-15 [10]
1-15 [20]	88333	97633	93228	59195	83020	105158	87761	15930	16,668	*	1.2		1.2		0.07		1-15 [20]
7-14W-27 [1]	70253	70984	41166	61477	61711	76735	63721	12511	-7,372		0.9		0.9		0.40		7-14W-27 [1]
7-14W-27 [10]	85592	83783	83239	61735	103552	53677	78596	18030	7,504	*	1.1		1.1		0.41		7-14W-27 [10]
7-14W-27 [20]	91906	56637	55676	54466	86080	82157	71154	17340	61		1.0		1.0		0.99		7-14W-27 [20]
71-90 [1]	50894	50587	63451	18353	51151	79030	52244	19987	-18,848		0.7		0.7		0.05	*	71-90 [1]
71-90 [10]	14797	82046	37927	73730	59934	70942	56563	25510	-14,530		0.8		0.8		0.14		71-90 [10]
71-90 [20]	65073	42042	16265	61594	55087	52523	48764	17811	-22,329		0.7		0.7		0.02	*	71-90 [20]
101-120 [1]	19906	5867	70508	27264	20462	61474	34247	25714	-36,846		0.5		0.5		0.00	*	101-120 [1]
101-120 [10]	21375	81767	100865	61976	47641	62297	62654	27406	-8,439		0.9		0.9		0.40		101-120 [10]
101-120 [20]	82554	69854	66394	95634	84571	64093	77183	12372	6,091	*	1.1		1.1		0.49		101-120 [20]
229-248 [1]	44025	22327	44574	9530	50219	84357	42505	25738	-28,587		0.6		0.6		0.01	*	229-248 [1]
229-248 [10]	70773	38803	63318	73691	77259	57833	63613	14058	-7,480		0.9		0.9		0.40		229-248 [10]
229-248 [20]	15519	39473	85289	29373	74531	65424	51602	27549	-19,491		0.7		0.7		0.06		229-248 [20]
267-286 [1]	36943	45226	35778	15491	61364	83668	46412	23545	-24,681		0.7		0.7		0.01	*	267-286 [1]
267-286 [10]	110469	72551	84686	10982	65936	71742	69394	32743	-1,698		1.0		1.0		0.87		267-286 [10]
267-286 [20]	83128	82665	93582	44870	63078	67364	72448	17546	1,355		1.0		1.0		0.88		267-286 [20]
307-326 [1]	63137	65195	64773	50953	66679	79429	65028	9076	-6,065		0.9		0.9		0.48		307-326 [1]
307-326 [10]	43213	79909	51962	52819	50992	61940	56806	12791	-14,287		0.8		0.8		0.11		307-326 [10]
307-326 [20]	48043	97781	84296	59689	82027	58149	71664	19147	571		1.0		1.0		0.95		307-326 [20]
sAg 10	58864	90343	95869	97492	78746	53181	79083	19117	7,990	*	1.1		1.1		0.39		sAg 10
sAg 100	71732	43584	45935	93525	84348	75184	69051	20301	-2,041		1.0		1.0		0.83		sAg 100
N	49	27	31	40	36	22	34	10	-71,059		0.0		0.0		0.00	*	N
N	46	39	40	42	32	31	38	6	-71,054		0.0		0.0		0.00	*	N
N	28	33	34	45	35	219	66	75	-71,027		0.0		0.0		0.00	*	N
3H	72981	61477	62821	31869	77061	74354	63427	16713	-7,666		0.9		0.9		0.40		3H
3H	93596	87434	56762	90824	86829	40880	76054	21821	4,961		1.1		1.1		0.60		3H
3H	82234	84878	77174	37413	95911	77448	75843	20032	4,750		1.1		1.1		0.61		3H
3H	47998	45804	110194	62049	68592	79643	69047	23824	-2,046		1.0		1.0		0.83		3H
	SMC																
N	51	49	35	54	27	102	53	26	-653		0.0		0.1		0.00	*	N
3H	489	498	809	941	359	1141	706	305	0		1.0		1.0		1.00		3H
PHA - 1	59991	50685	65478	58741	62816	64581	60382	5411	59,676	*	92.4	*	85.5	*	0.00	*	PHA - 1
PHA - 5	70629	71894	74061	73268	67490	77066	72401	3249	71,695	*	110.8	*	102.5	*	0.00	*	PHA - 5
PHA - 10	80413	51550	68453	64655	72263	69178	67752	9542	67,046	*	103.6	*	95.9	*	0.00	*	PHA - 10
LPS - 1	3922	7536	5024	4654	7570	6636	5890	1565	5,184	*	8.9	*	8.3	*	0.00	*	LPS - 1
LPS - 5	9742	6965	9222	8774	9898	10227	9138	1182	8,432	*	13.9	*	12.9	*	0.00	*	LPS - 5
LPS - 10	15479	22926	12378	13285	17619	16285	16329	3763	15,623	*	24.9	*	23.1	*	0.00	*	LPS - 10
LPS - 20	23641	34046	22671	25995	24131	28074	26426	4197	25,720	*	40.4	*	37.4	*	0.00	*	LPS - 20
LPS - 40	22259	39964	33478	29212	42814	27301	32505	7820	31,799	*	49.7	*	46.0	*	0.00	*	LPS - 40
	PBMC																
N	40	40	50	129	145	51	76	48	-45		0.0		0.6		0.10		N
3H	136	61	91	162	154	123	121	39	0		1.0		1.0		1.00		3H
PHA - 1	4924	5875	4515	4300	8052	4852	5420	1398	5,299	*	117.9	*	44.7	*	0.00	*	PHA - 1
PHA - 5	43896	50802	36063	63774	53315	71058	53151	12781	53,030	*	1170.8	*	438.7	*	0.00	*	PHA - 5
PHA - 10	70971	84411	69123	81342	65012	69960	73470	7625	73,349	*	1619.0	*	606.4	*	0.00	*	PHA - 10
LPS - 1	9650	10019	11531	9819	11182	13036	11006	1584	10,885	*	241.1	*	90.8	*	0.00	*	LPS - 1
LPS - 5	8545	9440	9957	11555	8273	10179	9658	1197	9,537	*	211.4	*	79.7	*	0.00	*	LPS - 5
LPS - 10	6859	9033	8817	8841	9045	9845	8740	995	8,619	*	191.1	*	72.1	*	0.00	*	LPS - 10
LPS - 20	8150	8751	7491	10276	8490	8774	8655	926	8,534	*	189.3	*	71.4	*	0.00	*	LPS - 20
LPS - 40	7050	7932	8056	8748	6345	8731	7810	952	7,689	*	170.6	*	64.5	*	0.00	*	LPS - 40
	prePBMC																
N	20	41	31				31	11	-31		0.0		0.5		0.16		N
3H	66	89	31				62	29	0		1.0		1.0		1.00		3H
1-15 [20]	143	97	85				108	31	46		2.5	*	1.7		0.13		1-15 [20]
7-14W-27 [20]	179	111	135				142	34	80		3.5	*	2.3	*	0.04	*	7-14W-27 [20]
71-90 [20]	50	47	53				50	3	-12		0.6		0.8		0.52		71-90 [20]
101-120 [20]	96	64	94				85	18	23		1.7		1.4		0.32		101-120 [20]
229-248 [20]	72	62	53				62	10	0		1.0		1.0		0.99		229-248 [20]
267-286 [20]	52	83	49				61	19	-1		1.0		1.0		0.98		267-286 [20]
307-326 [20]	77	85	299				154	126	92		3.9	*	2.5	*	0.29		307-326 [20]
sAg - 10	283	155	102				180	93	118		4.8	*	2.9	*	0.10		sAg - 10

Raw data for DNAvacc1 duck W133

W133	Mean	SD							CPM-3H		S.I.		P/N		t-Test		DNA vaccinated
Total N	48	22															
Total 3H	6144	7827															
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1		<0.05	
1-15 [1]	7804	6284	9669	417	6966	447	5265	3911	-879		0.9		0.9		0.79		1-15 [1]
1-15 [10]	13261	28800	7653	5287	7879	3297	11030	9326	4,886		1.8		1.8		0.20		1-15 [10]
1-15 [20]	8689	5280	1530	5476	3450	11341	5961	3550	-183		1.0		1.0		0.96		1-15 [20]
7-14W-27 [1]	253	2608	16668	5793	1368	2620	4885	6063	-1,259		0.8		0.8		0.72		7-14W-27 [1]
7-14W-27 [10]	5247	15276	48688	33491	25986	28462	26192	14983	20,048	*	4.3	*	4.3	*	0.00	*	7-14W-27 [10]
7-14W-27 [20]	73034	73597	54444	70798	18513	64530	59153	21164	53,009	*	9.7	*	9.6	*	0.00	*	7-14W-27 [20]
71-90 [1]	2439	320	6830	1271	4790	4832	3414	2477	-2,730		0.6		0.6		0.41		71-90 [1]
71-90 [10]	1083	130	1306	227	1323	2864	1156	989	-4,988		0.2		0.2		0.14		71-90 [10]
71-90 [20]	244	465	103	511	1130	11544	2333	4526	-3,811		0.4		0.4		0.27		71-90 [20]
101-120 [1]	338	2435	677	132	284	1153	837	864	-5,307		0.1		0.1		0.11		101-120 [1]
101-120 [10]	135	156	935	1006	7813	2136	2030	2926	-4,113		0.3		0.3		0.22		101-120 [10]
101-120 [20]	330	1196	7852	9392	11025	1592	5231	4718	-912		0.9		0.9		0.79		101-120 [20]
229-248 [1]	205	266	23269	1843	5779	191	5259	9082	-885		0.9		0.9		0.81		229-248 [1]
229-248 [10]	4667	2254	344	180	538	2312	1716	1731	-4,428		0.3		0.3		0.18		229-248 [10]
229-248 [20]	2975	795	2801	4040	4780	2321	2952	1386	-3,192		0.5		0.5		0.33		229-248 [20]
267-286 [1]	440	175	179	10418	674	3543	2572	4053	-3,572		0.4		0.4		0.29		267-286 [1]
267-286 [10]	4908	5619	153	3328	180	15171	4893	5537	-1,250		0.8		0.8		0.72		267-286 [10]
267-286 [20]	16160	1314	1705	1607	1052	940	3796	6064	-2,347		0.6		0.6		0.50		267-286 [20]
307-326 [1]	120	1124	195	3961	153	1155	1118	1474	-5,026		0.2		0.2		0.13		307-326 [1]
307-326 [10]	6206	1915	525	4047	618	2107	2570	2193	-3,574		0.4		0.4		0.28		307-326 [10]
307-326 [20]	10105	1006	574	968	4131	2661	3241	3620	-2,903		0.5		0.5		0.39		307-326 [20]
sAg 10	10665	6322	33486	1741	2327	2599	9523	12215	3,380		1.6		1.6		0.41		sAg 10
sAg 100	6134	24217	10494	14664	5513	4380	10900	7562	4,757		1.8		1.8		0.19		sAg 100
N	48	86	45	51	76	58	61	17	-6,083		0.0		0.0		0.07		N
N	41	36	59	98	45	33	52	24	-6,092		0.0		0.0		0.07		N
N	61	37	43	17	16	20	32	18	-6,111		0.0		0.0		0.07		N
3H	4484	23769	5940	20401	5053	9088	11456	8453	5,312	*	1.9		1.9		0.15		3H
3H	514	1858	12243	4053	6528	464	4277	4541	-1,867		0.7		0.7		0.58		3H
3H	991	1225	1349	3827	675	616	1447	1201	-4,696		0.2		0.2		0.16		3H
3H	652	1814	29244	6777	166	5716	7395	11040	1,251		1.2		1.2		0.75		3H
	SMC																
N	76	82	109	133	96	113	102	21	-526		0.0		0.2		0.00	*	N
3H	698	937	473	416	625	617	628	184	0		1.0		1.0		1.00		3H
PHA - 1	50882	42622	40565	46303	41411	47895	44946	4071	44,319	*	85.2	*	71.6	*	0.00	*	PHA - 1
PHA - 5	58185	69860	70720	67933	66926	75603	68205	5760	67,577	*	129.4	*	108.7	*	0.00	*	PHA - 5
PHA - 10	58537	66450	55785	62311	67182	82793	65510	9548	64,882	*	124.3	*	104.4	*	0.00	*	PHA - 10
LPS - 1	1148	1274	1558	1506	1723	1569	1463	212	835		2.6	*	2.3	*	0.00	*	LPS - 1
LPS - 5	2820	1935	1727	2487	1520	1711	2033	509	1,406		3.7	*	3.2	*	0.00	*	LPS - 5
LPS - 10	2900	2222	2940	5459	3311	2971	3301	1115	2,673		6.1	*	5.3	*	0.00	*	LPS - 10
LPS - 20	3700	6535	6507	6907	5883	5653	5864	1156	5,237	*	11.0	*	9.3	*	0.00	*	LPS - 20
LPS - 40	6686	9091	6549	7616	5908	6214	7011	1172	6,383	*	13.1	*	11.2	*	0.00	*	LPS - 40
	PBMC																
N	113	84	60	55	37	21	62	33	-63		0.0		0.5		0.02	*	N
3H	114	102	145	205	107	72	124	46	0		1.0		1.0		1.00		3H
PHA - 1	2213	1255	948	867	2881	4049	2036	1262	1,911		31.6	*	16.4	*	0.00	*	PHA - 1
PHA - 5	64406	76613	97881	93870	83902	86957	83938	12143	83,814	*	1342.0	*	676.0	*	0.00	*	PHA - 5
PHA - 10	87351	81721	91772	97040	86491	83745	88020	5589	87,896	*	1407.3	*	708.9	*	0.00	*	PHA - 10
LPS - 1	1043	697	577	812	532	1084	791	233	667		11.7	*	6.4	*	0.00	*	LPS - 1
LPS - 5	942	752	858	523	1168	1163	901	248	777		13.4	*	7.3	*	0.00	*	LPS - 5
LPS - 10	811	835	558	666	770	1033	779	161	655		11.5	*	6.3	*	0.00	*	LPS - 10
LPS - 20	584	530	798	758	803	1030	751	179	626		11.0	*	6.0	*	0.00	*	LPS - 20
LPS - 40	720	690	753	671	528	736	683	82	559		9.9	*	5.5	*	0.00	*	LPS - 40
	prePBMC																
N	61	64	53				59	6	-66		0.0		0.5		0.17		N
3H	66	109	200				125	68	0		1.0		1.0		1.00		3H
1-15 [20]	154	187	181				174	18	49		1.7		1.4		0.30		1-15 [20]
7-14W-27 [20]	589	58	46				231	310	106		2.6	*	1.8		0.59		7-14W-27 [20]
71-90 [20]	36	21	44				34	12	-91		-0.4		0.3		0.08		71-90 [20]
101-120 [20]	53	106	104				88	30	-37		0.4		0.7		0.44		101-120 [20]
229-248 [20]	127	114	115				119	7	-6		0.9		0.9		0.88		229-248 [20]
267-286 [20]	57	104	100				87	26	-38		0.4		0.7		0.42		267-286 [20]
307-326 [20]	45	89	66				67	22	-58		0.1		0.5		0.23		307-326 [20]
sAg - 10	134	850	577				520	361	395		7.0	*	4.2	*	0.14		sAg - 10

Raw data for Dv1 control duck G92

G92	Mean	SD															unvaccinated challenged
Total N	63	39															
Total 3H	11020	15365							CPM-3H		S.I.		P/N		t-Test		
	R1	R2	R3	R4	R5	R6	Mean	SD	>5000		>2.1		>2.1		<0.05		
1-15 [1]	4139	9661	1282	4088	13057	21565	8965	7511	-2,055		0.8		0.8		0.75		1-15 [1]
1-15 [10]	2013	260	10373	576	10651	3285	4526	4762	-6,494		0.4		0.4		0.32		1-15 [10]
1-15 [20]	1257	20264	5348	1407	2301	4392	5828	7260	-5,192		0.5		0.5		0.43		1-15 [20]
7-14W-27 [1]	15983	6851	10500	62873	3180	3237	17104	22939	6,084	*	1.6		1.6		0.44		7-14W-27 [1]
7-14W-27 [10]	24935	60887	22636	29924	33816	4037	29373	18548	18,353	*	2.7	*	2.7	*	0.02	*	7-14W-27 [10]
7-14W-27 [20]	59717	26204	28738	72028	19371	75704	46960	25065	35,940	*	4.3	*	4.3	*	0.00	*	7-14W-27 [20]
71-90 [1]	2828	5311	3818	6020	2546	6811	4556	1753	-6,464		0.4		0.4		0.32		71-90 [1]
71-90 [10]	10881	534	4891	1582	1043	12910	5307	5364	-5,713		0.5		0.5		0.38		71-90 [10]
71-90 [20]	1011	3753	5291	3691	2799	1758	3051	1537	-7,969		0.3		0.3		0.22		71-90 [20]
101-120 [1]	4245	7259	2026	1675	15335	7721	6377	5068	-4,643		0.6		0.6		0.48		101-120 [1]
101-120 [10]	1073	5471	2543	6399	1691	2920	3350	2125	-7,670		0.3		0.3		0.24		101-120 [10]
101-120 [20]	630	5061	952	2984	5167	4372	3194	2020	-7,826		0.3		0.3		0.23		101-120 [20]
229-248 [1]	1847	4822	2527	1618	4244	3284	3057	1295	-7,963		0.3		0.3		0.22		229-248 [1]
229-248 [10]	1538	3336	4425	23853	4703	2108	6661	8514	-4,359		0.6		0.6		0.51		229-248 [10]
229-248 [20]	4165	1561	4127	595	855	10665	3661	3774	-7,359		0.3		0.3		0.26		229-248 [20]
267-286 [1]	5270	6709	1922	999	4440	5644	4164	2237	-6,856		0.4		0.4		0.29		267-286 [1]
267-286 [10]	6833	907	2428	6490	3564	2700	3820	2364	-7,200		0.3		0.3		0.27		267-286 [10]
267-286 [20]	4307	38714	10213	10518	11014	17923	15448	12190	4,428		1.4		1.4		0.52		267-286 [20]
307-326 [1]	6112	2340	2238	2168	26775	7266	7817	9546	-3,203		0.7		0.7		0.63		307-326 [1]
307-326 [10]	6938	4939	327	3828	7372	9610	5502	3235	-5,518		0.5		0.5		0.39		307-326 [10]
307-326 [20]	3855	1514	6502	22915	4024	1716	6754	8123	-4,266		0.6		0.6		0.52		307-326 [20]
sAg 10	1401	2432	7859	1919	6294	19855	6627	6981	-4,393		0.6		0.6		0.50		sAg 10
sAg 100	9391	24930	3027	6746	4533	2374	8500	8450	-2,520		0.8		0.8		0.70		sAg 100
N	64	51	33	42	55	141	64	39	-10,956		0.0		0.0		0.10		N
N	125	27	25	40	35	78	55	39	-10,965		0.0		0.0		0.10		N
N	31	145	42	48	102	48	69	45	-10,951		0.0		0.0		0.10		N
3H	1103	4667	4819	440	1781	1251	2344	1908	-8,676		0.2		0.2		0.18		3H
3H	9456	8073	1098	2987	7622	16563	7633	5432	-3,387		0.7		0.7		0.60		3H
3H	28902	2493	656	37061	3257	8785	13526	15528	2,506		1.2		1.2		0.72		3H
3H	16646	68122	5206	3888	21686	7917	20578	24299	9,558	*	1.9		1.9		0.24		3H
	BMC																
N	52	60	132	113	171	65	99	48	-1,595		0.0		0.1		0.00	*	N
3H	2209	930	2145	1110	1571	2199	1694	577	0		1.0		1.0		1.00		3H
PHA - 1	83894	72968	70485	88689	93805	73104	80491	9668	78,797	*	50.4	*	47.5	*	0.00	*	PHA - 1
PHA - 5	96174	67459	67644	70716	71976	61016	72498	12205	70,804	*	45.4	*	42.8	*	0.00	*	PHA - 5
PHA - 10	83853	69736	68578	70183	79565	67907	73304	6699	71,610	*	45.9	*	43.3	*	0.00	*	PHA - 10
LPS - 1	2908	2318	1963	1895	1798	1837	2120	429	426		1.3		1.3		0.18		LPS - 1
LPS - 5	2282	1776	1199	1741	1907	2498	1901	455	207		1.1		1.1		0.51		LPS - 5
LPS - 10	1864	2454	1697	1488	1989	1749	1874	330	180		1.1		1.1		0.52		LPS - 10
LPS - 20	2450	2615	1743	2181	2396	1803	2198	358	504		1.3		1.3		0.10		LPS - 20
LPS - 40	2311	1663	1944	2554	3072	2671	2369	510	675		1.4		1.4		0.06		LPS - 40
	PBMC																
N	73	91	67	57	25	55	61	22	-86		0.0		0.4		0.06		N
3H	93	222	92	78	84	314	147	98	0		1.0		1.0		1.00		3H
PHA - 1	3881	5677	5769	5369	6445	3513	5109	1155	4,962		58.8	*	34.7	*	0.00	*	PHA - 1
PHA - 5	28090	33652	34160	51766	69580	59388	46106	16624	45,959	*	536.4	*	313.3	*	0.00	*	PHA - 5
PHA - 10	52539	58189	59573	61218	77637	61049	61701	8431	61,554	*	718.1	*	419.3	*	0.00	*	PHA - 10
LPS - 1	3992	4046	3643	3508	4479	4413	4014	393	3,866		46.0	*	27.3	*	0.00	*	LPS - 1
LPS - 5	4158	3134	3649	2813	3583	3687	3504	470	3,357		40.1	*	23.8	*	0.00	*	LPS - 5
LPS - 10	5215	4760	3250	2723	4346	4071	4061	933	3,914		46.6	*	27.6	*	0.00	*	LPS - 10
LPS - 20	3289	3994	3590	4054	4264	3159	3725	447	3,578		42.7	*	25.3	*	0.00	*	LPS - 20
LPS - 40	2639	2872	2486	2238	2489	1888	2435	339	2,288		27.7	*	16.5	*	0.00	*	LPS - 40
	prePBMC																
N	64	60	39				54	13	-31		0.0		0.6		0.04	*	N
3H	79	59	77				85	12	0		1.0		1.0		1.00		3H
1-15 [20]	3384	1709	553				1882	1423	1,797		59.6	*	22.1	*	0.09		1-15 [20]
7-14W-27 [20]	3507	4516	844				2956	1897	2,871		94.6	*	34.8	*	0.06		7-14W-27 [20]
71-90 [20]	426	1044	549				673	327	588		20.2	*	7.9	*	0.04	*	71-90 [20]
101-120 [20]	839	576	808				741	144	656		22.4	*	8.7	*	0.00	*	101-120 [20]
229-248 [20]	713	494	976				728	241	643		22.0	*	8.6	*	0.01	*	229-248 [20]
267-286 [20]	884	840	350				691	296	606		20.8	*	8.1	*	0.02	*	267-286 [20]
307-326 [20]	629	421	843				631	211	546		18.8	*	7.4	*	0.01	*	307-326 [20]
sAg - 10	1003	3423	5672				3366	2335	3,281		108.0	*	39.6	*	0.07		sAg - 10

Raw data for Dv1 control duck G93

G93	Mean	SD															unvaccinated challenged
Total N	253	270															
Total 3H	697	687							CPM-3H		S.I.		P/N		t-Test		
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1		<0.05	
1-15 [1]	477	494	1509	299	316	1017	685	480	-12		1.0		1.0		0.97		1-15 [1]
1-15 [10]	764	1033	737	416	433	1595	830	440	132		1.3		1.2		0.66		1-15 [10]
1-15 [20]	10944	1524	11632	2675	2630	2205	5268	4686	4,571		11.3	*	7.6	*	0.00	*	1-15 [20]
7-14W-27 [1]	287	282	508	628	930	613	541	244	-156		0.6		0.8		0.59		7-14W-27 [1]
7-14W-27 [10]	197	1257	259	52818	7600	7389	11587	20486	10,889	*	25.5	*	16.6	*	0.01	*	7-14W-27 [10]
7-14W-27 [20]	757	7664	48544	4465	26336	3359	15188	18744	14,490	*	33.7	*	21.8	*	0.00	*	7-14W-27 [20]
71-90 [1]	357	1334	117	2737	324	293	860	1016	163		1.4		1.2		0.64		71-90 [1]
71-90 [10]	571	207	416	967	2034	557	792	657	95		1.2		1.1		0.76		71-90 [10]
71-90 [20]	489	3461	251	706	3027	1028	1494	1387	796		2.8	*	2.1	*	0.05		71-90 [20]
101-120 [1]	696	369	112	553	205	312	375	218	-323		0.3		0.5		0.27		101-120 [1]
101-120 [10]	645	749	260	107	418	438	436	237	-261		0.4		0.6		0.37		101-120 [10]
101-120 [20]	389	1452	122	235	309	934	574	515	-124		0.7		0.8		0.68		101-120 [20]
229-248 [1]	1898	488	410	1375	252	146	762	708	64		1.1		1.1		0.84		229-248 [1]
229-248 [10]	681	686	5444	1771	765	2158	1918	1838	1,220		3.7	*	2.8	*	0.01	*	229-248 [10]
229-248 [20]	3686	2394	363	2607	4627	1713	2565	1491	1,868		5.2	*	3.7	*	0.00	*	229-248 [20]
267-286 [1]	445	306	293	2317	663	137	694	815	-4		1.0		1.0		0.99		267-286 [1]
267-286 [10]	534	398	553	6875	284	285	1488	2642	791		2.8	*	2.1	*	0.19		267-286 [10]
267-286 [20]	345	3769	9570	13522	439	418	4677	5619	3,980		10.0	*	6.7	*	0.00	*	267-286 [20]
307-326 [1]	439	390	546	709	1413	338	639	401	-58		0.9		0.9		0.85		307-326 [1]
307-326 [10]	633	829	427	344	658	360	542	195	-155		0.6		0.8		0.59		307-326 [10]
307-326 [20]	611	200	634	172	432	134	364	226	-333		0.2		0.5		0.26		307-326 [20]
sAg 10	855	355	191	550	784	117	475	306	-222		0.5		0.7		0.45		sAg 10
sAg 100	1167	358	1786	811	324	695	857	551	160		1.4		1.2		0.60		sAg 100
N	689	731	969	422	212	147	528	322	-169		0.6		0.8		0.57		N
N	89	195	168	139	35	16	107	72	-590		-0.3		0.2		0.05	*	N
N	92	174	139	77	204	64	125	56	-572		-0.3		0.2		0.05		N
3H	660	1023	2887	525	664	1308	1178	885	481		2.1		1.7		0.16		3H
3H	401	231	737	176	261	325	355	203	-342		0.2		0.5		0.24		3H
3H	985	330	362	51	2340	138	701	867	4		1.0		1.0		0.99		3H
3H	242	1279	617	433	528	230	555	387	-142		0.7		0.8		0.63		3H
	SMC																
N	17	61	74	44	35	66	50	21	-188		0.0		0.2		0.00	*	N
3H	221	273	178	325	214	212	237	53	0		1.0		1.0		1.00		3H
PHA - 1	3661	5108	3683	4982	8699	5389	5254	1843	5,017	*	27.7	*	22.2	*	0.00	*	PHA - 1
PHA - 5	27372	19012	22434	25053	26336	19736	23324	3484	23,087	*	124.0	*	98.3	*	0.00	*	PHA - 5
PHA - 10	42855	17778	36668	35121	39383	41182	35498	9133	35,261	*	188.9	*	149.7	*	0.00	*	PHA - 10
LPS - 1	585	643	1323	1017	1308	1645	1087	417	850		5.5	*	4.6	*	0.00	*	LPS - 1
LPS - 5	1094	802	964	1083	921	1067	989	115	751		5.0	*	4.2	*	0.00	*	LPS - 5
LPS - 10	1140	961	968	1054	1589	1576	1215	292	978		6.2	*	5.1	*	0.00	*	LPS - 10
LPS - 20	1033	909	1414	1251	1311	1201	1187	186	949		6.1	*	5.0	*	0.00	*	LPS - 20
LPS - 40	1000	680	1393	632	694	779	863	291	626		4.3	*	3.6	*	0.00	*	LPS - 40
	PBMC																
N	55	58	80	87	72	54	68	14	-114		0.0		0.4		0.00	*	N
3H	158	151	149	177	174	280	182	50	0		1.0		1.0		1.00		3H
PHA - 1	2183	1735	1594	1129	1742	1781	1694	341	1,513		14.3	*	9.3	*	0.00	*	PHA - 1
PHA - 5	19311	11903	17163	16449	17249	37332	19901	8884	19,720	*	174.2	*	109.6	*	0.00	*	PHA - 5
PHA - 10	25251	14346	15322	20657	21357	27088	20670	5123	20,489	*	181.0	*	113.9	*	0.00	*	PHA - 10
LPS - 1	1767	1077	954	1122	666	2198	1297	571	1,116		10.8	*	7.1	*	0.00	*	LPS - 1
LPS - 5	1064	1125	998	1544	1623	2003	1393	396	1,211		11.6	*	7.7	*	0.00	*	LPS - 5
LPS - 10	3415	786	1192	1403	1150	2112	1676	958	1,495		14.1	*	9.2	*	0.00	*	LPS - 10
LPS - 20	873	1078	888	973	1564	954	1055	260	874		8.7	*	5.8	*	0.00	*	LPS - 20
LPS - 40	283	301	218	183	224	214	237	45	56		1.5		1.3		0.07		LPS - 40
	prePBMC																
N	25	32	52				36	14	-66		0.0		0.4		0.01	*	N
3H	79	122	105				102	22	0		1.0		1.0		1.00		3H
1-15 [20]	147	151	159				152	6	50		1.8		1.5		0.02	*	1-15 [20]
7-14W-27 [20]	168	158	149				158	10	56		1.9		1.6		0.01	*	7-14W-27 [20]
71-90 [20]	40	125	77				81	43	-21		0.7		0.8		0.48		71-90 [20]
101-120 [20]	80	49	99				76	25	-26		0.6		0.7		0.25		101-120 [20]
229-248 [20]	91	70	78				80	11	-22		0.7		0.8		0.18		229-248 [20]
267-286 [20]	156	86	141				128	37	26		1.4		1.3		0.36		267-286 [20]
307-326 [20]	58	49	63				57	7	-45		0.3		0.6		0.03	*	307-326 [20]
sAg - 10	453	698	296				482	203	380		6.8	*	4.7	*	0.03	*	sAg - 10

Raw data for Dv1 control duck G100

G100	Mean	SD															unvaccinated challenged
Total N	139	65															
Total 3H	234	127							CPM-3H		S.I.		P/N		t-Test		
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1		<0.05	
1-15 [1]	469	132	414	325	324	2067	622	717	388		5.1	*	2.7	*	0.01	*	1-15 [1]
1-15 [10]	223	79	140	370	327	808	325	261	91		2.0		1.4		0.22		1-15 [10]
1-15 [20]	369	1940	193	3998	2539	2557	1933	1449	1,699		19.0	*	8.3	*	0.00	*	1-15 [20]
7-14W-27 [1]	263	2924	1137	169	317	280	848	1077	614		7.5	*	3.6	*	0.01	*	7-14W-27 [1]
7-14W-27 [10]	1663	3523	19301	903	7384	773	5591	7156	5,357	*	57.7	*	23.9	*	0.00	*	7-14W-27 [10]
7-14W-27 [20]	33308	72792	5199	2932	19665	8458	23726	26550	23,492	*	249.4	*	101.4	*	0.00	*	7-14W-27 [20]
71-90 [1]	720	224	186	167	255	228	297	210	63		1.7		1.3		0.35		71-90 [1]
71-90 [10]	307	178	248	374	294	244	274	67	40		1.4		1.2		0.47		71-90 [10]
71-90 [20]	339	400	412	220	1978	284	606	676	372		4.9	*	2.6	*	0.01	*	71-90 [20]
101-120 [1]	174	387	530	230	2820	898	840	1004	606		7.4	*	3.6	*	0.01	*	101-120 [1]
101-120 [10]	736	572	213	633	194	482	472	224	238		3.5	*	2.0		0.00	*	101-120 [10]
101-120 [20]	370	1035	472	506	295	166	474	301	240		3.5	*	2.0		0.00	*	101-120 [20]
229-248 [1]	198	185	265	321	254	213	239	51	5		1.1		1.0		0.92		229-248 [1]
229-248 [10]	202	134	172	151	227	250	189	45	-45		0.5		0.8		0.41		229-248 [10]
229-248 [20]	448	183	399	278	154	78	257	145	23		1.2		1.1		0.71		229-248 [20]
267-286 [1]	394	650	288	486	177	208	367	180	133		2.4	*	1.6		0.04	*	267-286 [1]
267-286 [10]	472	542	361	367	193	215	358	138	124		2.3	*	1.5		0.04	*	267-286 [10]
267-286 [20]	215	364	507	135	666	209	349	204	115		2.2	*	1.5		0.09		267-286 [20]
307-326 [1]	211	151	87	623	123	339	256	200	22		1.2		1.1		0.74		307-326 [1]
307-326 [10]	239	677	136	173	92	274	265	212	31		1.3		1.1		0.64		307-326 [10]
307-326 [20]	643	246	344	173	485	778	445	235	211		3.2	*	1.9		0.01	*	307-326 [20]
sAg 10	137	2080	244	206	280	3284	1039	1330	805		9.5	*	4.4	*	0.00	*	sAg 10
sAg 100	125	302	2908	485	393	10700	2486	4156	2,252		24.8	*	10.6	*	0.01	*	sAg 100
N	87	81	99	219	125	169	130	54	-104		-0.1		0.6		0.06		N
N	162	204	102	58	119	233	146	66	-88		0.1		0.6		0.12		N
N	294	116	157	110	135	40	142	84	-92		0.0		0.6		0.11		N
3H	135	162	92	287	145	134	159	67	-75		0.2		0.7		0.18		3H
3H	161	534	99	467	318	351	322	169	88		1.9		1.4		0.17		3H
3H	230	234	215	122	68	84	159	76	-75		0.2		0.7		0.18		3H
3H	364	306	401	187	339	181	296	92	62		1.7		1.3		0.27		3H
	SMC																
N	73	84	32	19	81	47	56	27	-1,678		0.0		0.0		0.00	*	N
3H	1604	1787	1249	2644	1246	1873	1734	518	0		1.0		1.0		1.00		3H
PHA - 1	57039	26989	53013	72177	90208	54191	58936	21143	57,202	*	35.1	*	34.0	*	0.00	*	PHA - 1
PHA - 5	39754	37234	35283	44699	40652	61101	43121	9373	41,387	*	25.7	*	24.9	*	0.00	*	PHA - 5
PHA - 10	38898	40533	29252	30714	33657	51354	37401	8147	35,668	*	22.3	*	21.6	*	0.00	*	PHA - 10
LPS - 1	727	733	601	552	449	949	669	175	-1,065		0.4		0.4		0.00	*	LPS - 1
LPS - 5	675	664	490	478	484	1037	638	216	-1,096		0.3		0.4		0.00	*	LPS - 5
LPS - 10	675	760	532	572	821	1045	734	187	-1,000		0.4		0.4		0.00	*	LPS - 10
LPS - 20	1010	1457	749	1228	1494	1576	1252	322	-482		0.7		0.7		0.08		LPS - 20
LPS - 40	1433	1047	1160	1593	1593	1684	1418	259	-316		0.8		0.8		0.21		LPS - 40
	PBMC																
N	78	23	118	109	104	232	111	69	-210		0.0		0.3		0.02	*	N
3H	225	182	208	290	412	605	320	162	0		1.0		1.0		1.00		3H
PHA - 1	2367	4712	4745	5832	4591	4886	4522	1147	4,202		21.0	*	14.1	*	0.00	*	PHA - 1
PHA - 5	11573	13038	17329	14157	22301	48306	21117	13855	20,797	*	100.2	*	65.9	*	0.00	*	PHA - 5
PHA - 10	19257	41350	27850	35874	46663	65391	39398	16023	39,077	*	187.4	*	123.0	*	0.00	*	PHA - 10
LPS - 1	4942	5773	7030	6795	7851	7949	6723	1179	6,403	*	31.5	*	21.0	*	0.00	*	LPS - 1
LPS - 5	5245	5561	5489	8004	5571	6453	6054	1040	5,734	*	28.3	*	18.9	*	0.00	*	LPS - 5
LPS - 10	4508	5206	6238	6174	6655	5158	5657	821	5,336	*	26.5	*	17.7	*	0.00	*	LPS - 10
LPS - 20	3367	3370	4863	5285	5257	3870	4335	907	4,015		20.1	*	13.5	*	0.00	*	LPS - 20
LPS - 40	3031	2472	3658	3995	4822	3503	3614	815	3,293		16.7	*	11.3	*	0.00	*	LPS - 40
	prePBMC																
N	42	30	55				42	13	-29		0.0		0.6		0.09		N
3H	53	90	70				71	19	0		1.0		1.0		1.00		3H
1-15 [20]	54	97	121				91	34	20		1.7		1.3		0.43		1-15 [20]
7-14W-27 [20]	285	330	244				286	43	215		8.5	*	4.0	*	0.00	*	7-14W-27 [20]
71-90 [20]	76	70	41				62	19	-9		0.7		0.9		0.60		71-90 [20]
101-120 [20]	83	50	123				85	37	14		1.5		1.2		0.58		101-120 [20]
229-248 [20]	148	278	88				171	97	100		4.5	*	2.4	*	0.15		229-248 [20]
267-286 [20]	78	83	109				90	17	19		1.7		1.3		0.26		267-286 [20]
307-326 [20]	73	65	59				66	7	-5		0.8		0.9		0.67		307-326 [20]
sAg - 10	265	108	170				181	79	110		4.8	*	2.5	*	0.08		sAg - 10

Raw data for Dv1 control duck W42

W42	Mean	SD							CPM-3H	>5000	S.I.	>2.1	P/N	>2.1	t-Test	<0.05	
Total N	49	19															
Total 3H	20610	21104															
	R1	R2	R3	R4	R5	R6	Mean	SD									
1-15 [1]	3176	1576	6770	9351	3272	1160	4218	3199	-16,392		0.2		0.2		0.07		1-15 [1]
1-15 [10]	9682	9521	1368	722	1276	1431	4000	4346	-16,61C		0.2		0.2		0.07		1-15 [10]
1-15 [20]	38419	1832	3933	15607	38017	8911	17787	16522	-2,823		0.9		0.9		0.76		1-15 [20]
7-14W-27 [1]	61817	6581	499	24053	46433	7397	24463	24753	3,854		1.2		1.2		0.70		7-14W-27 [1]
7-14W-27 [10]	74059	35848	38417	95963	111360	62926	69762	30380	49,153	*	3.4	*	3.4	*	0.00	*	7-14W-27 [10]
7-14W-27 [20]	44831	42929	112865	63145	64701	125940	75735	35244	55,126	*	3.7	*	3.7	*	0.00	*	7-14W-27 [20]
71-90 [1]	377	509	2044	403	291	541	694	667	-19,915		0.0		0.0		0.03	*	71-90 [1]
71-90 [10]	1217	1455	58219	34521	2002	1765	16530	24300	-4,080		0.8		0.8		0.68		71-90 [10]
7I-90 [20]	864	12202	1455	1333	1221	9623	4450	5076	-16,16C		0.2		0.2		0.08		71-90 [20]
101-120 [1]	55834	4524	2440	12514	11059	3391	14960	20452	-5,649		0.7		0.7		0.56		101-120 [1]
101-120 [10]	976	17774	50704	294	877	27412	16340	20190	-4,270		0.8		0.8		0.66		101-120 [10]
101-120 [20]	68094	15819	2046	27012	34655	45193	32137	23099	11,527	*	1.6		1.6		0.25		101-120 [20]
229-248 [1]	66072	10608	4992	13134	50512	6447	25294	26191	4,685		1.2		1.2		0.65		229-248 [1]
229-248 [10]	22394	914	8751	1210	1859	83677	19801	32352	-809		1.0		1.0		0.94		229-248 [10]
229-248 [20]	2314	187	413	595	6089	572	1695	2284	-18,915		0.1		0.1		0.04	*	229-248 [20]
267-286 [1]	532	10718	25877	3208	714	87942	21499	33930	889		1.0		1.0		0.94		267-286 [1]
267-286 [10]	1372	18001	3633	3020	403	42423	11475	16480	-9,134		0.6		0.6		0.33		267-286 [10]
267-286 [20]	36620	3880	34538	5004	5379	50164	22598	20275	1,988		1.1		1.1		0.84		267-286 [20]
307-326 [1]	1239	376	27581	2008	1072	5355	6272	10585	-14,338		0.3		0.3		0.12		307-326 [1]
307-326 [10]	29003	945	4036	67736	3747	7158	18771	26068	-1,839		0.9		0.9		0.86		307-326 [10]
307-326 [20]	2098	10251	73423	37245	52009	16102	31855	27435	11,245	*	1.5		1.5		0.28		307-326 [20]
sAg 10	22298	3656	1134	2950	689	6512	6207	8152	-14,403		0.3		0.3		0.12		sAg 10
sAg 100	1174	16084	2713	1988	770	1270	4000	5960	-16,61C		0.2		0.2		0.07		sAg 100
N	66	32	64	71	79	53	61	17	-20,549		0.0		0.0		0.03	*	N
N	38	34	38	31	84	22	41	22	-20,568		0.0		0.0		0.03	*	N
N	28	39	38	47	42	68	44	13	-20,566		0.0		0.0		0.03	*	N
3H	4388	4624	4198	41253	1075	16142	11947	15267	-8,663		0.6		0.6		0.36		3H
3H	17780	9112	7232	3119	33642	7418	13051	11190	-7,559		0.6		0.6		0.41		3H
3H	16598	28766	7676	82982	26361	70756	38857	30628	18,247	*	1.9		1.9		0.09		3H
3H	16224	14253	19932	14321	44074	2703	18585	13757	-2,025		0.9		0.9		0.83		3H
	SMC																
N	122	50	44	24	115	134	82	47	-971		0.0		0.1		0.00	*	N
3H	882	974	572	742	1247	1900	1053	473	0		1.0		1.0		1.00		3H
PHA - 1	96712	74865	69753	72612	73910	114736	83765	18022	82,712	*	86.2	*	79.6	*	0.00	*	PHA - 1
PHA - 5	109605	65432	57334	65534	66535	127273	81952	29000	80,899	*	84.3	*	77.8	*	0.00	*	PHA - 5
PHA - 10	95765	65020	49152	56614	69355	113470	74896	24693	73,843	*	77.0	*	71.1	*	0.00	*	PHA - 10
LPS - 1	15708	13208	15922	6223	22188	22093	15890	5980	14,838	*	16.3	*	15.1	*	0.00	*	LPS - 1
LPS - 5	52074	42822	32530	47607	56474	53751	47543	8792	46,490	*	48.9	*	45.2	*	0.00	*	LPS - 5
LPS - 10	63927	55901	55798	61337	67350	67658	61995	5300	60,942	*	63.7	*	58.9	*	0.00	*	LPS - 10
LPS - 20	80647	76708	52730	67144	75468	79180	71980	10540	70,927	*	74.0	*	68.4	*	0.00	*	LPS - 20
LPS - 40	69407	62840	60920	61836	67184	76550	66456	5936	65,403	*	68.3	*	63.1	*	0.00	*	LPS - 40
	PBMC																
N	37	25	184	142	183	114	114	70	-6		0.0		1.0		0.87		N
3H	119	53	118	157	190	82	120	49	0		1.0		1.0		1.00		3H
PHA - 1	1373	1474	908	1114	1329	684	1147	305	1,027		182.3	*	9.6	*	0.00	*	PHA - 1
PHA - 5	5007	11010	12256	11148	20268	7555	11207	5197	11,088	*	1957.6	*	93.5	*	0.00	*	PHA - 5
PHA - 10	14166	24509	24329	25761	27404	20979	22858	4755	22,738	*	4013.6	*	190.7	*	0.00	*	PHA - 10
LPS - 1	476	632	840	362	1367	845	754	357	634		112.9	*	6.3	*	0.00	*	LPS - 1
LPS - 5	1822	475	1276	973	916	1346	1135	457	1,015		180.1	*	9.5	*	0.00	*	LPS - 5
LPS - 10	1111	739	865	1104	737	1067	937	179	817		145.2	*	7.8	*	0.00	*	LPS - 10
LPS - 20	523	414	524	793	476	480	535	133	415		74.3	*	4.5	*	0.00	*	LPS - 20
LPS - 40	326	337	260	407	279	343	325	52	206		37.3	*	2.7	*	0.00	*	LPS - 40
	prePBMC																
N	17	93	69				60	39	-253		0.0		0.2		0.01	*	N
3H	275	403	260				313	79	0		1.0		1.0		1.00		3H
1-15 [20]	220	259	215				231	24	-81		0.7		0.7		0.16		1-15 [20]
7-14W-27 [20]	1916	230	200				782	982	469		2.9	*	2.5	*	0.46		7-14W-27 [20]
71-90 [20]	202	2107	366				892	1056	579		3.3	*	2.9	*	0.40		71-90 [20]
101-120 [20]	283	583	341				402	159	90		1.4		1.3		0.43		101-120 [20]
229-248 [20]	125	111	105				114	10	-199		0.2		0.4		0.01	*	229-248 [20]
267-286 [20]	108	62	91				87	23	-226		0.1		0.3		0.01	*	267-286 [20]
307-326 [20]	144	1759	474				792	853	480		2.9	*	2.5	*	0.39		307-326 [20]
sAg - 10	1795	422	1566				1261	736	948		4.7	*	4.0	*	0.09		sAg - 10

unvaccinated challenged

Raw data for Dv1 control duck W118

W118	Mean	SD							CPM-3H		S.I.		P/N		t-Test		unvaccinated challenged
Total N	45	21															
Total 3H	2231	3898															
	R1	R2	R3	R4	R5	R6	Mean	SD	>5000		>2.1		>2.1		<0.05		
1-15 [1]	529	552	500	1939	314	2189	1004	829	-1,227		0.4		0.4		0.46		1-15 [1]
1-15 [10]	18483	1090	2151	1121	3131	2416	4732	6782	2,501		2.1	*	2.1	*	0.24		1-15 [10]
1-15 [20]	4704	3312	6590	1842	6314	3681	4407	1833	2,176		2.0		2.0		0.20		1-15 [20]
7-14W-27 [1]	722	688	3227	24295	2405	3567	5817	9134	3,586		2.6	*	2.6	*	0.14		7-14W-27 [1]
7-14W-27 [10]	26346	13092	18673	31896	32395	23988	24398	7546	22,167	*	11.1	*	10.9	*	0.00	*	7-14W-27 [10]
7-14W-27 [20]	32216	17371	20155	43612	32727	54396	33413	13995	31,182	*	15.3	*	15.0	*	0.00	*	7-14W-27 [20]
71-90 [1]	772	531	825	617	8561	25677	6164	10066	3,933		2.8	*	2.8	*	0.13		71-90 [1]
71-90 [10]	13106	830	739	1179	1031	25636	7087	10308	4,856		3.2	*	3.2	*	0.07		71-90 [10]
71-90 [20]	609	1392	696	919	824	2110	1092	569	-1,139		0.5		0.5		0.49		71-90 [20]
101-120 [1]	13844	5685	10359	8930	25953	1621	11065	8397	8,834	*	5.0	*	5.0	*	0.00	*	101-120 [1]
101-120 [10]	9525	1722	879	14728	2779	38678	11385	14404	9,154	*	5.2	*	5.1	*	0.01	*	101-120 [10]
101-120 [20]	6374	6484	1303	11393	2093	18992	7773	6584	5,542	*	3.5	*	3.5	*	0.01	*	101-120 [20]
229-248 [1]	13137	3047	4221	11104	16560	21414	11581	7087	9,349	*	5.3	*	5.2	*	0.00	*	229-248 [1]
229-248 [10]	2954	13680	8268	8123	27383	32550	15493	11827	13,262	*	7.1	*	6.9	*	0.00	*	229-248 [10]
229-248 [20]	15609	6459	4977	1380	4288	18653	8561	6908	6,330	*	3.9	*	3.8	*	0.01	*	229-248 [20]
267-286 [1]	7926	1326	4735	1033	1887	42166	9846	16050	7,614	*	4.5	*	4.4	*	0.04	*	267-286 [1]
267-286 [10]	1024	890	1455	1259	1196	10864	2781	3964	550		1.3		1.2		0.76		267-286 [10]
267-286 [20]	3379	7089	4784	1969	1334	12260	5136	4056	2,905		2.3	*	2.3	*	0.12		267-286 [20]
307-326 [1]	9433	1515	772	1158	4335	31131	8057	11761	5,826	*	3.7	*	3.6	*	0.05	*	307-326 [1]
307-326 [10]	8326	3338	1001	5512	4109	25170	7909	8797	5,678	*	3.6	*	3.5	*	0.02	*	307-326 [10]
307-326 [20]	5716	2572	4840	3966	11935	43415	12074	15691	9,843	*	5.5	*	5.4	*	0.01	*	307-326 [20]
sAg 10	632	690	433	22840	742	995	4389	9041	2,158		2.0		2.0		0.37		sAg 10
sAg 100	466	1505	879	1339	897	5361	1741	1811	-490		0.8		0.8		0.77		sAg 100
N	21	22	60	45	33	46	38	15	-2,193		0.0		0.0		0.18		N
N	38	30	34	32	106	35	46	30	-2,185		0.0		0.0		0.19		N
N	58	42	71	41	30	59	50	15	-2,181		0.0		0.0		0.19		N
3H	3981	476	468	589	439	456	1068	1428	-1,163		0.5		0.5		0.48		3H
3H	2626	405	768	493	1051	4291	1606	1545	-625		0.7		0.7		0.71		3H
3H	1697	19051	1819	648	2194	6372	5297	7023	3,066		2.4	*	2.4	*	0.16		3H
3H	266	282	484	565	2481	1645	954	906	-1,277		0.4		0.4		0.44		3H
	SMC																
N	189	157	196	214	266	44	178	75	-9,330		0.0		0.0		0.00	*	N
3H	16471	9017	6125	7595	12241	5598	9508	4161	0		1.0		1.0		1.00		3H
PHA - 1	133828	95115	93875	77699	87740	115639	100649	20471	91,142	*	10.8	*	10.6	*	0.00	*	PHA - 1
PHA - 5	137824	86229	75385	66083	91271	102882	93279	25259	83,771	*	10.0	*	9.8	*	0.00	*	PHA - 5
PHA - 10	109511	63823	54611	50269	59838	79831	69647	22021	60,139	*	7.4	*	7.3	*	0.00	*	PHA - 10
LPS - 1	14571	11568	10901	8824	9186	15628	11780	2789	2,272		1.2		1.2		0.29		LPS - 1
LPS - 5	20729	9161	11641	12074	13413	12036	13176	3952	3,668		1.4		1.4		0.15		LPS - 5
LPS - 10	26543	14315	15902	16951	11463	23282	18076	5704	8,568	*	1.9		1.9		0.01	*	LPS - 10
LPS - 20	22985	24871	20188	22299	17902	32484	23455	5032	13,947	*	2.5	*	2.5	*	0.00	*	LPS - 20
LPS - 40	33359	21313	18694	20069	21320	20166	22487	5414	12,979	*	2.4	*	2.4	*	0.00	*	LPS - 40
	PBMC																
N	200	127	93	89	42	59	102	56	2		0.0		1.0		0.95		N
3H	168	121	101	64	63	82	100	40	0		1.0		1.0		1.00		3H
PHA - 1	2217	2482	5620	8699	3958	2449	4238	2540	4,138		-2255.9		42.4	*	0.00	*	PHA - 1
PHA - 5	74278	51705	66240	66651	67695	66209	65463	7410	65,363	*	-35651.6		655.7	*	0.00	*	PHA - 5
PHA - 10	80813	54526	62703	66835	67973	74814	67944	9182	67,844	*	-37004.9		680.6	*	0.00	*	PHA - 10
LPS - 1	9703	5263	8618	11570	4876	4498	7421	2951	7,322	*	-3992.5		74.3	*	0.00	*	LPS - 1
LPS - 5	8965	7490	11851	9834	6826	7505	8745	1880	8,645	*	-4714.6		87.6	*	0.00	*	LPS - 5
LPS - 10	4679	3715	6509	9006	9537	7240	6781	2309	6,681	*	-3643.3		67.9	*	0.00	*	LPS - 10
LPS - 20	8433	5729	7001	8329	5889	7024	7068	1153	6,968	*	-3799.5		70.8	*	0.00	*	LPS - 20
LPS - 40	6165	4436	5029	2928	8320	2956	4972	2057	4,873		-2656.7		49.8	*	0.00	*	LPS - 40
	prePBMC																
N	40	27	49				39	11	-8		0.0		0.8		0.38		N
3H	45	39	56				47	9	0		1.0		1.0		1.00		3H
1-15 [20]	107	63	69				80	24	33		5.1	*	1.7		0.09		1-15 [20]
7-14W-27 [20]	240	230	225				232	8	185		24.1	*	5.0	*	0.00	*	7-14W-27 [20]
71-90 [20]	72	46	63				60	13	14		2.7	*	1.3		0.21		71-90 [20]
101-120 [20]	88	132	47				89	43	42		6.3	*	1.9		0.17		101-120 [20]
229-248 [20]	137	72	73				94	37	47		6.9	*	2.0		0.10		229-248 [20]
267-286 [20]	510	96	115				240	234	194		25.2	*	5.2	*	0.22		267-286 [20]
307-326 [20]	94	49	55				66	24	19		3.4	*	1.4		0.27		307-326 [20]
sAg - 10	6866	128	74				2356	3906	2,309		289.7	*	50.5	*	0.36		sAg - 10

Raw data for Dv1 control duck W120

W120	Mean	SD																unvaccinated challenged
Total N	65	28																
Total 3H	28496	17198							CPM-3H		S.I.		P/N		t-Test			
	R1	R2	R3	R4	R5	R6	Mean	SD	>5000		>2.1		>2.1		<0.05			
1-15 [1]	3249	387	32246	48678	39146	2421	21021	21480	-7,475		0.7		0.7		0.37			1-15 [1]
1-15 [10]	61331	19528	50568	47275	23967	33244	39319	16362	10,823	*	1.4		1.4		0.18			1-15 [10]
1-15 [20]	61936	56339	56370	46419	42342	51504	52485	7210	23,989	*	1.8		1.8		0.00	*		1-15 [20]
7-14W-27 [1]	52030	2841	45780	51197	46468	46194	40752	18768	12,255	*	1.4		1.4		0.14			7-14W-27 [1]
7-14W-27 [10]	73786	63886	53487	64155	63026	66350	64115	6523	35,619	*	2.3	*	2.2	*	0.00	*		7-14W-27 [10]
7-14W-27 [20]	82300	59467	58357	65730	57606	56706	63361	9817	34,865	*	2.2	*	2.2	*	0.00	*		7-14W-27 [20]
71-90 [1]	1792	31215	65270	3407	5878	15801	20561	24483	-7,936		0.7		0.7		0.36			71-90 [1]
71-90 [10]	55210	44226	49597	45241	5380	21931	36931	19169	8,435	*	1.3		1.3		0.30			71-90 [10]
71-90 [20]	9425	43995	12680	52050	23101	11054	25384	18353	-3,112		0.9		0.9		0.70			71-90 [20]
101-120 [1]	10450	26507	29838	44878	41318	28996	30331	12200	1,835		1.1		1.1		0.81			101-120 [1]
101-120 [10]	21903	49078	13152	3849	5890	16037	18318	16460	-10,178		0.6		0.6		0.20			101-120 [10]
101-120 [20]	2674	62772	60349	48105	19874	36408	38364	23637	9,867	*	1.3		1.3		0.25			101-120 [20]
229-248 [1]	2615	6027	62025	5249	1744	36041	18950	24759	-9,546		0.7		0.7		0.27			229-248 [1]
229-248 [10]	1232	3868	60210	54345	10901	35493	27675	25993	-821		1.0		1.0		0.93			229-248 [10]
229-248 [20]	17071	59578	43766	56947	56703	23308	42896	18535	14,399	*	1.5		1.5		0.08			229-248 [20]
267-286 [1]	3660	13052	9648	44913	32355	29797	22238	15885	-6,259		0.8		0.8		0.43			267-286 [1]
267-286 [10]	74033	33870	48835	48022	7781	46329	43145	21712	14,649	*	1.5		1.5		0.09			267-286 [10]
267-286 [20]	59378	86613	29322	45599	47905	52533	53558	19028	25,062	*	1.9		1.9		0.00	*		267-286 [20]
307-326 [1]	47881	1883	21060	31417	51683	39212	32189	18539	3,693		1.1		1.1		0.65			307-326 [1]
307-326 [10]	7425	7768	11891	41136	14064	20297	17097	12689	-11,399		0.6		0.6		0.14			307-326 [10]
307-326 [20]	11831	18194	12451	50671	9146	50746	25507	19743	-2,990		0.9		0.9		0.71			307-326 [20]
sAg 10	43703	32991	21511	59023	46197	47959	41897	13013	13,401	*	1.5		1.5		0.09			sAg 10
sAg 100	19318	2180	58193	51102	42125	11207	30688	22915	2,191		1.1		1.1		0.80			sAg 100
N	132	52	34	27	49	72	61	38	-28,435		0.0		0.0		0.00	*		N
N	76	49	68	52	49	55	58	11	-28,438		0.0		0.0		0.00	*		N
N	79	129	83	58	44	58	75	30	-28,421		0.0		0.0		0.00	*		N
3H	34462	6134	29832	49721	47865	42779	35132	16142	6,636	*	1.2		1.2		0.40			3H
3H	16277	28681	46855	12740	40743	8717	25669	15671	-2,827		0.9		0.9		0.72			3H
3H	9927	56408	40695	61631	7727	28568	34159	22845	5,663	*	1.2		1.2		0.50			3H
3H	7628	8543	10687	30878	28910	27501	19025	11130	-9,472		0.7		0.7		0.21			3H
	sMC																	
N	114	102	122	99	114	70	104	18	-448		0.0		0.2		0.00	*		N
3H	495	676	414	468	825	430	551	164	0		1.0		1.0		1.00			3H
PHA - 1	72674	82007	74339	69307	75024	69279	73772	4712	73,220	*	164.5	*	133.8	*	0.00	*		PHA - 1
PHA - 5	56714	56056	50729	52340	56622	62030	55749	3951	55,197	*	124.3	*	101.1	*	0.00	*		PHA - 5
PHA - 10	50678	40550	42174	45568	54513	54497	47997	6118	47,445	*	106.9	*	87.1	*	0.00	*		PHA - 10
LPS - 1	3037	3946	4001	3162	2895	2456	3250	610	2,698		7.0	*	5.9	*	0.00	*		LPS - 1
LPS - 5	2261	2338	3022	2570	2314	2435	2490	282	1,939		5.3	*	4.5	*	0.00	*		LPS - 5
LPS - 10	2314	2749	3388	2717	2535	2039	2624	459	2,072		5.6	*	4.8	*	0.00	*		LPS - 10
LPS - 20	3829	2830	3968	4434	3242	2584	3481	714	2,930		7.5	*	6.3	*	0.00	*		LPS - 20
LPS - 40	3372	5450	4277	4215	4734	3548	4266	767	3,715		9.3	*	7.7	*	0.00	*		LPS - 40
	PBMC																	
N	112	130	111	47	25	40	78	45	-9		0.0		0.9		0.72			N
3H	120	102	81	74	30	109	86	32	0		1.0		1.0		1.00			3H
PHA - 1	879	824	748	935	1329	815	922	209	836		99.3	*	10.7	*	0.00	*		PHA - 1
PHA - 5	4383	6050	9099	11873	5888	8040	7556	2697	7,470	*	879.8	*	87.9	*	0.00	*		PHA - 5
PHA - 10	7351	22051	20507	21054	20286	24743	19332	6091	19,246	*	2265.2	*	224.8	*	0.00	*		PHA - 10
LPS - 1	1732	2700	3341	3289	4302	2721	3014	857	2,928		345.5	*	35.0	*	0.00	*		LPS - 1
LPS - 5	2103	3034	2621	4003	4616	4416	3466	1027	3,380		398.6	*	40.3	*	0.00	*		LPS - 5
LPS - 10	1912	2232	2045	3695	3969	3373	2871	911	2,785		328.6	*	33.4	*	0.00	*		LPS - 10
LPS - 20	1376	1825	2035	2158	2652	2246	2049	428	1,963		231.9	*	23.8	*	0.00	*		LPS - 20
LPS - 40	1380	1472	1627	2175	1740	1631	1671	278	1,585		187.5	*	19.4	*	0.00	*		LPS - 40
	prePBMC																	
N	53	114	64				77	33	8		0.0		1.1		0.71			N
3H	70	85	51				69	17	0		1.0		1.0		1.00			3H
1-15 [20]	131	74	99				101	29	33		-2.9		1.5		0.16			1-15 [20]
7-14W-27 [20]	281	176	199				219	55	150		-17.0		3.2	*	0.01	*		7-14W-27 [20]
71-90 [20]	94	71	97				87	14	19		-1.2		1.3		0.22			71-90 [20]
101-120 [20]	181	67	102				117	58	48		-4.8		1.7		0.24			101-120 [20]
229-248 [20]	103	63	64				77	23	8		0.0		1.1		0.65			229-248 [20]
267-286 [20]	301	66	238				202	122	133		-15.0		2.9	*	0.13			267-286 [20]
307-326 [20]	419	71	60				183	204	115		-12.8		2.7	*	0.39			307-326 [20]
sAg - 10	157	364	201				241	109	172		-19.6		3.5	*	0.05			sAg - 10

Raw data for Dv1 control duck W124

W124	Mean	SD													unvaccinated challenged
Total N	70	73													
Total 3H	11852	14377							CPM-3H		S.I.		P/N		t-Test		
	R1	R2	R3	R4	R5	R6	Mean	SD	>5000		>2.1		>2.1		<0.05		
1-15 [1]	3829	1407	2867	779	1081	4673	2439	1598	-9,412		0.2		0.2		0.13		1-15 [1]
1-15 [10]	20763	3948	3587	6735	17942	835	8968	8306	-2,883		0.8		0.8		0.64		1-15 [10]
1-15 [20]	7210	507	10231	11206	8528	5750	7239	3844	-4,613		0.6		0.6		0.45		1-15 [20]
7-14W-27 [1]	59238	25898	860	15625	6924	139	18114	22379	6,262	*	1.5		1.5		0.40		7-14W-27 [1]
7-14W-27 [10]	38465	33087	72427	69700	64624	86260	60761	20708	48,909	*	5.2	*	5.1	*	0.00	*	7-14W-27 [10]
7-14W-27 [20]	37649	58564	67471	60453	10919	84696	53292	25716	41,440	*	4.5	*	4.5	*	0.00	*	7-14W-27 [20]
71-90 [1]	9749	9665	7178	2107	761	491	4992	4376	-6,860		0.4		0.4		0.26		71-90 [1]
71-90 [10]	58107	53581	2274	5308	15203	3496	22995	25890	11,143	*	1.9		1.9		0.16		71-90 [10]
71-90 [20]	206	1465	2633	1305	1372	35557	7090	13967	-4,762		0.6		0.6		0.47		71-90 [20]
101-120 [1]	1634	221	842	4739	394	22555	5064	8728	-6,788		0.4		0.4		0.28		101-120 [1]
101-120 [10]	2003	1295	1743	13933	2951	2105	4005	4894	-7,847		0.3		0.3		0.20		101-120 [10]
101-120 [20]	3365	791	7559	22482	8818	2557	7595	7908	-4,256		0.6		0.6		0.49		101-120 [20]
229-248 [1]	909	1229	1957	4435	9728	8930	4531	3925	-7,320		0.4		0.4		0.23		229-248 [1]
229-248 [10]	5011	2717	2784	13638	3061	26641	8975	9613	-2,876		0.8		0.8		0.65		229-248 [10]
229-248 [20]	693	664	2789	3848	2291	3235	2253	1323	-9,598		0.2		0.2		0.12		229-248 [20]
267-286 [1]	976	284	360	425	310	5246	1267	1966	-10,585		0.1		0.1		0.09		267-286 [1]
267-286 [10]	1693	6177	176	1233	861	1516	1943	2143	-9,909		0.2		0.2		0.11		267-286 [10]
267-286 [20]	799	111	1952	905	3209	2716	1615	1208	-10,236		0.1		0.1		0.10		267-286 [20]
307-326 [1]	1441	12557	202	1174	245	2992	3102	4743	-8,750		0.3		0.3		0.16		307-326 [1]
307-326 [10]	494	617	640	458	1187	2015	902	606	-10,950		0.1		0.1		0.08		307-326 [10]
307-326 [20]	201	320	858	2956	10281	7016	3605	4161	-8,246		0.3		0.3		0.18		307-326 [20]
sAg 10	12791	5209	29542	13559	15568	5054	13621	8968	1,769		1.2		1.1		0.78		sAg 10
sAg 100	22775	5161	1247	3134	7636	33788	12290	13041	438		1.0		1.0		0.95		sAg 100
N	20	25	20	57	71	12	34	24	-11,819		0.0		0.0		0.06		N
N	55	18	20	21	99	298	85	109	-11,767		0.0		0.0		0.06		N
N	101	147	25	151	12	109	91	60	-11,761		0.0		0.0		0.06		N
3H	7582	12490	688	49607	6387	1163	12986	18467	1,134		1.1		1.1		0.87		3H
3H	2027	938	32969	28937	2382	8066	12553	14523	701		1.1		1.1		0.92		3H
3H	638	4807	11822	32951	23828	12730	14463	12032	2,611		1.2		1.2		0.69		3H
3H	938	1073	3681	280	865	37592	7405	14836	-4,447		0.6		0.6		0.51		3H
	SMC																
N	99	90	108	123	60	34	86	33	-445		0.0		0.2		0.00	*	N
3H	804	173	509	440	537	720	531	222	0		1.0		1.0		1.00		3H
PHA - 1	54368	38644	57971	48325	64147	62061	54253	9502	53,722	*	121.8	*	102.3	*	0.00	*	PHA - 1
PHA - 5	62204	51635	57323	42421	58639	61658	55647	7509	55,116	*	124.9	*	104.9	*	0.00	*	PHA - 5
PHA - 10	46538	37169	57695	46337	65947	59958	52274	10693	51,744	*	117.3	*	98.5	*	0.00	*	PHA - 10
LPS - 1	834	1354	1041	1219	1604	1298	1225	265	695		2.6	*	2.3	*	0.00	*	LPS - 1
LPS - 5	1077	769	1122	915	1266	1370	1087	221	556		2.2	*	2.0		0.00	*	LPS - 5
LPS - 10	969	2675	1758	1117	1490	1973	1664	623	1,133		3.5	*	3.1	*	0.00	*	LPS - 10
LPS - 20	1690	1537	1569	2518	2196	1506	1836	421	1,306		3.9	*	3.5	*	0.00	*	LPS - 20
LPS - 40	1311	1140	1633	1366	1356	1517	1387	171	857		2.9	*	2.6	*	0.00	*	LPS - 40
	PBMC																
N	185	177	91	63	30	41	98	68	2		0.0		1.0		0.95		N
3H	110	88	121	85	63	108	96	21	0		1.0		1.0		1.00		3H
PHA - 1	2671	4708	8392	5168	4406	10397	5957	2865	5,861	*	-2929.6		62.2	*	0.00	*	PHA - 1
PHA - 5	30153	54806	46790	53469	55543	54034	49133	9817	49,037	*	-24517.3		512.7	*	0.00	*	PHA - 5
PHA - 10	30128	49222	36357	50774	51908	52299	45115	9465	45,019	*	-22508.4		470.8	*	0.00	*	PHA - 10
LPS - 1	1042	1694	1680	2645	2984	3279	2221	877	2,125		-1061.4		23.2	*	0.00	*	LPS - 1
LPS - 5	654	1287	1893	2241	1938	2989	1834	801	1,738		-867.9		19.1	*	0.00	*	LPS - 5
LPS - 10	608	1038	1152	2211	2497	2524	1672	837	1,576		-786.9		17.4	*	0.00	*	LPS - 10
LPS - 20	688	1399	1331	1976	1679	2289	1560	558	1,465		-731.3		16.3	*	0.00	*	LPS - 20
LPS - 40	396	562	902	1360	914	1401	923	407	827		-412.3		9.6	*	0.00	*	LPS - 40
	prePBMC																
N	49	32	18				33	16	-64		0.0		0.3		0.06		N
3H	133	103	56				97	39	0		1.0		1.0		1.00		3H
1-15 [20]	247	163	450				287	148	189		3.9	*	2.9	*	0.10		1-15 [20]
7-14W-27 [20]	319	5927	821				2356	3103	2,258		36.1	*	24.2	*	0.28		7-14W-27 [20]
71-90 [20]	164	137	100				134	32	36		1.6		1.4		0.28		71-90 [20]
101-120 [20]	7847	4479	3193				5173	2403	5,076	*	79.9	*	53.1	*	0.02	*	101-120 [20]
229-248 [20]	247	146	173				189	52	91		2.4	*	1.9		0.07		229-248 [20]
267-286 [20]	225	208	213				215	9	118		2.8	*	2.2	*	0.01	*	267-286 [20]
307-326 [20]	16322	10918	6959				11400	4700	11,302	*	176.7	*	117.1	*	0.01	*	307-326 [20]
sAg - 10	36649	61573	31274				43165	16166	43,068	*	670.5	*	443.5	*	0.01	*	sAg - 10

Raw data for Bursectomy duck W101

W101	Mean	SD											
Total N	1114	3336											
Total 3H	992	2025							CPM-3H		S.I.	P/N	t-Test
	R1	R2	R3	R4	R5	R6	Mean	SD	>5000		>2.1	>2.1	<0.05
37-56 [1]													
37-56 [10]													
37-56 [20]	28	34	45	78	214	114	86	71	-907		8.4 *	0.1	0.30
54-73 [1]													
54-73 [10]													
54-73 [20]													
71-90 [1]	58	1229	1429	2600	1135	122	1096	940	104		0.2	1.1	0.91
71-90 [10]	66	1141	1174	1958	4506	97	1490	1643	498		-3.1	1.5	0.61
71-90 [20]	109	326	842	4394	595	71	1056	1661	64		0.5	1.1	0.95
87-106 [1]	75	2151	622	874	85	185	665	796	-327		3.7 *	0.7	0.71
87-106 [10]	231	425	506	783	729	49	454	283	-538		5.4 *	0.5	0.53
87-106 [20]	55	1764	631	2091	144	47	789	914	-203		2.7 *	0.8	0.82
101-120 [1]													
101-120 [10]													
101-120 [20]													
116-130 [1]	29	536	1585	2866	288	48	892	1125	-100		1.8	0.9	0.91
116-130 [10]	29	286	5327	430	458	61	1099	2079	107		0.1	1.1	0.92
116-130 [20]	33	921	1394	7426	382	199	1726	2837	734		-5.0	1.7	0.53
126-140 [1]	38	251	1325	6233	278	52	1363	2433	371		-2.0	1.4	0.74
126-140 [10]	27	372	1245	787	101	36	428	494	-564		5.6 *	0.4	0.52
126-140 [20]	45	231	568	21795	1538	42	4037	8718	3,045		-24.0	4.1 *	0.25
136-150 [1]	53	2984	341	307	614	62	727	1125	-265		3.2 *	0.7	0.77
136-150 [10]	45	3189	285	447	6889	61	1819	2759	827		-5.8	1.8	0.48
136-150 [20]	38	147	1671	500	402	44	467	620	-525		5.3 *	0.5	0.55
146-160 [1]													
146-160 [10]													
146-160 [20]													
156-170 [1]	35	7705	255	5943	386	11315	4273	4762	3,281		-25.9	4.3 *	0.05
156-170 [10]	31	686	324	9911	450	57	1910	3927	918		-6.5	1.9	0.52
156-170 [20]	66	333	313	2508	7733	196	1858	3021	866		-6.1	1.9	0.48
166-180 [1]													
166-180 [10]													
166-180 [20]													
176-195 [1]													
176-195 [10]													
176-195 [20]													
191-210 [1]	73	5748	11851	19146	762	114	6282	7795	5,290 *		-42.4	6.3 *	0.04 *
191-210 [10]	302	702	7542	1299	1817	961	2104	2714	1,112		-8.1	2.1 *	0.34
191-210 [20]	236	879	14808	872	9330	834	4493	6123	3,501		-27.7	4.5 *	0.08
210-229 [1]	32	31	33	32	37	45	35	5	-957		8.9 *	0.0	0.27
210-229 [10]	30	201	9882	323	52	44	1755	3983	763		-5.3	1.8	0.59
210-229 [20]	35	307	397	1247	658	61	451	453	-541		5.4 *	0.5	0.53
229-248 [1]													
229-248 [10]													
229-248 [20]													
248-267 [1]	54	45	36	37	57	37	44	9	-948		8.8 *	0.0	0.28
248-267 [10]	65	28	474	304	321	41	206	186	-787		7.5 *	0.2	0.36
248-267 [20]	47	65	186	10119	223	38	1780	4086	788		-5.5	1.8	0.59
267-286 [1]	43	378	2503	2093	1420	63	1083	1074	91		0.3	1.1	0.92
267-286 [10]	32	332	758	10798	292	36	2041	4298	1,049		-7.6	2.1	0.48
267-286 [20]	32	49	315	1843	2402	55	783	1058	-209		2.7 *	0.8	0.82
287-306 [1]	30	885	373	3468	512	270	923	1279	-69		1.6	0.9	0.94
287-306 [10]	33	197	414	1100	595	95	406	399	-586		5.8 *	0.4	0.50
287-306 [20]	35	3755	2512	8013	948	92	2559	3041	1,567		-11.9	2.6 *	0.21
307-326 [1]													
307-326 [10]													
307-326 [20]													
sAg 10	44	140	5346	998	801	101	1238	2052	246		-1.0	1.2	0.81
sAg 100	34	930	25238	5527	1692	439	5643	9801	4,651		-37.2	5.7 *	0.12
N	37	32	27	49	71	13	38	20	-954		8.8 *	0.0	0.27
N	46	11691	271	342	658	129	2190	4660	1,198		-8.8	2.2 *	0.45
3H	31	120	327	506	743	76	301	281	-692		6.7 *	0.3	0.42
3H	48	7286	664	1486	492	125	1684	2792	692		-4.7	1.7	0.55
SMC													
N													
3H													
PHA - 1													
PHA - 5													
PHA - 10													
LPS - 1													
LPS - 5													
LPS - 10													
LPS - 20													
LPS - 40													
PBMC													
N													
3H													
PHA - 1													
PHA - 5													
PHA - 10													
LPS - 1													
LPS - 5													
LPS - 10													
LPS - 20													
LPS - 40													

Raw data for Bursectomy duck W109

W109	Mean	SD														
Total N	1147	1148														
Total 3H	1792	1724							CPM-3H		S.I.		P/N		t-Test	
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1		<0.05	
37-56 [1]	263	176	283	1205	353	489	462	379	-1,330		-1.1		0.3		0.08		37-56 [1]
37-56 [10]	788	1743	1293	361	365	1215	961	553	-831		-0.3		0.5		0.27		37-56 [10]
37-56 [20]	558	1573	1339	406	436	997	885	496	-907		-0.4		0.5		0.23		37-56 [20]
54-73 [1]	993	1735	856	509	764	4275	1522	1411	-270		0.6		0.8		0.75		54-73 [1]
54-73 [10]	2830	517	1008	3746	4224	1866	2365	1489	574		1.9		1.3		0.50		54-73 [10]
54-73 [20]	731	832	1255	8058	8174	5025	4013	3555	2,221		4.4	*	2.2	*	0.09		54-73 [20]
71-90 [1]	1202	1358	538	919	545	281	807	422	-985		-0.5		0.5		0.19		71-90 [1]
71-90 [10]	1682	398	440	529	593	141	631	538	-1,161		-0.8		0.4		0.13		71-90 [10]
71-90 [20]	384	829	639	1776	847	169	774	557	-1,018		-0.6		0.4		0.18		71-90 [20]
87-106 [1]	617	752	1109	741	1228	990	906	239	-886		-0.4		0.5		0.24		87-106 [1]
87-106 [10]	361	620	623	4379	732	951	1278	1531	-514		0.2		0.7		0.55		87-106 [10]
87-106 [20]	1066	763	914	1110	1865	2661	1397	727	-395		0.4		0.8		0.60		87-106 [20]
101-120 [1]	1567	857	857	541	3479	719	1337	1106	-455		0.3		0.7		0.57		101-120 [1]
101-120 [10]	538	486	710	545	869	505	609	150	-1,183		-0.8		0.3		0.12		101-120 [10]
101-120 [20]	1850	487	996	523	823	798	913	498	-879		-0.4		0.5		0.24		101-120 [20]
116-130 [1]	764	1408	660	2310	766	1931	1307	695	-485		0.2		0.7		0.52		116-130 [1]
116-130 [10]	656	444	600	1011	505	6505	1620	2401	-172		0.7		0.9		0.86		116-130 [10]
116-130 [20]	418	743	968	361	632	1358	747	372	-1,045		-0.6		0.4		0.17		116-130 [20]
126-140 [1]	434	243	266	1126	41	31	357	406	-1,435		-1.2		0.2		0.06		126-140 [1]
126-140 [10]	317	1375	387	46	32	33	365	519	-1,427		-1.2		0.2		0.07		126-140 [10]
126-140 [20]	959	570	566	76	69	67	385	372	-1,407		-1.2		0.2		0.07		126-140 [20]
136-150 [1]	539	1622	1298	2863	610	1265	1366	846	-426		0.3		0.8		0.58		136-150 [1]
136-150 [10]	1602	587	749	964	2070	720	1115	590	-676		0.0		0.6		0.37		136-150 [10]
136-150 [20]	565	347	826	2064	395	4599	1466	1661	-326		0.5		0.8		0.71		136-150 [20]
146-160 [1]	4677	16138	9196	8461	15473	29098	13841	8663	12,049	*	19.7	*	7.7	*	0.00	*	146-160 [1]
146-160 [10]	6601	4855	1095	2722	12196	11086	6426	4464	4,634		8.2	*	3.6	*	0.01	*	146-160 [10]
146-160 [20]	3762	6452	5534	5237	21212	6212	8068	6508	6,277	*	10.7	*	4.5	*	0.01	*	146-160 [20]
156-170 [1]	534	429	1008	642	599	1542	792	416	-999		-0.5		0.4		0.19		156-170 [1]
156-170 [10]	592	472	703	1328	511	605	702	317	-1,090		-0.7		0.4		0.15		156-170 [10]
156-170 [20]	562	460	509	2728	555	595	902	896	-890		-0.4		0.5		0.26		156-170 [20]
166-180 [1]	255	11819	783	1252	1767	1619	2916	4397	1,124		2.7	*	1.6		0.44		166-180 [1]
166-180 [10]	300	400	366	2195	636	1169	844	734	-947		-0.5		0.5		0.22		166-180 [10]
166-180 [20]	213	583	259	535	342	5492	1237	2090	-554		0.1		0.7		0.56		166-180 [20]
176-195 [1]	359	271	414	429	721	1727	654	547	-1,138		-0.8		0.4		0.14		176-195 [1]
176-195 [10]	844	358	485	552	661	1049	658	252	-1,134		-0.8		0.4		0.13		176-195 [10]
176-195 [20]	312	210	354	2327	742	2215	1027	981	-765		-0.2		0.6		0.33		176-195 [20]
191-210 [1]	372	1640	526	1491	433	690	859	560	-933		-0.4		0.5		0.22		191-210 [1]
191-210 [10]	578	1332	1012	893	722	537	846	300	-946		-0.5		0.5		0.21		191-210 [10]
191-210 [20]	1787	1722	2075	1745	605	628	1427	641	-365		0.4		0.8		0.63		191-210 [20]
210-229 [1]	557	850	455	474	548	2033	820	611	-972		-0.5		0.5		0.20		210-229 [1]
210-229 [10]	553	1152	627	1897	1030	3451	1452	1091	-340		0.5		0.8		0.67		210-229 [10]
210-229 [20]	720	4192	682	941	609	901	1341	1403	-451		0.3		0.7		0.59		210-229 [20]
229-248 [1]																	229-248 [1]
229-248 [10]																	229-248 [10]
229-248 [20]																	229-248 [20]
248-267 [1]	578	2778	1129	511	1910	539	1241	926	-551		0.1		0.7		0.48		248-267 [1]
248-267 [10]	2656	4050	873	1006	1354	2119	2010	1210	218		1.3		1.1		0.79		248-267 [10]
248-267 [20]	1436	1879	9895	922	2279	11247	4610	4659	2,818		5.4	*	2.6	*	0.08		248-267 [20]
267-286 [1]	272	429	1438	53	36	116	391	534	-1,401		-1.2		0.2		0.07		267-286 [1]
267-286 [10]	456	1064	446	38	36	44	347	405	-1,444		-1.2		0.2		0.06		267-286 [10]
267-286 [20]	1928	417	449	39	39	31	484	734	-1,308		-1.0		0.3		0.10		267-286 [20]
287-306 [1]	427	555	220	477	579	655	486	153	-1,306		-1.0		0.3		0.09		287-306 [1]
287-306 [10]	441	601	1604	5350	539	3129	1944	1957	152		1.2		1.1		0.87		287-306 [10]
287-306 [20]	1748	939	2135	356	573	4847	1766	1657	-25		1.0		1.0		0.98		287-306 [20]
307-326 [1]																	307-326 [1]
307-326 [10]																	307-326 [10]
307-326 [20]																	307-326 [20]
sAg 10	1006	1580	1786	463	4039	350	1537	1354	-254		0.6		0.9		0.76		sAg 10
sAg 100	789	901	3471	7503	1381	205	2375	2753	583		1.9		1.3		0.59		sAg 100
N	262	246	439	4230	1034	483	1116	1552	-676		0.0		0.6		0.43		N
N	1465	646	780	2438	1134	603	1178	699	-614		0.0		0.7		0.42		N
3H	226	220	194	3699	1913	962	1202	1395	-589		0.1		0.7		0.48		3H
3H	1083	779	5840	1890	1269	3425	2381	1938	589		1.9		1.3		0.52		3H
	SMC																
N	121	123	187	147	106	268	159	61	17		0.0		1.1		0.59		N
3H	108	118	113	142	157	215	142	40	0		1.0		1.0		1.00		3H
PHA - 1	4080	4252	3843	4244	4674	5029	4354	428	4,212		-254.2		30.6	*	0.00	*	PHA - 1
PHA - 5	7297	5196	5100	5424	6464	6659	6023	907	5,881	*	-355.4		42.4	*	0.00	*	PHA - 5
PHA - 10	4209	6249	4195	5627	4925	5454	5110	821	4,968		-300.1		35.9	*	0.00	*	PHA - 10
LPS - 1	340	238	161	162	240	221	227	66	85		-4.1		1.6		0.02	*	LPS - 1
LPS - 5	159	110	159	134	139	192	149	28	7		0.6		1.0		0.75		LPS - 5
LPS - 10	147	92	138	118	99	127	120	22	-22		2.3	*	0.8		0.27		LPS - 10
LPS - 20	105	142	101	155	149	127	130	23	-12		1.7		0.9		0.53		LPS - 20
LPS - 40	154	68	99	108	92	106	105	28	-38		3.3	*	0.7		0.09		LPS - 40
	PBMC																
N	616	749	1024	849	835	934	835	142	-237		0.0		0.8		0.01	*	N
3H	1165	1149	1021	1201	880	1015	1072	122	0		1.0		1.0		1.00		3H
PHA - 1	16399	19611	19981	19953	15300	16459	17951	2124	16,879	*	72.1	*	16.7	*	0.00	*	PHA - 1
PHA - 5	32299	39475	48001	40945	30812	23888	35903	8577	34,832	*	147.8	*	33.5	*	0.00	*	PHA - 5
PHA - 10	36117	48308	56195	53191	41113	28815	43957	10515	42,885	*	181.7	*	41.0	*	0.00	*	PHA - 10
LPS - 1	1693	1509	1497	1136	1177	1104	1353	245	281		2.2	*	1.3		0.03	*	LPS - 1
LPS - 5	1503	1080	1214	1342	1113	1149	1234	161	162		1.7		1.2		0.08		LPS - 5
LPS - 10	1837	1929	1574	1127	805	861	1356	492	284		2.2	*	1.3		0.20		LPS - 10
LPS - 20	880	720	836	873	708	705	787	85	-285		-0.2		0.7		0.00	*	LPS - 20
LPS - 40	765	879	845	1109	791	651	840	153	-232		0.0		0.8		0.02	*	LPS - 40

Raw data for Bursectomy duck W121

W121	Mean	SD												
Total N	1292	2100												
Total 3H	1100	933							CPM-3H		S.I.		P/N	t-Test
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1	<0.05	
37-56 [1]	1610	2341	692	2208	1591	2283	1788	632	687		-2.6		1.6	0.13		37-56 [1]
37-56 [10]	621	312	1142	972	6368	2797	2035	2291	935		-3.9		1.8	0.23		37-56 [10]
37-56 [20]	1549	167	261	271	477	694	570	516	-531		3.8	*	0.5	0.22		37-56 [20]
54-73 [1]	1001	1915	150	462	708	483	787	621	-314		2.6	*	0.7	0.47		54-73 [1]
54-73 [10]	562	1013	356	239	953	329	575	334	-525		3.7	*	0.5	0.21		54-73 [10]
54-73 [20]	740	1311	130	399	506	1759	808	613	-293		2.5	*	0.7	0.50		54-73 [20]
71-90 [1]	1982	161	293	673	1522	701	889	716	-212		2.1	*	0.8	0.63		71-90 [1]
71-90 [10]	3526	129	868	430	458	357	961	1279	-139		1.7		0.9	0.80		71-90 [10]
71-90 [20]	288	1794	2654	727	809	255	1088	948	-13		1.1		1.0	0.98		71-90 [20]
87-106 [1]	261	195	125	955	909	2513	826	903	-274		2.4	*	0.8	0.56		87-106 [1]
87-106 [10]	1259	234	1720	2159	4332	506	1702	1477	601		-2.1		1.5	0.30		87-106 [10]
87-106 [20]	348	685	1362	177	134	177	481	478	-620		4.2	*	0.4	0.15		87-106 [20]
101-120 [1]	1253	264	1001	145	404	547	602	436	-498		3.6	*	0.5	0.24		101-120 [1]
101-120 [10]	2306	1507	286	340	6225	169	1806	2324	705		-2.7		1.6	0.36		101-120 [10]
101-120 [20]	187	85	284	1161	1876	291	647	716	-453		3.4	*	0.6	0.31		101-120 [20]
116-130 [1]	263	490	132	237	1580	997	617	564	-484		3.5	*	0.6	0.26		116-130 [1]
116-130 [10]	1308	721	459	1085	731	842	858	299	-243		2.3	*	0.8	0.55		116-130 [10]
116-130 [20]	647	955	297	510	162	4352	1154	1591	53		0.7		1.0	0.93		116-130 [20]
126-140 [1]	152	286	557	892	716	700	551	281	-550		3.9	*	0.5	0.18		126-140 [1]
126-140 [10]	331	798	1534	387	354	1032	739	482	-361		2.9	*	0.7	0.39		126-140 [10]
126-140 [20]	2364	1067	695	550	508	1273	1076	699	-24		1.1		1.0	0.96		126-140 [20]
136-150 [1]	728	1852	2378	1156	1060	370	1257	738	157		0.2		1.1	0.73		136-150 [1]
136-150 [10]	5355	1713	345	393	847	1911	1761	1879	660		-2.4		1.6	0.33		136-150 [10]
136-150 [20]	695	487	3923	605	1362	2060	1522	1317	422		-1.2		1.4	0.44		136-150 [20]
146-160 [1]	408	939	1147	446	1944	665	925	575	-176		1.9		0.8	0.68		146-160 [1]
146-160 [10]	588	977	186	650	1955	638	832	605	-268		2.4	*	0.8	0.53		146-160 [10]
146-160 [20]	391	241	220	839	933	690	552	310	-548		3.9	*	0.5	0.19		146-160 [20]
156-170 [1]	565	3082	476	1299	1101	449	1162	1004	62		0.7		1.1	0.90		156-170 [1]
156-170 [10]	192	967	5343	540	3100	1368	1918	1960	818		-3.3		1.7	0.24		156-170 [10]
156-170 [20]	4203	1778	2980	1713	818	815	2051	1321	951		-4.0		1.9	0.09		156-170 [20]
166-180 [1]	3771	439	1009	866	3421	3191	2116	1497	1,016		-4.3		1.9	0.09		166-180 [1]
166-180 [10]	1967	2409	1721	503	1502	4232	2056	1241	955		-4.0		1.9	0.08		166-180 [10]
166-180 [20]	350	1514	1747	1416	1072	1006	1184	494	84		0.6		1.1	0.84		166-180 [20]
176-195 [1]	545	216	924	492	8381	3076	2272	3167	1,172		-5.1		2.1	0.24		176-195 [1]
176-195 [10]	1124	1713	859	837	2267	1149	1325	559	224		-0.2		1.2	0.60		176-195 [10]
176-195 [20]	17669	1303	1503	1046	239	7756	4919	6816	3,819		-19.0		4.5	0.07	*	176-195 [20]
191-210 [1]	651	609	1100	2197	489	1006	1009	629	-92		1.5		0.9	0.83		191-210 [1]
191-210 [10]	181	333	858	124	1053	2174	787	776	-313		2.6	*	0.7	0.49		191-210 [10]
191-210 [20]	800	438	754	160	2010	368	755	661	-345		2.8	*	0.7	0.43		191-210 [20]
210-229 [1]	92	269	1419	381	276	198	439	489	-661		4.5	*	0.4	0.13		210-229 [1]
210-229 [10]	307	2461	746	591	263	807	863	814	-238		2.2	*	0.8	0.60		210-229 [10]
210-229 [20]	287	1017	252	178	839	3955	1088	1446	-12		1.1		1.0	0.98		210-229 [20]
229-248 [1]																229-248 [1]
229-248 [10]																229-248 [10]
229-248 [20]																229-248 [20]
248-267 [1]	139	280	314	316	161	77	215	102	-886		5.6	*	0.2	0.04	*	248-267 [1]
248-267 [10]	255	293	347	449	1465	460	545	458	-556		3.9	*	0.5	0.19		248-267 [10]
248-267 [20]	267	1355	403	316	998	168	585	478	-516		3.7	*	0.5	0.23		248-267 [20]
267-286 [1]	247	171	426	1548	345	624	560	509	-540		3.8	*	0.5	0.21		267-286 [1]
267-286 [10]	609	428	271	1419	534	751	669	402	-432		3.3	*	0.6	0.30		267-286 [10]
267-286 [20]	3923	3466	3119	926	1669	1877	2497	1175	1,396		-6.3		2.3	0.01	*	267-286 [20]
287-306 [1]	2699	733	133	155	679	2728	1188	1208	87		0.5		1.1	0.87		287-306 [1]
287-306 [10]	1726	394	694	478	764	380	739	508	-361		2.9	*	0.7	0.39		287-306 [10]
287-306 [20]	300	1197	597	584	561	6298	1590	2326	489		-1.6		1.4	0.53		287-306 [20]
307-326 [1]																307-326 [1]
307-326 [10]																307-326 [10]
307-326 [20]																307-326 [20]
sAg 10	2433	305	4841	582	1768	510	1740	1733	639		-2.3		1.6	0.32		sAg 10
sAg 100	252	517	274	267	426	235	329	115	-772		5.0	*	0.3	0.06		sAg 100
N	282	446	1066	215	293	1859	694	651	-407		3.1	*	0.6	0.36		N
N	996	237	457	7779	917	955	1890	2901	790		-3.1		1.7	0.39		N
3H	367	667	416	631	811	572	577	165	-523		3.7	*	0.5	0.20		3H
3H	200	3406	1732	871	1361	2171	1624	1109	523		-1.7		1.5	0.31		3H
	SMC															
N	55	37	33	31	33	45	39	9	-2		0.0		1.0	0.75		N
3H	36	34	35	45	45	48	41	6	0		1.0		1.0	1.00		3H
PHA - 1	344	102	72	92	25	33	111	118	71		48.2	*	2.7	0.17	*	PHA - 1
PHA - 5	298	276	274	168	24	18	176	129	136		91.6	*	4.4	0.03	*	PHA - 5
PHA - 10	1091	553	321	481	43	136	438	375	397		265.7	*	10.8	0.03	*	PHA - 10
LPS - 1	652	78	75	62	209	44	187	235	146		98.4	*	4.6	0.16		LPS - 1
LPS - 5	232	95	81	87	267	28	132	95	91		61.8	*	3.3	0.04	*	LPS - 5
LPS - 10	181	111	74	80	66	23	89	53	49		33.4	*	2.2	0.05		LPS - 10
LPS - 20	144	83	58	52	118	26	80	44	40		27.4	*	2.0	0.05		LPS - 20
LPS - 40	165	48	47	55	41	23	63	51	23		16.1	*	1.6	0.31		LPS - 40
	PBMC															
N	1321	2001	1528	1485	1569	1919	1637	265	-625		0.0		0.7	0.01	*	N
3H	2506	2563	2144	2122	1657	2579	2262	361	0		1.0		1.0	1.00		3H
PHA - 1	64353	63665	60681	51135	63592	73363	62798	7154	60,536	*	97.9	*	27.8	0.00	*	PHA - 1
PHA - 5	78062	85291	76355	68453	73551	72469	75697	5755	73,435	*	118.6	*	33.5	0.00	*	PHA - 5
PHA - 10	62974	79143	80056	68440	75043	73447	73184	6527	70,922	*	114.5	*	32.4	0.00	*	PHA - 10
LPS - 1	3167	3141	3136	2072	2740	3759	3003	561	741		2.2	*	1.3	0.02	*	LPS - 1
LPS - 5	3639	3353	3060	2834	3763	2616	3211	453	949		2.5	*	1.4	0.00	*	LPS - 5
LPS - 10	3315	5602	6156	5333	5579	4772	5126	994	2,864		5.6	*	2.3	0.00	*	LPS - 10
LPS - 20	6728	9745	7533	6592	9380	8080	8010	1325	5,748	*	10.2	*	3.5	0.00	*	LPS - 20
LPS - 40	8558	11042	10467	10179	12709	10728	10614	1343	8,352	*	14.4	*	4.7	0.00	*	LPS - 40

Raw data for Bursectomy duck W130

W130	Mean	SD													
Total N	274	103													
Total 3H	372	85							CPM-3H		S.I.		P/N	t-Test	
	R1	R2	R3	R4	R5	R6	Mean	SD	>5000		>2.1		>2.1	<0.05	
37-56 [1]	111	119	43	1023			324	467	-48		0.5		0.9	0.72	37-56 [1]
37-56 [10]	48	57	33	1030			292	492	-80		0.2		0.8	0.58	37-56 [10]
37-56 [20]	224		463	449	955	473	513	268	141		2.4	*	1.4	0.11	37-56 [20]
54-73 [1]	246	390	523	505	510	406	430	106	59		1.6		1.2	0.22	54-73 [1]
54-73 [10]	289	376	373	651	457	317	411	131	39		1.4		1.1	0.45	54-73 [10]
54-73 [20]	267	402	417	362	439	643	422	124	50		1.5		1.1	0.33	54-73 [20]
71-90 [1]	348	420	432	492	513	724	488	129	117		2.2	*	1.3	0.03	* 71-90 [1]
71-90 [10]	231	359	349	378	392	591	383	117	12		1.1		1.0	0.81	71-90 [10]
71-90 [20]	280	266	429	392	429	399	366	74	-6		0.9		1.0	0.89	71-90 [20]
87-106 [1]	616	448	401	552	441	558	503	84	131		2.3	*	1.4	0.01	* 87-106 [1]
87-106 [10]	373	382	401	528	448	449	430	58	59		1.6		1.2	0.15	87-106 [10]
87-106 [20]	433	401	452	444	416	445	432	20	60		1.6		1.2	0.11	87-106 [20]
101-120 [1]	288	448	437	533	420	430	426	79	55		1.6		1.1	0.21	101-120 [1]
101-120 [10]	273	327	344	446	391	370	359	59	-13		0.9		1.0	0.74	101-120 [10]
101-120 [20]	210	340	434	337	1990	242	592	689	221		3.3	*	1.6	0.28	101-120 [20]
116-130 [1]	270	544	508	485	871	470	525	195	153		2.6	*	1.4	0.03	* 116-130 [1]
116-130 [10]	230	528	426	465	541	557	458	122	86		1.9		1.2	0.10	116-130 [10]
116-130 [20]	217	554	452	420	522	521	448	123	76		1.8		1.2	0.14	116-130 [20]
126-140 [1]	163	168	337				223	99	-149		-0.5		0.6	0.02	* 126-140 [1]
126-140 [10]	157	162	501				273	197	-98		0.0		0.7	0.19	126-140 [10]
126-140 [20]	103	147	401				217	161	-155		-0.6		0.6	0.03	* 126-140 [20]
136-150 [1]	491	656	687	1010	1283		825	317	454		5.6	*	2.2	* 0.00	* 136-150 [1]
136-150 [10]	533	677	808	831	1334		837	302	465		5.7	*	2.3	* 0.00	* 136-150 [10]
136-150 [20]	524	518	702	902	1343		798	343	426		5.4	*	2.1	* 0.00	* 136-150 [20]
146-160 [1]	183	472	2342	1302	2518	2392	1535	1036	1,163		12.9	*	4.1	* 0.00	* 146-160 [1]
146-160 [10]	279	702	1453	7624	14484	2976	4586	5536	4,215		44.0	*	12.3	* 0.02	* 146-160 [10]
146-160 [20]	1575	7848	2422	6804	3748	5736	4689	2501	4,317		45.1	*	12.6	* 0.00	* 146-160 [20]
156-170 [1]	501	824	820	853	1671		934	437	562		6.7	*	2.5	* 0.00	* 156-170 [1]
156-170 [10]	571	684	807	947	2021		1006	584	635		7.5	*	2.7	* 0.00	* 156-170 [10]
156-170 [20]	686	925	2060	1780	2447		1580	750	1,208		13.3	*	4.3	* 0.00	* 156-170 [20]
166-180 [1]	161	445	709	1169			621	428	250		3.5	*	1.7	0.06	166-180 [1]
166-180 [10]	215	387	467	1055			531	365	160		2.6	*	1.4	0.16	166-180 [10]
166-180 [20]	77	392	385	1155			502	459	131		2.3	*	1.4	0.33	166-180 [20]
176-195 [1]	33	227	27	1179			367	550	-5		0.9		1.0	0.97	176-195 [1]
176-195 [10]	309	183	289	1592			593	668	222		3.3	*	1.6	0.25	176-195 [10]
176-195 [20]	148	147	179	3702			1044	1772	673		7.9	*	2.8	* 0.18	176-195 [20]
191-210 [1]	212	307	321	419	390	498	358	100	-14		0.9		1.0	0.76	191-210 [1]
191-210 [10]	292	404	422	460	408	495	414	69	42		1.4		1.1	0.31	191-210 [10]
191-210 [20]	212	357	359	469	611	591	433	154	62		1.6		1.2	0.28	191-210 [20]
210-229 [1]	324	310	332	370	361	447	357	49	-14		0.9		1.0	0.71	210-229 [1]
210-229 [10]	236	308	305	321	379	462	335	77	-36		0.6		0.9	0.39	210-229 [10]
210-229 [20]	276	307	423	423	385	450	377	70	6		1.1		1.0	0.89	210-229 [20]
229-248 [1]															229-248 [1]
229-248 [10]															229-248 [10]
229-248 [20]															229-248 [20]
248-267 [1]	134	408	457	619	820	307	458	240	86		1.9		1.2	0.27	248-267 [1]
248-267 [10]	231	544	495	753	272	281	429	204	58		1.6		1.2	0.40	248-267 [10]
248-267 [20]	336	537	846	1162	3233	445	1093	1091	722		8.4	*	2.9	* 0.03	* 248-267 [20]
267-286 [1]	447	216	460				374	137	3		1.0		1.0	0.96	267-286 [1]
267-286 [10]	378	239	376				331	80	-41		0.6		0.9	0.47	267-286 [10]
267-286 [20]	384	254	252				297	76	-75		0.2		0.8	0.19	267-286 [20]
287-306 [1]	448	619	452	528	580	443	512	76	140		2.4	*	1.4	0.00	* 287-306 [1]
287-306 [10]	246	553	613	635	579	525	525	142	154		2.6	*	1.4	0.01	* 287-306 [10]
287-306 [20]	351	712	536	589	660	523	562	126	190		2.9	*	1.5	0.00	* 287-306 [20]
307-326 [1]															307-326 [1]
307-326 [10]															307-326 [10]
307-326 [20]															307-326 [20]
sAg 10	685	1229	1397	1285	1881		1295	427	924		10.4	*	3.5	* 0.00	* sAg 10
sAg 100	716	1044	775	2201	1770		1301	655	930		10.5	*	3.5	* 0.00	* sAg 100
N	171	399	240				270	117	-102		0.0		0.7	0.11	N
N	176	401	254				277	114	-95		0.0		0.7	0.13	N
3H	227	341	313	374	448	490	366	95	-6		0.9		1.0	0.89	3H
3H	215	421	398	440	378	413	378	82	6		1.1		1.0	0.89	3H
	SMC														
N	126	148	185	121	166	214	160	36	-11		0.0		0.9	0.63	N
3H	152	158	235	133	149	197	171	38	0		1.0		1.0	1.00	3H
PHA - 1	14858	13520	12148	5760	7739	20558	12431	5286	12,260	*	1150.4	*	72.8	* 0.00	* PHA - 1
PHA - 5	36504	29162	34179	24538	34713	36480	32596	4776	32,425	*	3040.9	*	191.0	* 0.00	* PHA - 5
PHA - 10	33003	22235	31051	26270	29454	58228	33374	12753	33,203	*	3113.8	*	195.5	* 0.00	* PHA - 10
LPS - 1	790	578	623	383	476	584	572	138	402		38.7	*	3.4	* 0.00	* LPS - 1
LPS - 5	621	560	595	483	612	557	571	51	401		38.6	*	3.3	* 0.00	* LPS - 5
LPS - 10	434	465	555	590	512	658	536	83	365		35.2	*	3.1	* 0.00	* LPS - 10
LPS - 20	433	736	632	482	579	640	584	111	413		39.7	*	3.4	* 0.00	* LPS - 20
LPS - 40	486	658	567	537	644	645	590	70	419		40.3	*	3.5	* 0.00	* LPS - 40
	PBMC														
N	421	866	823	544	690	793	690	175	-100		0.0		0.9	0.28	N
3H	935	803	605	838	681	877	790	124	0		1.0		1.0	1.00	3H
PHA - 1	4209	4346	3082	3522	2518	3192	3478	700	2,688		27.8	*	4.4	* 0.00	* PHA - 1
PHA - 5	4308	3402	2573	3450	2097	3005	3139	769	2,349		24.4	*	4.0	* 0.00	* PHA - 5
PHA - 10	4080	2143	2465	2077	2121	2172	2510	782	1,720		18.1	*	3.2	* 0.00	* PHA - 10
LPS - 1	1418	781	761	612	392	732	783	343	-7		0.9		1.0	0.96	LPS - 1
LPS - 5	894	788	625	498	470	584	643	167	-147		-0.5		0.8	0.11	LPS - 5
LPS - 10	520	584	365	519	444	513	491	76	-299		-2.0		0.6	0.00	* LPS - 10
LPS - 20	416	455	358	378	360	353	387	41	-403		-3.0		0.5	0.00	* LPS - 20
LPS - 40	316	318	186	229	261	189	250	59	-540		-4.4		0.3	0.00	* LPS - 40

Raw data for Bursectomy duck W131

W131	Mean	SD												
Total N	152	128												
Total 3H	555	439							CPM-3H	S.I.	P/N	t-Test		
	R1	R2	R3	R4	R5	R6	Mean	SD	>5000	>2.1	>2.1	<0.05		
37-56 [1]	351	957	246	236	547	848	531	311	-24	0.9	1.0	0.91		37-56 [1]
37-56 [10]	620	672	285	319	958	314	528	269	-27	0.9	1.0	0.89		37-56 [10]
37-56 [20]	475	1794	655	943	773	1306	991	484	436	2.1	1.8	0.07		37-56 [20]
54-73 [1]	27	131	89	105	94	76	87	35	-468	-0.2	0.2	0.02	*	54-73 [1]
54-73 [10]	174			364	46		195	160	-360	0.1	0.4	0.20		54-73 [10]
54-73 [20]	121	167		480	263	178	242	143	-313	0.2	0.4	0.15		54-73 [20]
71-90 [1]	192	218	678	527	892	802	552	295	-3	1.0	1.0	0.99		71-90 [1]
71-90 [10]	65	555	813	638	5557	704	1389	2058	834	3.1 *	2.5 *	0.19		71-90 [10]
71-90 [20]	60	637	646	979	2820	1640	1130	976	575	2.4 *	2.0	0.10		71-90 [20]
87-106 [1]	81	151	132	193	181	183	154	42	-401	0.0	0.3	0.04	*	87-106 [1]
87-106 [10]	236	51	118	132	179	193	152	65	-403	0.0	0.3	0.04	*	87-106 [10]
87-106 [20]	165	128	135	110	128	140	134	18	-421	0.0	0.2	0.03	*	87-106 [20]
101-120 [1]	90	562	418	528	421	120	357	203	-198	0.5	0.6	0.31		101-120 [1]
101-120 [10]	73	141	842	538	342	62	333	310	-222	0.4	0.6	0.29		101-120 [10]
101-120 [20]	20	63	210	124	75	73	94	66	-461	-0.1	0.2	0.02	*	101-120 [20]
116-130 [1]	418	607	873	658	1371	534	744	342	189	1.5	1.3	0.37		116-130 [1]
116-130 [10]	996	1574	3650	1010	857	1191	1546	1060	991	3.5 *	2.8 *	0.01	*	116-130 [10]
116-130 [20]	518	3773	1822	531	369	1853	1478	1310	923	3.3 *	2.7 *	0.04	*	116-130 [20]
126-140 [1]	320	12148	719	428	99	60	2296	4833	1,741	5.3 *	4.1 *	0.22		126-140 [1]
126-140 [10]	651	4305	4477	3003	176	64	2113	2063	1,558	4.9 *	3.8 *	0.02	*	126-140 [10]
126-140 [20]	11461	1311	1396	2837	529	361	2983	4245	2,428	7.0 *	5.4 *	0.06		126-140 [20]
136-150 [1]	3309	579	472	9245	776	4033	3069	3386	2,514	7.2 *	5.5 *	0.02	*	136-150 [1]
136-150 [10]	1241	6971	547	609	599	56	1671	2624	1,116	3.8 *	3.0 *	0.16		136-150 [10]
136-150 [20]	43	419	408	1068	427	99	411	365	-144	0.6	0.7	0.50		136-150 [20]
146-160 [1]	48	469	430	625	654	212	406	237	-149	0.6	0.7	0.45		146-160 [1]
146-160 [10]	38	328	637	378	308	161	308	204	-247	0.4	0.6	0.21		146-160 [10]
146-160 [20]	50	240	677	561	384	861	462	297	-93	0.8	0.8	0.65		146-160 [20]
156-170 [1]	520	1863	571	22704	6596	68	5387	8820	4,832	13.0 *	9.7 *	0.07		156-170 [1]
156-170 [10]	3993	1969	1764	1080	472	85	1561	1394	1,006	3.5 *	2.8 *	0.03	*	156-170 [10]
156-170 [20]	57	47	96	819	4729	87	973	1865	418	2.0	1.8	0.46		156-170 [20]
166-180 [1]	49	169	421	312	580	577	351	216	-204	0.5	0.6	0.30		166-180 [1]
166-180 [10]	32	81	249	456	185	359	227	162	-328	0.2	0.4	0.10		166-180 [10]
166-180 [20]	338	345	62	94	554	1545	490	548	-65	0.8	0.9	0.79		166-180 [20]
176-195 [1]	521	316	139	237	295	208	286	131	-269	0.3	0.5	0.17		176-195 [1]
176-195 [10]	768	318	152	359	838	2076	752	702	197	1.5	1.4	0.47		176-195 [10]
176-195 [20]	341	1276	229	326	374	438	497	388	-58	0.9	0.9	0.79		176-195 [20]
191-210 [1]	32	138	771	280	304	712	373	303	-182	0.5	0.7	0.38		191-210 [1]
191-210 [10]	70	370	2596	244	667	682	772	925	217	1.5	1.4	0.50		191-210 [10]
191-210 [20]	89	407	573	625	689	525	485	216	-70	0.8	0.9	0.72		191-210 [20]
210-229 [1]	191	281	164	308	359	344	275	80	-280	0.3	0.5	0.15		210-229 [1]
210-229 [10]	283	443	418	1052	684	214	516	309	-39	0.9	0.9	0.85		210-229 [10]
210-229 [20]	279	141	198	387	331	200	256	93	-299	0.3	0.5	0.12		210-229 [20]
229-248 [1]														229-248 [1]
229-248 [10]														229-248 [10]
229-248 [20]														229-248 [20]
248-267 [1]	44	129	525	424	88	35	208	212	-347	0.1	0.4	0.09		248-267 [1]
248-267 [10]	485	3146	1535	1885	1050	75	1363	1096	808	3.0 *	2.5 *	0.04	*	248-267 [10]
248-267 [20]	757	854	20431	1649	694	31	4069	8032	3,514	9.7 *	7.3 *	0.14		248-267 [20]
267-286 [1]	373	471	441	499	351	28	361	172	-194	0.5	0.6	0.32		267-286 [1]
267-286 [10]	276	516	500	565	627	61	424	214	-131	0.7	0.8	0.50		267-286 [10]
267-286 [20]	176	532	643	324	376	51	350	219	-205	0.5	0.6	0.30		267-286 [20]
287-306 [1]	136	283	229	372	764	78	310	245	-245	0.4	0.6	0.23		287-306 [1]
287-306 [10]	287	583	584	632	677	106	478	228	-77	0.8	0.9	0.70		287-306 [10]
287-306 [20]	454	295	291	209	147	30	238	145	-317	0.2	0.4	0.11		287-306 [20]
307-326 [1]														307-326 [1]
307-326 [10]														307-326 [10]
307-326 [20]														307-326 [20]
sAg 10	135	161	203	363	184	157	201	83	-354	0.1	0.4	0.07		sAg 10
sAg 100	172	233	416	333	163	84	234	122	-321	0.2	0.4	0.10		sAg 100
N	52	349	373	104	40	41	160	158	-395	0.0	0.3	0.05		N
N	54	47	122	291	253	92	143	104	-412	0.0	0.3	0.04	*	N
3H	1685	672	704	563	195	64	647	571	92	1.2	1.2	0.71		3H
3H	40	249	436	648	626	777	463	277	-92	0.8	0.8	0.65		3H
	SMC													
N														N
3H														3H
PHA - 1														PHA - 1
PHA - 5														PHA - 5
PHA - 10														PHA - 10
LPS - 1														LPS - 1
LPS - 5														LPS - 5
LPS - 10														LPS - 10
LPS - 20														LPS - 20
LPS - 40														LPS - 40
	PBMC													
N														N
3H														3H
PHA - 1														PHA - 1
PHA - 5														PHA - 5
PHA - 10														PHA - 10
LPS - 1														LPS - 1
LPS - 5														LPS - 5
LPS - 10														LPS - 10
LPS - 20														LPS - 20
LPS - 40														LPS - 40

Raw data for Bursectomy duck W132

W132	Mean	SD													
Total N	444	262													
Total 3H	646	258							C2H-3H		S.I.		P/N	t-Test	
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1	<0.05	
37-56 [1]	387	172	2362	265	298	226	618	857	-28		0.9		1.0	0.92		37-56 [1]
37-56 [10]	317	869	1831	962	276	243	750	616	104		1.5		1.2	0.62		37-56 [10]
37-56 [20]	618	595	3018	545	498	2805	1347	1215	701		4.5	*	2.1	0.07		37-56 [20]
54-73 [1]	370	310	5205	593	672	752	1317	1912	671		4.3	*	2.0	0.24		54-73 [1]
54-73 [10]	524	1129	399	527	479	520	596	265	-50		0.8		0.9	0.71		54-73 [10]
54-73 [20]	915	886	5062	2879	1936	1125	2134	1627	1,488		8.4	*	3.3	*	0.01	* 54-73 [20]
71-90 [1]	536	254	277	2117	342	315	640	730	-6		1.0		1.0	0.98		71-90 [1]
71-90 [10]	838	1574	1422	203	314	561	819	572	173		1.9		1.3	0.38		71-90 [10]
71-90 [20]	793	264	17534	555	494	223	3311	6971	2,665		14.2	*	5.1	*	0.19	71-90 [20]
87-106 [1]	3182	416	2614	574	363	968	1353	1229	707		4.5	*	2.1	0.07		87-106 [1]
87-106 [10]	881	315	399	1612	418	4322	1325	1547	679		4.4	*	2.1	0.15		87-106 [10]
87-106 [20]	645	272	830	258	308	3974	1048	1452	402		3.0	*	1.6	0.35		87-106 [20]
101-120 [1]	998	2419	2654	626	367	806	1312	974	666		4.3	*	2.0	0.04	*	101-120 [1]
101-120 [10]	3749	541	11531	1856	601	4452	3788	4119	3,143		16.6	*	5.9	*	0.02	* 101-120 [10]
101-120 [20]	306	490	300	1224	1189	813	720	420	75		1.4		1.1	0.65		101-120 [20]
116-130 [1]	1067	4371	334	895	1661	5656	2331	2159	1,685		9.4	*	3.6	*	0.01	* 116-130 [1]
116-130 [10]	328	561	940	1330	2167	569	983	679	337		2.7	*	1.5	0.14		116-130 [10]
116-130 [20]	1238	2615	381	1836	1286	451	1301	847	655		4.3	*	2.0	0.02	*	116-130 [20]
126-140 [1]	364	399	272	255	3416	698	901	1243	255		2.3	*	1.4	0.49		126-140 [1]
126-140 [10]	477	276	1383	343	236	218	489	448	-157		0.2		0.8	0.35		126-140 [10]
126-140 [20]	2757	5996	567	1284	326	302	1872	2222	1,226		7.1	*	2.9	*	0.07	126-140 [20]
136-150 [1]	384	891	361	600	603	615	576	192	-70		0.7		0.9	0.57		136-150 [1]
136-150 [10]	522	401	353	424	464	949	519	218	-127		0.4		0.8	0.32		136-150 [10]
136-150 [20]	373	2809	1285	801	3111	790	1528	1150	882		5.4	*	2.4	*	0.02	* 136-150 [20]
146-160 [1]	266	239	1424	579	1024	631	694	458	48		1.2		1.1	0.78		146-160 [1]
146-160 [10]	878	2018	821	4144	788	573	1537	1376	891		5.4	*	2.4	*	0.04	* 146-160 [10]
146-160 [20]	295	1027	1740	2856	1358	620	1316	913	670		4.3	*	2.0	0.03	*	146-160 [20]
156-170 [1]	3337	1934	301	270	1156	607	1268	1192	622		4.1	*	2.0	0.09		156-170 [1]
156-170 [10]	682	1150	362	557	929	4243	1321	1458	675		4.3	*	2.0	0.13		156-170 [10]
156-170 [20]	464	573	3534	489	1430	661	1192	1203	546		3.7	*	1.8	0.14		156-170 [20]
166-180 [1]	372	977	242	157	381	434	427	288	-219		-0.1		0.7	0.12		166-180 [1]
166-180 [10]	5635	204	219	183	292	289	1137	2204	491		3.4	*	1.8	0.44		166-180 [10]
166-180 [20]	216	178	266	4081	218	255	869	1574	223		2.1	*	1.3	0.63		166-180 [20]
176-195 [1]	158	206	1569	1329	232	236	622	646	-24		0.9		1.0	0.91		176-195 [1]
176-195 [10]	355	925	223	308	149	244	367	282	-279		-0.4		0.6	0.05		176-195 [10]
176-195 [20]	682	1351	157	208	7528	338	1711	2884	1,065		6.3	*	2.6	*	0.21	176-195 [20]
191-210 [1]	1835	2422	3955	514	1104	3491	2220	1339	1,574		8.8	*	3.4	*	0.00	* 191-210 [1]
191-210 [10]	624	599	704	1896	1522	574	987	574	341		2.7	*	1.5	0.10		191-210 [10]
191-210 [20]	2893	2324	2978	453	1582	584	1802	1113	1,157		6.7	*	2.8	*	0.00	* 191-210 [20]
210-229 [1]	225	612	514	548	613	1689	700	505	54		1.3		1.1	0.76		210-229 [1]
210-229 [10]	1079	369	1816	1398	9486	1320	2578	3418	1,932		10.6	*	4.0	*	0.06	210-229 [10]
210-229 [20]	1241	1157	2001	588	3484	3520	1999	1248	1,353		7.7	*	3.1	*	0.00	* 210-229 [20]
229-248 [1]																229-248 [1]
229-248 [10]																229-248 [10]
229-248 [20]																229-248 [20]
248-267 [1]	2875	3264	352	585	853	1693	1604	1229	958		5.8	*	2.5	*	0.02	* 248-267 [1]
248-267 [10]	1746	8172	4304	1991	1603	2327	3357	2557	2,711		14.5	*	5.2	*	0.00	* 248-267 [10]
248-267 [20]	1036	809	2569	337	435	893	1013	809	367		2.8	*	1.6	0.16		248-267 [20]
267-286 [1]	375	229	225	133	410	283	276	103	-370		-0.8		0.4	0.00	*	267-286 [1]
267-286 [10]	338	471	955	2176	364	341	774	726	128		1.6		1.2	0.58		267-286 [10]
267-286 [20]	301	280	235	281	328	214	273	42	-373		-0.8		0.4	0.00	*	267-286 [20]
287-306 [1]	2445	735	9543	1995	3404	521	3107	3332	2,461		13.2	*	4.8	*	0.02	* 287-306 [1]
287-306 [10]	393	303	1125	845	934	1649	875	495	229		2.1	*	1.4	0.21		287-306 [10]
287-306 [20]	1238	354	574	3208	3867	6799	2673	2473	2,028		11.1	*	4.1	*	0.01	* 287-306 [20]
307-326 [1]																307-326 [1]
307-326 [10]																307-326 [10]
307-326 [20]																307-326 [20]
sAg 10	8416	451	835	802	510	2217	2205	3110	1,559		8.7	*	3.4	*	0.09	sAg 10
sAg 100	1015	13626	768	326	1608		3469	5697	2,823		15.0	*	5.4	*	0.09	sAg 100
N	438	235	353	306	1201	454	498	354	-148		0.3		0.8	0.33		N
N	425	213	538	232	452	485	391	136	-255		-0.3		0.6	0.04	*	N
3H	277	397	752	394	1070	978	645	336	-1		1.0		1.0	0.99		3H
3H	907	526	404	621	813	611	647	185	1		1.0		1.0	0.99		3H
	SMC															
N																N
3H																3H
PHA - 1																PHA - 1
PHA - 5																PHA - 5
PHA - 10																PHA - 10
LPS - 1																LPS - 1
LPS - 5																LPS - 5
LPS - 10																LPS - 10
LPS - 20																LPS - 20
LPS - 40																LPS - 40
	PBMC															
N																N
3H																3H
PHA - 1																PHA - 1
PHA - 5																PHA - 5
PHA - 10																PHA - 10
LPS - 1																LPS - 1
LPS - 5																LPS - 5
LPS - 10																LPS - 10
LPS - 20																LPS - 20
LPS - 40																LPS - 40

Raw data for Bursectomy duck W145

W145	Mean	SD											
Total N	259	329											
Total 3H	734	569							CPM-3H	S.I.	P/N	t-Test	
	R1	R2	R3	R4	R5	R6	Mean	SD	>5000	>2.1	>2.1	<0.05	
37-56 [1]	199	572	532	1549	1373	482	785	543	50	1.1	1.1	0.86	37-56 [1]
37-56 [10]	92	547	730	864	400	86	453	323	-281	0.4	0.6	0.28	37-56 [10]
37-56 [20]	109	456	632	1464	483		629	505	-105	0.8	0.9	0.73	37-56 [20]
54-73 [1]	391	502	1114	178	491	51	455	369	-280	0.4	0.6	0.29	54-73 [1]
54-73 [10]	1063	537	489	428	536	1155	701	320	-33	0.9	1.0	0.90	54-73 [10]
54-73 [20]	405	389	1116	427	514	1039	648	336	-86	0.8	0.9	0.74	54-73 [20]
71-90 [1]	79	634	830	713	1410	1316	830	488	96	1.2	1.1	0.73	71-90 [1]
71-90 [10]	269	1356	467	194	1537	690	752	568	18	1.0	1.0	0.95	71-90 [10]
71-90 [20]	333	747	705	402	930	1073	698	289	-36	0.9	1.0	0.89	71-90 [20]
87-106 [1]	307	1221	575	198	1406	790	750	487	15	1.0	1.0	0.96	87-106 [1]
87-106 [10]	292	712	839	820	942	719	721	227	-14	1.0	1.0	0.96	87-106 [10]
87-106 [20]	353	657	1232	1244	846	1365	950	398	215	1.5	1.3	0.42	87-106 [20]
101-120 [1]	162	486	997	1234	724	1233	806	430	72	1.2	1.1	0.79	101-120 [1]
101-120 [10]	74	675	551	1455	458	149	560	496	-174	0.6	0.8	0.53	101-120 [10]
101-120 [20]	59	727	417	428	533	94	376	258	-358	0.2	0.5	0.17	101-120 [20]
116-130 [1]	339	491	445	516	605		479	98	-255	0.5	0.7	0.34	116-130 [1]
116-130 [10]	579	657	517	708	779		648	103	-86	0.8	0.9	0.75	116-130 [10]
116-130 [20]	60		27	32	20		35	18	-700	-0.5	0.0	0.03 *	116-130 [20]
126-140 [1]	485	506	575	16	380	233	366	209	-368	0.2	0.5	0.15	126-140 [1]
126-140 [10]	1074	1052	925	13	752	100	653	477	-82	0.8	0.9	0.77	126-140 [10]
126-140 [20]	354	688	347	166	596	308	410	194	-324	0.3	0.6	0.20	126-140 [20]
136-150 [1]	780	841	801	1096	868		877	127	143	1.3	1.2	0.59	136-150 [1]
136-150 [10]	626	632	882	849	764		751	119	16	1.0	1.0	0.95	136-150 [10]
136-150 [20]	545	883	646	779	259		622	240	-112	0.8	0.8	0.68	136-150 [20]
146-160 [1]	410	372	1291	457	1009	1063	767	400	33	1.1	1.0	0.90	146-160 [1]
146-160 [10]	973	357	575	758	563	438	611	224	-124	0.7	0.8	0.62	146-160 [10]
146-160 [20]	380	487	429	888	988	388	593	272	-141	0.7	0.8	0.58	146-160 [20]
156-170 [1]	773	1499	748	737	726		897	337	162	1.3	1.2	0.56	156-170 [1]
156-170 [10]	505	799	1141	980	622		809	258	75	1.2	1.1	0.78	156-170 [10]
156-170 [20]	139	824	393	757	575		538	279	-197	0.6	0.7	0.48	156-170 [20]
166-180 [1]	51	393	581	1417	255	15	452	518	-282	0.4	0.6	0.32	166-180 [1]
166-180 [10]	854	542	1059	860	847	14	696	373	-38	0.9	0.9	0.88	166-180 [10]
166-180 [20]	1001	581	694	784	817	790	778	139	44	1.1	1.1	0.86	166-180 [20]
176-195 [1]	526	597	909	1099	759	447	723	248	-11	1.0	1.0	0.96	176-195 [1]
176-195 [10]	535	993	598	769	710	450	676	194	-58	0.9	0.9	0.81	176-195 [10]
176-195 [20]	457	519	741	1632	911	1880	1023	595	289	1.6	1.4	0.33	176-195 [20]
191-210 [1]	322	796	1294	477	870	1451	868	442	134	1.3	1.2	0.62	191-210 [1]
191-210 [10]	166	628	904	922	1158	1277	843	401	108	1.2	1.1	0.68	191-210 [10]
191-210 [20]	58	675	538	1056	1288	1247	810	477	76	1.2	1.1	0.78	191-210 [20]
210-229 [1]	113	1472	576	564	91	110	488	533	-247	0.5	0.7	0.39	210-229 [1]
210-229 [10]	489	1323	465	802	1289	1047	903	379	168	1.4	1.2	0.52	210-229 [10]
210-229 [20]	755	435	642	829	1354	630	774	314	40	1.1	1.1	0.88	210-229 [20]
229-248 [1]													229-248 [1]
229-248 [10]													229-248 [10]
229-248 [20]													229-248 [20]
248-267 [1]													248-267 [1]
248-267 [10]													248-267 [10]
248-267 [20]													248-267 [20]
267-286 [1]	71	285	244	397	424	176	266	133	-468	0.0	0.4	0.07	267-286 [1]
267-286 [10]	60	43	38	34	42	86	51	20	-684	-0.4	0.1	0.01 *	267-286 [10]
267-286 [20]	25	25	43	37	26	37	32	8	-702	-0.5	0.0	0.01 *	267-286 [20]
287-306 [1]	485	279	473	429	617		457	122	-278	0.4	0.6	0.31	287-306 [1]
287-306 [10]	559	635	571	471	1313		710	342	-24	0.9	1.0	0.93	287-306 [10]
287-306 [20]	443	379	587	397	995		560	256	-174	0.6	0.8	0.53	287-306 [20]
307-326 [1]													307-326 [1]
307-326 [10]													307-326 [10]
307-326 [20]													307-326 [20]
sAg 10	170	764	736	190	262		424	299	-310	0.3	0.6	0.27	sAg 10
sAg 100	867	954	549	809	392		714	235	-20	1.0	1.0	0.94	sAg 100
N	64	90	49	54	627	92	163	228	-572	-0.2	0.2	0.03 *	N
N	53	207	208	325	1164	180	356	405	-378	0.2	0.5	0.17	N
3H	1194	236	691	23	1832	116	682	712	-52	0.9	0.9	0.87	3H
3H	179	1148	603	639	705	1445	787	446	52	1.1	1.1	0.85	3H
	SMC												
N													N
3H													3H
PHA - 1													PHA - 1
PHA - 5													PHA - 5
PHA - 10													PHA - 10
LPS - 1													LPS - 1
LPS - 5													LPS - 5
LPS - 10													LPS - 10
LPS - 20													LPS - 20
LPS - 40													LPS - 40
	PBMC												
N													N
3H													3H
PHA - 1													PHA - 1
PHA - 5													PHA - 5
PHA - 10													PHA - 10
LPS - 1													LPS - 1
LPS - 5													LPS - 5
LPS - 10													LPS - 10
LPS - 20													LPS - 20
LPS - 40													LPS - 40

Raw data for Thymectomy duck W122

W122	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	123	112															
Total 3H	330	293															
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1		<0.05	
1-15 [1]	332	544	406	444	532	1116	562	283	232		2.1	*	1.7		0.09		1-15 [1]
1-15 [10]	626	245	249	703	406	331	427	195	96		1.5		1.3		0.45		1-15 [10]
1-15 [20]	257	252	385	417	353	354	336	68	6		1.0		1.0		0.96		1-15 [20]
7-14W-27 [1]	1295	150	573	369	240	949	596	445	266		2.3	*	1.8		0.09		7-14W-27 [1]
7-14W-27 [10]	180	160	455	148	150	526	270	173	-60		0.7		0.8		0.64		7-14W-27 [10]
7-14W-27 [20]	318	383	296	173	424	261	309	89	-21		0.9		0.9		0.87		7-14W-27 [20]
7-14R-27 [1]	169	3290	1231	887	109	253	990	1213	660		4.2	*	3.0	*	0.02	*	7-14R-27 [1]
7-14R-27 [10]	1162	678	231	119	3312	2704	1368	1337	1,037		6.0	*	4.1	*	0.00	*	7-14R-27 [10]
7-14R-27 [20]	510	827	427	394	499	594	542	156	212		2.0		1.6		0.10		7-14R-27 [20]
22-41 [1]	875	866	688	538	316	268	592	264	262		2.3	*	1.8		0.06		22-41 [1]
22-41 [10]	478	539	1064	440	355	416	549	260	218		2.1		1.7		0.11		22-41 [10]
22-41 [20]	913	621	4019	892	1816	5297	2260	1944	1,929		10.3	*	6.8	*	0.00	*	22-41 [20]
37-56 [1]	2024	609	502	583	742	527	831	590	501		3.4	*	2.5	*	0.01	*	37-56 [1]
37-56 [10]	352	736	156	372	79	202	316	235	-14		0.9		1.0		0.91		37-56 [10]
37-56 [20]	551	281	315	315	290	285	340	105	9		1.0		1.0		0.94		37-56 [20]
54-73 [1]	254	447	454	458	283	387	381	91	50		1.2		1.2		0.68		54-73 [1]
54-73 [10]	698	408	613	411	657	344	522	151	192		1.9		1.6		0.14		54-73 [10]
54-73 [20]	451	253	438	294	523	399	393	102	63		1.3		1.2		0.61		54-73 [20]
71-90 [1]	715	427	445	154	210	418	395	199	65		1.3		1.2		0.62		71-90 [1]
71-90 [10]	243	396	794	280	529	45	381	259	51		1.2		1.2		0.70		71-90 [10]
71-90 [20]	111	565	849	641	363	775	551	274	220		2.1		1.7		0.11		71-90 [20]
87-106 [1]	609	468	400	212	461	697	475	169	144		1.7		1.4		0.26		87-106 [1]
87-106 [10]	323	580	749	535	383	901	579	218	248		2.2	*	1.8		0.06		87-106 [10]
87-106 [20]	500	465	1174	317	524	367	558	312	228		2.1		1.7		0.11		87-106 [20]
101-120 [1]	541	353	271	324	214	439	357	118	27		1.1		1.1		0.83		101-120 [1]
101-120 [10]	552	473	342	280	400	1108	526	301	196		1.9		1.6		0.16		101-120 [10]
101-120 [20]	611	282	189	338	164	259	307	162	-23		0.9		0.9		0.86		101-120 [20]
116-130 [1]	131	153	830	529	619	203	411	290	81		1.4		1.2		0.55		116-130 [1]
116-130 [10]	193	163	286	163	748	310	311	223	-20		0.9		0.9		0.88		116-130 [10]
116-130 [20]	266	555	334	307	365	350	363	100	33		1.2		1.1		0.79		116-130 [20]
126-140 [1]	342	200	218	565	327	229	314	137	-17		0.9		0.9		0.89		126-140 [1]
126-140 [10]	472	457	218	206	575	307	373	151	42		1.2		1.1		0.74		126-140 [10]
126-140 [20]	436	228	163	406	264	331	305	106	-26		0.9		0.9		0.84		126-140 [20]
136-150 [1]	447	345	168	343	533	468	384	129	54		1.3		1.2		0.67		136-150 [1]
136-150 [10]	132	1155	762	720	533	81	564	408	234		2.1	*	1.7		0.12		136-150 [10]
136-150 [20]	831	878	394	234	181	1493	669	500	338		2.6	*	2.0		0.04	*	136-150 [20]
146-160 [1]	458	282	136	432	259	402	328	124	-2		1.0		1.0		0.99		146-160 [1]
146-160 [10]	375	327	315	75	190	730	335	222	5		1.0		1.0		0.97		146-160 [10]
146-160 [20]	343	259	375	426	282	627	385	133	55		1.3		1.2		0.66		146-160 [20]
156-170 [1]	296	157	272	242	398	194	260	85	-70		0.7		0.8		0.57		156-170 [1]
156-170 [10]	325	371	237	194	413	418	326	93	-4		1.0		1.0		0.98		156-170 [10]
156-170 [20]	593	394	398	225	238	523	395	148	65		1.3		1.2		0.61		156-170 [20]
166-180 [1]	586	922	1176	822	199	1568	879	472	549		3.6	*	2.7	*	0.00	*	166-180 [1]
166-180 [10]	641	327	166	976	218	546	479	306	149		1.7		1.5		0.28		166-180 [10]
166-180 [20]	933	489	497	838	464	655	646	200	316		2.5	*	2.0		0.02	*	166-180 [20]
176-195 [1]	666	429	585	277	283	323	427	165	97		1.5		1.3		0.45		176-195 [1]
176-195 [10]	307	335	197	158	274	236	251	67	-79		0.6		0.8		0.52		176-195 [10]
176-195 [20]	475	194	161	246	134	370	263	133	-67		0.7		0.8		0.59		176-195 [20]
191-210 [1]	932	324	471	388	245	173	422	271	92		1.4		1.3		0.49		191-210 [1]
191-210 [10]	214	567	602	546	906	1811	774	553	444		3.1	*	2.3	*	0.01	*	191-210 [10]
191-210 [20]	1251	682	451	969	300	180	639	411	309		2.5	*	1.9		0.04	*	191-210 [20]
210-229 [1]	590	323	460	324	439	307	407	111	77		1.4		1.2		0.54		210-229 [1]
210-229 [10]	608	375	253	280	439	505	410	136	80		1.4		1.2		0.53		210-229 [10]
210-229 [20]	622	404	246	497	699	339	468	172	138		1.7		1.4		0.28		210-229 [20]
229-248 [1]	435	368	490	146	448	404	382	123	52		1.2		1.2		0.68		229-248 [1]
229-248 [10]	454	268	434	252	145	617	362	171	31		1.2		1.1		0.80		229-248 [10]
229-248 [20]	778	327	319	377	270	525	433	190	102		1.5		1.3		0.43		229-248 [20]
248-267 [1]	36	444	346	559	653	92	355	249	25		1.1		1.1		0.85		248-267 [1]
248-267 [10]	621	487	167	234	313	138	327	191	-4		1.0		1.0		0.98		248-267 [10]
248-267 [20]	147	425	364	150	695	518	383	213	53		1.3		1.2		0.68		248-267 [20]
267-286 [1]	581	185	364	635	185	280	372	196	41		1.2		1.1		0.75		267-286 [1]
267-286 [10]	322	201	292	262	600	457	356	147	25		1.1		1.1		0.84		267-286 [10]
267-286 [20]	205	218	120	418	476	596	339	185	9		1.0		1.0		0.95		267-286 [20]
287-306 [1]	1841	635	975	390	334	[redacted]	835	617	505		3.4	*	2.5	*	0.01	*	287-306 [1]
287-306 [10]	1020	431	162	141	177	173	351	345	20		1.1		1.1		0.88		287-306 [10]
287-306 [20]	531	528	218	96	489	406	378	181	48		1.2		1.1		0.71		287-306 [20]
307-326 [1]	376	591	488	614	392	917	563	199	233		2.1	*	1.7		0.08		307-326 [1]
307-326 [10]	157	194	274	124	509	591	308	196	-22		0.9		0.9		0.86		307-326 [10]
307-326 [20]	224	356	345	131	210	404	278	105	-52		0.7		0.8		0.68		307-326 [20]
sAg 10	3202	314	580	173	326	268	811	1179	480		3.3	*	2.5	*	0.08		sAg 10
sAg 100	162	7288	541	831	802	136	1627	2790	1,296		7.3	*	4.9	*	0.03	*	sAg 100
N	87	16	79	239	289	177	148	105	-182		0.1		0.4		0.15		N
N	21	27	27	113	63	337	98	122	-232		-0.1		0.3		0.07		N
3H	465	411	316	264	224	442	354	100	23		1.1		1.1		0.85		3H
3H	52	80	[redacted]	374	47	256	162	146	-168		0.2		0.5		0.23		3H
3H	172	149	65	202	213	682	247	219	-83		0.6		0.7		0.52		3H
3H	61	1396	617	304	514	288	530	466	200		2.0		1.6		0.20		3H
	SMC																
N	76	68	64	52	60	28	58	17	-382		0.0		0.1		0.00	*	N
3H	311	465	337	473	572	484	440	98	0		1.0		1.0		1.00		3H
PHA - 1	672	669	660	485	811	565	644	111	203		1.5		1.5		0.01	*	PHA - 1
PHA - 5	826	670	435	464	889	743	671	187	231		1.6		1.5		0.02	*	PHA - 5
PHA - 10	557	622	574	564	723	626	611	62	171		1.4		1.4		0.00	*	PHA - 10
LPS - 1	424	500	523	515	543	539	507	44	67		1.2		1.2		0.16		LPS - 1
LPS - 5	361	325	193	420	261	345	318	80	-123		0.7		0.7		0.04	*	LPS - 5
LPS - 10	74	125	124	152	96	133	117	28	-323		0.2		0.3		0.00	*	LPS - 10
LPS - 20	94	273	234	202	219	179	200	61	-240		0.4		0.5		0.00	*	LPS - 20
LPS - 40	119	94	88	107	98	63	95	19	-346		0.1		0.2		0.00	*	LPS - 40
	PBMC																
N	47	69	85	119	114	241	113	69	-16		0.0		0.9		0.61		N
3H	113	125	96	123	160	153	128	24	0		1.0		1.0		1.00		3H
PHA - 1	9515	11062	7689	8189	9458	11578	9582	1531	9,454	*	598.1	*	74.7	*	0.00	*	PHA - 1
PHA - 5	2372	3284	1652	2948	2464	887	2268	875	2,140		136.1	*	17.7	*	0.00	*	PHA - 5
PHA - 10	210	208	212	214	278	515	273	122	145		10.1	*	2.1	*	0.02	*	PHA - 10
LPS - 1	83	105	110	124	113	151	114	22	-14		0.1		0.9		0.32		LPS - 1
LPS - 5	151	101	75	195	107	177	134	47	6		1.4		1.0		0.79		LPS - 5
LPS - 10	183	102	121	172	120	121	137	33	8		1.5		1.1		0.63		LPS - 10
LPS - 20	182	186	139	97	124	135	144	34	16		2.0		1.1		0.39		LPS - 20
LPS - 40	71	86	188	131	92	187	126	52	-3		0.8		1.0		0.92		LPS - 40

Raw data for Thymectomy duck W125

W125	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	169	136															
Total 3H	1382	1624															
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1		<0.05	
1-15 [1]	925	538	755	334	521	981	676	253	-706		0.4		0.5		0.30		1-15 [1]
1-15 [10]	2081	752	179	3415	400	613	1240	1257	-142		0.9		0.9		0.84		1-15 [10]
1-15 [20]	6538	7301	1033	1577	2459	794	3284	2884	1,902		2.6	*	2.4	*	0.03	*	1-15 [20]
7-14W-27 [1]	371	829	80	1177	296	372	521	404	-861		0.3		0.4		0.21		7-14W-27 [1]
7-14W-27 [10]	844	1808	17947	3326	7914	23214	9176	9312	7,794	*	7.4	*	6.6	*	0.00	*	7-14W-27 [10]
7-14W-27 [20]	69961	5014	13837	769	13881	66274	28289	31289	26,908	*	23.2	*	20.5	*	0.00	*	7-14W-27 [20]
7-14R-27 [1]	194	3640	301	857	8629	333	2326	3354	944		1.8		1.7		0.29		7-14R-27 [1]
7-14R-27 [10]	2072	489	758	316	737	2495	1145	907	-237		0.8		0.8		0.73		7-14R-27 [10]
7-14R-27 [20]	817	881	977	4194	326		1439	1560	57		1.0		1.0		0.94		7-14R-27 [20]
22-41 [1]	441	560	235	1597	208	925	661	527	-721		0.4		0.5		0.29		22-41 [1]
22-41 [10]	389	2962	1586	329	219	328	969	1102	-413		0.7		0.7		0.56		22-41 [10]
22-41 [20]	1508	3733	370	1378	574	2798	1727	1305	345		1.3		1.2		0.63		22-41 [20]
37-56 [1]	706	5992	3704	595	2108	249	2226	2248	844		1.7		1.6		0.28		37-56 [1]
37-56 [10]	1804	529	1258	2162	690	390	1139	726	-243		0.8		0.8		0.72		37-56 [10]
37-56 [20]	1891	1146	1141	750	755	491	1029	492	-353		0.7		0.7		0.61		37-56 [20]
54-73 [1]	1007	568	640	1035	892	831	829	191	-553		0.5		0.6		0.42		54-73 [1]
54-73 [10]	454	407	430	605	447	1965	718	615	-664		0.5		0.5		0.34		54-73 [10]
54-73 [20]	329	377	1087	364	699	879	623	316	-759		0.4		0.5		0.27		54-73 [20]
71-90 [1]	2224	415	900	230	344	2897	1168	1123	-213		0.8		0.8		0.76		71-90 [1]
71-90 [10]	245	1149	676	3279	656	307	1052	1138	-330		0.7		0.8		0.64		71-90 [10]
71-90 [20]	47	588	753	316	1548	3488	1123	1265	-258		0.8		0.8		0.72		71-90 [20]
87-106 [1]	1784	223	540	1960	484	2810	1300	1035	-82		0.9		0.9		0.91		87-106 [1]
87-106 [10]	2242	393	549	698	571	819	879	683	-503		0.6		0.6		0.47		87-106 [10]
87-106 [20]	550	521	791	470	1182	592	684	268	-697		0.4		0.5		0.31		87-106 [20]
101-120 [1]	592	1252	1380	701	200	918	841	438	-541		0.6		0.6		0.43		101-120 [1]
101-120 [10]	291	364	1877	237	334	174	546	656	-836		0.3		0.4		0.23		101-120 [10]
101-120 [20]	3060	2053	5569	1522	848	450	2250	1868	869		1.7		1.6		0.25		101-120 [20]
116-130 [1]	333	5733	2557	1412	755	167	1826	2102	444		1.4		1.3		0.56		116-130 [1]
116-130 [10]	313	183	270	3513	736	3004	1337	1510	-45		1.0		1.0		0.95		116-130 [10]
116-130 [20]	4818	836	470	5261	986	1003	2229	2190	847		1.7		1.6		0.28		116-130 [20]
126-140 [1]	394	8	705	769	202	260	390	297	-992		0.2		0.3		0.15		126-140 [1]
126-140 [10]	865	1839	340	312	634	292	714	596	-668		0.4		0.5		0.33		126-140 [10]
126-140 [20]	695	777	396	696	820	2201	931	640	-451		0.6		0.7		0.51		126-140 [20]
136-150 [1]	2760	1090	215	204	761	701	955	948	-427		0.6		0.7		0.54		136-150 [1]
136-150 [10]	679	456	2783	1211	2044	7860	2506	2763	1,124		1.9		1.8		0.18		136-150 [10]
136-150 [20]	5834	2268	1099	557	629	16763	4525	6312	3,143		3.6	*	3.3	*	0.02	*	136-150 [20]
146-160 [1]	891	943	801	543	4322	2645	1691	1493	309		1.3		1.2		0.67		146-160 [1]
146-160 [10]	4668	487	1814	868	286	2281	1734	1632	352		1.3		1.3		0.63		146-160 [10]
146-160 [20]	2120	516	460	785	1038	397	886	650	-496		0.6		0.6		0.47		146-160 [20]
156-170 [1]	748	1110	1413	1254	394	4273	1532	1392	150		1.1		1.1		0.83		156-170 [1]
156-170 [10]	340	331	583	1088	718	278	556	311	-825		0.3		0.4		0.23		156-170 [10]
156-170 [20]	2727	597	774	2557	2788	206	1608	1202	226		1.2		1.2		0.75		156-170 [20]
166-180 [1]	139	1100	866	536	1375	1465	914	509	-468		0.6		0.7		0.49		166-180 [1]
166-180 [10]	128	1123	1018	1846	1836	2378	1388	798	6		1.0		1.0		0.99		166-180 [10]
166-180 [20]	14613	1209	792	1051	3919	1122	3784	5430	2,403		3.0	*	2.7	*	0.04	*	166-180 [20]
176-195 [1]	920	802	977	1249	438	1628	1002	405	-379		0.7		0.7		0.58		176-195 [1]
176-195 [10]	1132	1631	632	747	745	2569	1243	747	-139		0.9		0.9		0.84		176-195 [10]
176-195 [20]	1616	456	1706	1162	423	1719	1180	609	-201		0.8		0.9		0.77		176-195 [20]
191-210 [1]	1983	172	326	913	626	1274	882	670	-499		0.6		0.6		0.47		191-210 [1]
191-210 [10]	233	3272	1710	1491	1864	148	1453	1161	71		1.1		1.1		0.92		191-210 [10]
191-210 [20]	194	13058	1027	1003	194	6057	3589	5139	2,207		2.8	*	2.6	*	0.05		191-210 [20]
210-229 [1]	5289	807	632	661	836	337	1427	1900	45		1.0		1.0		0.95		210-229 [1]
210-229 [10]	5686	3835	8830	2600	1286	891	3855	3002	2,473		3.0	*	2.8	*	0.01	*	210-229 [10]
210-229 [20]	15881	6311	4135	6881	4502	3726	6906	4571	5,524	*	5.6	*	5.0	*	0.00	*	210-229 [20]
229-248 [1]	826	467	283	1291	696	1051	769	371	-613		0.5		0.6		0.37		229-248 [1]
229-248 [10]	11440	1132	1064	1011	631	888	2694	4288	1,313		2.1		1.9		0.20		229-248 [10]
229-248 [20]	410	622	1330	766	1097	2536	1127	765	-255		0.8		0.8		0.71		229-248 [20]
248-267 [1]	12347	258	533	349	874	28	2398	4882	1,016		1.8		1.7		0.35		248-267 [1]
248-267 [10]	128	21	22	67	122	129	82	52	-1,300		-0.1		0.1		0.06		248-267 [10]
248-267 [20]	1744	771	2125	14927	668	7695	4655	5668	3,273		3.7	*	3.4	*	0.01	*	248-267 [20]
267-286 [1]	2615	395	1772	345	10662	140	2655	4041	1,273		2.0		1.9		0.20		267-286 [1]
267-286 [10]	1140	538	298	1141	671	8546	2056	3197	674		1.6		1.5		0.44		267-286 [10]
267-286 [20]	1483	1807	237	398	254	3317	1249	1215	-132		0.9		0.9		0.85		267-286 [20]
287-306 [1]	458	543	2113	1096	526	3240	1329	1126	-52		1.0		1.0		0.94		287-306 [1]
287-306 [10]	317	780	713	222	1405	3283	1120	1139	-262		0.8		0.8		0.71		287-306 [10]
287-306 [20]	1517	299	2518	4344	1575	615	1811	1468	430		1.4		1.3		0.55		287-306 [20]
307-326 [1]	577	377	1917	766	875	446	826	566	-555		0.5		0.6		0.42		307-326 [1]
307-326 [10]	1419	1981	2045	2046	3617	825	1989	931	607		1.5		1.4		0.38		307-326 [10]
307-326 [20]	680	606	663	2514	490	2969	1320	1112	-61		0.9		1.0		0.93		307-326 [20]
sAg 10	7374	548	303	534	573	6814	2691	3417	1,309		2.1		1.9		0.15		sAg 10
sAg 100	133	23283	3644	507	3431	130	5188	9011	3,806		4.1	*	3.8	*	0.03	*	sAg 100
N	218	142	81	52	98	421	169	136	-1,213		0.0		0.1		0.08		N
3H	2232	2475	2041	431	1931	2789	1983	821	601		1.5		1.4		0.39		3H
3H	506	378	480	391	442	2521	786	851	-595		0.5		0.6		0.39		3H
3H	990	2822	74	144	1370	228	938	1060	-444		0.6		0.7		0.53		3H
3H	316	201	492	943	249	414	436	270	-946		0.2		0.3		0.17		3H
3H	549	5232	2788	7151	664	208	2765	2870	1,384		2.1	*	2.0		0.11		3H
	SMC																
N	217	127	214	91	54	21	121	82	-741		0.0		0.1		0.00	*	N
3H	765	693	842	784	1030	1053	861	148	0		1.0		1.0		1.00		3H
PHA - 1	83310	51061	45122	46653	73145	59838	59855	15463	58,994	*	80.7	*	69.5	*	0.00	*	PHA - 1
PHA - 5	101646	104427	92514	69663	76927	81793	87828	13955	86,967	*	118.4	*	102.0	*	0.00	*	PHA - 5
PHA - 10	95817	95932	105551	81272	85137	84094	91301	9332	90,439	*	123.1	*	106.0	*	0.00	*	PHA - 10
LPS - 1	1493	1215	1022	958	1289	1777	1292	305	431		1.6		1.5		0.01	*	LPS - 1
LPS - 5	2577	1168	1646	1171	1500	1221	1547	541	686		1.9		1.8		0.01	*	LPS - 5
LPS - 10	2299	2447	1712	2088	2469	1076	2015	539	1,154		2.6	*	2.3	*	0.00	*	LPS - 10
LPS - 20	2659	4886	2597	4215	4383	2844	3597	1011	2,736		4.7	*	4.2	*	0.00	*	LPS - 20
LPS - 40	210	4624	5090	5979	6102	491	3749	2691	2,888		4.9	*	4.4	*	0.03	*	LPS - 40
	PBMC																
N	68	211	189	219	178	996	310	340	133		0.0		1.7		0.37		N
3H	227	128	203	190	133	185	178	39	0		1.0		1.0		1.00		3H
PHA - 1	697	313	1031	283	487	342	526	291	348		-1.6		3.0	*	0.02	*	PHA - 1
PHA - 5	41430	22631	52804	4813	2724	36959	26894	20366	26,716	*	-200.6		151.4	*	0.01	*	PHA - 5
PHA - 10	49067	43584	39325	36499	46544	61319	46056	8775	45,879	*	-345.3		259.2	*	0.00	*	PHA - 10
LPS - 1	2305	2407	1706	1928	2152	2302	2133	268	1,956		-13.8		12.0	*	0.00	*	LPS - 1
LPS - 5	1120	1025	127	280	532	3319	1067	1172	890		-5.7		6.0	*	0.09		LPS - 5
LPS - 10	468	421	207	202	527	382	368	135	190		-0.4		2.1		0.01	*	LPS - 10
LPS - 20	787	561	499	564	725	602	623	110	445		-2.4		3.5	*	0.00	*	LPS - 20
LPS - 40	356	299	259	354	205	263	289	59	112		0.2		1.6		0.00	*	LPS - 40

Raw data for Thymectomy duck W126

W126	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	169	174															
Total 3H	327	186															
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1		<0.05	
1-15 [1]	515	547	398	612	506	502	513	70	186		2.2	*	1.6		0.02	*	1-15 [1]
1-15 [10]	853	552	500	797	556	1181	740	260	413		3.6	*	2.3	*	0.00	*	1-15 [10]
1-15 [20]	279	763	450	650	514	926	597	232	270		2.7	*	1.8		0.01	*	1-15 [20]
7-14W-27 [1]	283	125	173	437	292	106	236	126	-91		0.4		0.7		0.27		7-14W-27 [1]
7-14W-27 [10]	371	235	154	458	160	188	261	125	-66		0.6		0.8		0.42		7-14W-27 [10]
7-14W-27 [20]	577	612	692	420	556	237	516	163	189		2.2	*	1.6		0.03	*	7-14W-27 [20]
7-14R-27 [1]	284	172	430	368	593	437	381	144	54		1.3		1.2		0.52		7-14R-27 [1]
7-14R-27 [10]	148	351	205	281	451	386	304	114	-23		0.9		0.9		0.77		7-14R-27 [10]
7-14R-27 [20]	250	857	648	335	384	166	440	262	113		1.7		1.3		0.23		7-14R-27 [20]
22-41 [1]	523	286	409	343	190	130	314	144	-13		0.9		1.0		0.87		22-41 [1]
22-41 [10]	327	768	272	287	250	258	360	202	33		1.2		1.1		0.70		22-41 [10]
22-41 [20]	1767	1021	1123	586	634	886	1003	429	676		5.3	*	3.1	*	0.00	*	22-41 [20]
37-56 [1]	566	815	582	230	1145	566	651	306	324		3.1	*	2.0		0.00	*	37-56 [1]
37-56 [10]	682	805	548	455	382	389	544	171	217		2.4	*	1.7		0.02	*	37-56 [10]
37-56 [20]	460	444	439	269	459	444	419	74	92		1.6		1.3		0.25		37-56 [20]
54-73 [1]	297	794	399	920	411	350	529	261	202		2.3	*	1.6		0.04	*	54-73 [1]
54-73 [10]	796	335	424	162	597	621	489	227	162		2.0		1.5		0.08		54-73 [10]
54-73 [20]	387	393	157	334	232	63	261	134	-66		0.6		0.8		0.42		54-73 [20]
71-90 [1]	387	266	144	88	240	158	214	107	-113		0.3		0.7		0.17		71-90 [1]
71-90 [10]	153	263	202	411	464	65	260	153	-67		0.6		0.8		0.42		71-90 [10]
71-90 [20]	561	389	302	381	225	764	437	195	110		1.7		1.3		0.21		71-90 [20]
87-106 [1]	378	315	394	87	144	248	261	125	-66		0.6		0.8		0.42		87-106 [1]
87-106 [10]	530	634	833	290	163	302	459	252	132		1.8		1.4		0.16		87-106 [10]
87-106 [20]	580	332	535	267	247	152	352	170	25		1.2		1.1		0.77		87-106 [20]
101-120 [1]	178	223	150	157	148	368	204	85	-123		0.2		0.6		0.13		101-120 [1]
101-120 [10]	288	150	272	130	120	295	209	84	-118		0.3		0.6		0.15		101-120 [10]
101-120 [20]	302	423	156	84	282	198	241	120	-86		0.5		0.7		0.29		101-120 [20]
116-130 [1]	398	325	538	380	297	69	335	155	8		1.0		1.0		0.93		116-130 [1]
116-130 [10]	468	411	553	256	392	187	378	135	51		1.3		1.2		0.54		116-130 [10]
116-130 [20]	270	257	168	174	100	106	179	72	-148		0.1		0.5		0.07		116-130 [20]
126-140 [1]	1025	87	211	287	134	172	319	352	-8		1.0		1.0		0.94		126-140 [1]
126-140 [10]	619	470	210	233	273	516	387	171	60		1.4		1.2		0.48		126-140 [10]
126-140 [20]	220	432	487	789	219	464	435	211	108		1.7		1.3		0.22		126-140 [20]
136-150 [1]	386	138	209	360	270	467	305	122	-22		0.9		0.9		0.79		136-150 [1]
136-150 [10]	369	385	469	275	302	290	348	74	21		1.1		1.1		0.79		136-150 [10]
136-150 [20]	326	317	314	479	607	499	424	123	97		1.6		1.3		0.24		136-150 [20]
146-160 [1]	626	121	366	715	438	994	543	303	216		2.4	*	1.7		0.03	*	146-160 [1]
146-160 [10]	737	228	736	458	617	659	573	198	246		2.6	*	1.8		0.01	*	146-160 [10]
146-160 [20]	451	289	380	479	416	612	438	108	111		1.7		1.3		0.18		146-160 [20]
156-170 [1]	141	100	394	431	371	326	294	139	-33		0.8		0.9		0.69		156-170 [1]
156-170 [10]	203	348	145	203	287	616	300	170	-27		0.8		0.9		0.75		156-170 [10]
156-170 [20]	94	130	208	400	277	405	252	132	-75		0.5		0.8		0.37		156-170 [20]
166-180 [1]	390	484	99	190	540	429	355	173	28		1.2		1.1		0.74		166-180 [1]
166-180 [10]	274	261	376	184	475	742	385	202	58		1.4		1.2		0.50		166-180 [10]
166-180 [20]	264	561	292	147	384	392	340	141	13		1.1		1.0		0.87		166-180 [20]
176-195 [1]	567	455	575	416	163	367	424	152	97		1.6		1.3		0.25		176-195 [1]
176-195 [10]	413	224	116	371	514	378	336	143	9		1.1		1.0		0.91		176-195 [10]
176-195 [20]	363	355	204	254	348	308	305	64	-22		0.9		0.9		0.78		176-195 [20]
191-210 [1]	598	274	323	230	370	356	359	128	32		1.2		1.1		0.70		191-210 [1]
191-210 [10]	196	180	219	728	417	59	300	239	-27		0.8		0.9		0.77		191-210 [10]
191-210 [20]	927	757	964	413	448	190	617	313	290		2.8	*	1.9		0.01	*	191-210 [20]
210-229 [1]	663	904	662	357	170	192	491	297	164		2.0		1.5		0.10		210-229 [1]
210-229 [10]	548	1434	439	266	594	408	615	417	288		2.8	*	1.9		0.02	*	210-229 [10]
210-229 [20]	487	1150	502	298	872	537	641	311	314		3.0	*	2.0		0.00	*	210-229 [20]
229-248 [1]	452	812	353	112	365	764	476	267	149		1.9		1.5		0.12		229-248 [1]
229-248 [10]	494	352	222	358	170	567	361	152	34		1.2		1.1		0.69		229-248 [10]
229-248 [20]	282	328	283	126	87	382	248	116	-79		0.5		0.8		0.33		229-248 [20]
248-267 [1]	375	509	852	225	123	71	359	291	32		1.2		1.1		0.74		248-267 [1]
248-267 [10]	177	344	113	125	88	180	171	92	-156		0.0		0.5		0.06		248-267 [10]
248-267 [20]	161	271	158	167	250	342	225	75	-102		0.4		0.7		0.20		248-267 [20]
267-286 [1]	694	664	540	157	230	155	407	254	80		1.5		1.2		0.39		267-286 [1]
267-286 [10]	245	352	164	181	213	299	242	72	-85		0.5		0.7		0.29		267-286 [10]
267-286 [20]	300	164	250	155	539	245	276	140	-51		0.7		0.8		0.53		267-286 [20]
287-306 [1]	124	196	353	421	101	93	215	140	-112		0.3		0.7		0.18		287-306 [1]
287-306 [10]	88	243	568	561	90	221	295	218	-32		0.8		0.9		0.72		287-306 [10]
287-306 [20]	279	280	246	246	236	401	281	61	-46		0.7		0.9		0.56		287-306 [20]
307-326 [1]	237	480	238	394	255	162	294	118	-33		0.8		0.9		0.69		307-326 [1]
307-326 [10]	134	614	290	304	389	261	332	161	5		1.0		1.0		0.95		307-326 [10]
307-326 [20]	181	159	352	511	827	268	383	252	56		1.4		1.2		0.54		307-326 [20]
sAg 10	349	352	322	209	152	269	276	81	-51		0.7		0.8		0.52		sAg 10
sAg 100	763	733	495	268	233	200	449	254	122		1.8		1.4		0.19		sAg 100
N	103	164	398	516	424	38	274	197	-53		0.7		0.8		0.54		N
N	67	77	133	51	41	21	65	39	-262		-0.7		0.2		0.00	*	N
3H	93	272	484	290	537	259	323	163	-4		1.0		1.0		0.96		3H
3H	151	678	264	348	651	265	393	220	66		1.4		1.2		0.46		3H
3H	521	527	607	436	204	310	434	151	107		1.7		1.3		0.20		3H
3H	252	145	298	101	75	79	158	95	-169		-0.1		0.5		0.04	*	3H
	BMC																
N	83	28	73	180	102	40	84	54	-36		0.0		0.7		0.18		N
3H	130	123	79	144	148	96	120	27	0		1.0		1.0		1.00		3H
PHA - 1	275	185	255	295	181	173	227	54	107		4.0	*	1.9		0.00	*	PHA - 1
PHA - 5	657	465	548	647	559	479	559	81	439		13.3	*	4.7	*	0.00	*	PHA - 5
PHA - 10	1738	2047	1201	1743	1386	1252	1561	334	1,441		41.4	*	13.0	*	0.00	*	PHA - 10
LPS - 1	154	226	304	254	189	254	230	53	110		4.1	*	1.9		0.00	*	LPS - 1
LPS - 5	195	257	350	246	184	210	240	61	120		4.4	*	2.0		0.00	*	LPS - 5
LPS - 10	159	224	205	188	247	203	204	30	84		3.4	*	1.7		0.00	*	LPS - 10
LPS - 20	267	299	269	390	486	321	339	85	219		7.1	*	2.8	*	0.00	*	LPS - 20
LPS - 40	377	424	395	467	466	287	403	67	283		8.9	*	3.4	*	0.00	*	LPS - 40
	PBMC																
N	50	27	61	47	41	187	69	59	2		0.0		1.0		0.94		N
3H	26	41	53	65	109	107	67	34	0		1.0		1.0		1.00		3H
PHA - 1	846	282	182	243	290	185	338	253	271		-134.6		5.1	*	0.03	*	PHA - 1
PHA - 5	1172	635	249	519	1163	1318	843	433	776		-386.9		12.6	*	0.00	*	PHA - 5
PHA - 10	737	674	464	604	545	619	607	96	540		-269.2		9.1	*	0.00	*	PHA - 10
LPS - 1	270	454	361	393	415	328	370	66	303		-150.7		5.5	*	0.00	*	LPS - 1
LPS - 5	140	384	315	297	339	123	266	109	200		-98.8		4.0	*	0.00	*	LPS - 5
LPS - 10	207	153	231	260	264	83	200	70	133		-65.4		3.0	*	0.00	*	LPS - 10
LPS - 20	124	106	177	235	289	121	175	73	109		-53.3		2.6	*	0.01	*	LPS - 20
LPS - 40	94	81	123	216	276	147	156	76	89		-43.7		2.3	*	0.03	*	LPS - 40

Raw data for Thymectomy duck W147

W147	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	52	30															
Total 3H	92	39															
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1		<0.05	
1-15 [1]	85	62	61	52	56		63	13	-29		0.3		0.7		0.12		1-15 [1]
1-15 [10]	139	183	288	105	65		156	86	64		2.6	*	1.7		0.02	*	1-15 [10]
1-15 [20]	231	153	289	293	43		202	105	110		3.7	*	2.2	*	0.00	*	1-15 [20]
7-14W-27 [1]	35	134	101	57	35		72	44	-20		0.5		0.8		0.33		7-14W-27 [1]
7-14W-27 [10]	46	284	300	106	37		155	128	63		2.6	*	1.7		0.05		7-14W-27 [10]
7-14W-27 [20]	103	114	569	138	41		193	213	101		3.5	*	2.1		0.04	*	7-14W-27 [20]
7-14R-27 [1]	43	73	71	183	104		95	54	3		1.1		1.0		0.89		7-14R-27 [1]
7-14R-27 [10]	21	41	132	90	105		78	46	-14		0.6		0.8		0.48		7-14R-27 [10]
7-14R-27 [20]	60	133	166	181	90		126	51	34		1.8		1.4		0.11		7-14R-27 [20]
22-41 [1]	49	95	88	202	112		109	57	17		1.4		1.2		0.42		22-41 [1]
22-41 [10]	48	217	129	135	80		122	64	30		1.7		1.3		0.19		22-41 [10]
22-41 [20]	31	71	97	269	66		107	94	15		1.4		1.2		0.57		22-41 [20]
37-56 [1]	135	146	66	31	33		82	55	-10		0.8		0.9		0.64		37-56 [1]
37-56 [10]	68	81	40	36	41		53	20	-39		0.0		0.6		0.04	*	37-56 [10]
37-56 [20]	65	182	107	37	80		94	55	2		1.1		1.0		0.92		37-56 [20]
54-73 [1]	35	153	50	58	77		75	46	-17		0.6		0.8		0.39		54-73 [1]
54-73 [10]	147	202	72	87	25		107	69	15		1.4		1.2		0.52		54-73 [10]
54-73 [20]	73	102	169	24	20		78	62	-14		0.6		0.8		0.51		54-73 [20]
71-90 [1]	85	83	57	43	45		63	20	-29		0.3		0.7		0.12		71-90 [1]
71-90 [10]	115	197	116	63	57		110	56	18		1.4		1.2		0.41		71-90 [10]
71-90 [20]	121	147	143	127	142		136	11	44		2.1		1.5		0.02	*	71-90 [20]
87-106 [1]	129	81	191	70	110		116	48	24		1.6		1.3		0.24		87-106 [1]
87-106 [10]	217	208	305	202	140		214	59	122		4.1	*	2.3	*	0.00	*	87-106 [10]
87-106 [20]	154	114	163	147	59		127	42	35		1.9		1.4		0.08		87-106 [20]
101-120 [1]	50	64	75	97	80		73	18	-19		0.5		0.8		0.31		101-120 [1]
101-120 [10]	85	41	38	48	33		49	21	-43		-0.1		0.5		0.03	*	101-120 [10]
101-120 [20]	62	65	83	97	62		74	16	-18		0.5		0.8		0.32		101-120 [20]
116-130 [1]	82	22	75	28	114		64	39	-28		0.3		0.7		0.16		116-130 [1]
116-130 [10]	40	25	24	31	67		37	18	-55		-0.4		0.4		0.01	*	116-130 [10]
116-130 [20]	50	36	57	126	107		75	39	-17		0.6		0.8		0.40		116-130 [20]
126-140 [1]	42	44	77	269	97		106	94	14		1.3		1.2		0.60		126-140 [1]
126-140 [10]	75	73	96	59	44		69	19	-23		0.4		0.8		0.23		126-140 [10]
126-140 [20]	80	41	42	62	44		54	17	-38		0.0		0.6		0.05	*	126-140 [20]
136-150 [1]	30	27	32	68	82		48	25	-44		-0.1		0.5		0.03	*	136-150 [1]
136-150 [10]	82	60	120	107	104		95	24	3		1.1		1.0		0.89		136-150 [10]
136-150 [20]	143	129	122	51	26		94	52	2		1.1		1.0		0.92		136-150 [20]
146-160 [1]	197	91	349	40	142		164	119	72		2.8	*	1.8		0.02	*	146-160 [1]
146-160 [10]	184	151	226	57	73		138	72	46		2.2	*	1.5		0.05		146-160 [10]
146-160 [20]	101	81	57	53	64		71	20	-21		0.5		0.8		0.27		146-160 [20]
156-170 [1]	53	88	97	45	23		61	31	-31		0.2		0.7		0.12		156-170 [1]
156-170 [10]	47	55	198	100	55		91	63	-1		1.0		1.0		0.96		156-170 [10]
156-170 [20]	74	68	63	25	46		55	20	-37		0.1		0.6		0.05		156-170 [20]
166-180 [1]	39	88	111	68	83		78	27	-14		0.6		0.8		0.45		166-180 [1]
166-180 [10]	39	73	87	43	84		65	23	-27		0.3		0.7		0.16		166-180 [10]
166-180 [20]	97	135	203	78	97		122	50	30		1.7		1.3		0.15		166-180 [20]
176-195 [1]	61	200	207	168	191		165	60	73		2.8	*	1.8		0.00	*	176-195 [1]
176-195 [10]	76	151	114	121	48		102	40	10		1.2		1.1		0.61		176-195 [10]
176-195 [20]	32	96	163	85	42		84	52	-8		0.8		0.9		0.69		176-195 [20]
191-210 [1]	41	79	86	157	83		89	42	-3		0.9		1.0		0.89		191-210 [1]
191-210 [10]	23	81	141	137	72		91	49	-1		1.0		1.0		0.95		191-210 [10]
191-210 [20]	126	50	18	49	63		61	40	-31		0.2		0.7		0.13		191-210 [20]
210-229 [1]	148	91	79	130	82		106	31	14		1.3		1.2		0.47		210-229 [1]
210-229 [10]	257	578	792	763	99		498	308	406		11.1	*	5.4	*	0.00	*	210-229 [10]
210-229 [20]	272	233	951	466	350		454	292	362		10.0	*	4.9	*	0.00	*	210-229 [20]
229-248 [1]	68	101	43	67	25		61	29	-31		0.2		0.7		0.11		229-248 [1]
229-248 [10]	102	123	85	94	71		95	19	3		1.1		1.0		0.87		229-248 [10]
229-248 [20]	86	103	98	106	90		97	8	5		1.1		1.1		0.80		229-248 [20]
248-267 [1]	58	161	105	121	102	160	118	39	26		1.6		1.3		0.17		248-267 [1]
248-267 [10]	38	159	79	129	167	90	110	50	18		1.5		1.2		0.35		248-267 [10]
248-267 [20]	64	133	138	64	31	40	78	46	-14		0.7		0.9		0.47		248-267 [20]
267-286 [1]	67	115	65	148	55	76	88	36	-4		0.9		1.0		0.81		267-286 [1]
267-286 [10]	60	288	129	135	146	60	136	83	44		2.1	*	1.5		0.07		267-286 [10]
267-286 [20]	78	145	195	74	126	45	111	55	19		1.5		1.2		0.36		267-286 [20]
287-306 [1]	83	119	167	138	90		119	35	27		1.7		1.3		0.16		287-306 [1]
287-306 [10]	35	68	82	108	45		68	29	-24		0.4		0.7		0.21		287-306 [10]
287-306 [20]	101	152	261	113	166		159	63	67		2.7	*	1.7		0.01	*	287-306 [20]
307-326 [1]	185	120	189	101	77		134	50	42		2.1		1.5		0.05	*	307-326 [1]
307-326 [10]	104	101	185	164	84		128	44	36		1.9		1.4		0.09		307-326 [10]
307-326 [20]	29	61	48	93	171		80	56	-12		0.7		0.9		0.59		307-326 [20]
sAg 10	45	118	83	156	149		110	47	18		1.5		1.2		0.37		sAg 10
sAg 100	62	188	112	137	90		118	48	26		1.6		1.3		0.21		sAg 100
N	72	20	32	91	102		63	36	-29		0.3		0.7		0.15		N
N	74	20	31	39	38		40	20	-52		-0.3		0.4		0.01	*	N
3H	38	81	153	105	111		98	42	6		1.1		1.1		0.78		3H
3H	67	91	127	103	177		113	42	21		1.5		1.2		0.30		3H
3H	114	123	95	133	108	73	108	21	16		1.4		1.2		0.36		3H
3H	24	39	50	82	92	38	54	27	-38		0.1		0.6		0.04	*	3H
	EMC																
N	106	84	43				78	32	-160		0.0		0.3		0.02	*	N
3H	293	171	248				237	62	0		1.0		1.0		1.00		3H
PHA - 1	1265	714	436				805	422	568		4.6	*	3.4	*	0.08		PHA - 1
PHA - 5	6284	6848	3745				5626	1653	5,388	*	34.7	*	23.7	*	0.00	*	PHA - 5
PHA - 10	11497	9871	9998				10455	904	10,218	*	65.0	*	44.1	*	0.00	*	PHA - 10
LPS - 1	3077	2236	2246				2520	483	2,282		15.3	*	10.6	*	0.00	*	LPS - 1
LPS - 5	4369	4329	4649				4449	174	4,212		27.4	*	18.7	*	0.00	*	LPS - 5
LPS - 10	9374	14902	8887				11054	3341	10,817	*	68.7	*	46.6	*	0.00	*	LPS - 10
LPS - 20	11336	10802	14318				12152	1895	11,915	*	75.6	*	51.2	*	0.00	*	LPS - 20
LPS - 40	12029	11805	16069				13301	2400	13,064	*	82.8	*	56.0	*	0.00	*	LPS - 40
	PBMC																
N	104	192	116	163	186	171	155	37	46		0.0		1.4		0.09		N
3H	65	125	189	77	76	124	109	47	0		1.0		1.0		1.00		3H
PHA - 1	749	696	729	634	976	1246	838	231	729		-14.8		7.7	*	0.00	*	PHA - 1
PHA - 5	3202	2621	2092	5364	7771	5891	4490	2209	4,381		-94.2		41.1	*	0.00	*	PHA - 5
PHA - 10	4498	3658	3218	3041	3836	4089	3723	543	3,614		-77.6		34.1	*	0.00	*	PHA - 10
LPS - 1	1613	1225	1012	975	1199	1557	1264	268	1,154		-24.1		11.6	*	0.00	*	LPS - 1
LPS - 5	552	294	426	449	345	448	419	90	310		-5.7		3.8	*	0.00	*	LPS - 5
LPS - 10	612	290	325	358	319	442	391	120	282		-5.1		3.6	*	0.00	*	LPS - 10
LPS - 20	164	448	454	529	397	83	346	179	237		-4.1		3.2	*	0.01	*	LPS - 20
LPS - 40	396	154	196	186	167	140	207	95	97		-1.1		1.9		0.05	*	LPS - 40

Raw data for Thymectomy duck W151

W151	Mean	SD							CPM-3H		S.I.		P/N		t-Test	
Total N	403	586														
Total 3H	2835	7337														
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1		<0.05	
1-15 [1]	77	127	86	219	547	935	332	344	-2,503		0.0		0.1		0.42		1-15 [1]
1-15 [10]	120	340	157	91	88	7610	1401	3043	-1,434		0.4		0.5		0.65		1-15 [10]
1-15 [20]	140	499	736	855	152	3520	984	1277	-1,851		0.2		0.3		0.55		1-15 [20]
7-14W-27 [1]	1326	346	225	182	143	17417	3273	6943	438		1.2		1.2		0.90		7-14W-27 [1]
7-14W-27 [10]	5350	876	5683	2269	1369		3109	2256	275		1.1		1.1		0.94		7-14W-27 [10]
7-14W-27 [20]	10051	16231	2403	5953	1071	59	5961	6223	3,127		2.3	*	2.1	*	0.35		7-14W-27 [20]
7-14R-27 [1]	70	19074	2655	23120	510	147	7596	10578	4,761		3.0	*	2.7	*	0.20		7-14R-27 [1]
7-14R-27 [10]	2541	523	300	577	661		920	916	-1,914		0.2		0.3		0.57		7-14R-27 [10]
7-14R-27 [20]	201	129	121	420	455	26343	4612	10647	1,777		1.7		1.6		0.63		7-14R-27 [20]
22-41 [1]	392	107	164	123	94	767	275	265	-2,560		-0.1		0.1		0.41		22-41 [1]
22-41 [10]	744	132	881	120	890	2270	840	785	-1,995		0.2		0.3		0.52		22-41 [10]
22-41 [20]	2206	351	398	1788	2618	757	1353	979	-1,482		0.4		0.5		0.63		22-41 [20]
37-56 [1]	112	193	5683	3225	25975	210	5900	10086	3,065		2.3	*	2.1		0.40		37-56 [1]
37-56 [10]	7409	299	432	228	101	1358	1638	2863	-1,197		0.5		0.6		0.70		37-56 [10]
37-56 [20]	4685	186	127	626	1153	3404	1697	1899	-1,138		0.5		0.6		0.71		37-56 [20]
54-73 [1]	218	145	243	165	136	99	168	54	-2,667		-0.1		0.1		0.39		54-73 [1]
54-73 [10]	761	187	133	712	198	734	454	310	-2,381		0.0		0.2		0.44		54-73 [10]
54-73 [20]	152	221	269	167	509	389	285	139	-2,550		0.0		0.1		0.41		54-73 [20]
71-90 [1]	523	407	97	87	121	163	233	185	-2,602		-0.1		0.1		0.40		71-90 [1]
71-90 [10]	105	1703	1466	98	10623	270	2378	4101	-457		0.8		0.8		0.89		71-90 [10]
71-90 [20]	119	2670	856	103	18865	90	3784	7455	949		1.4		1.3		0.78		71-90 [20]
87-106 [1]	246	241	114	74	185	418	213	121	-2,622		-0.1		0.1		0.40		87-106 [1]
87-106 [10]	482	136	170	144	253	326	252	134	-2,583		-0.1		0.1		0.40		87-106 [10]
87-106 [20]	276	285	125	208	166	1663	454	596	-2,381		0.0		0.2		0.44		87-106 [20]
101-120 [1]	150	135	84	183	618	131	217	199	-2,618		-0.1		0.1		0.40		101-120 [1]
101-120 [10]	745	127	139	71	305	253	273	247	-2,562		-0.1		0.1		0.41		101-120 [10]
101-120 [20]	6323	85	301	133	76	13501	3403	5530	568		1.2		1.2		0.86		101-120 [20]
116-130 [1]	92	6589	11062	28704	21470	68	11331	11665	8,496	*	4.5	*	4.0	*	0.03	*	116-130 [1]
116-130 [10]	1332	92	150	71	816	24175	4439	9682	1,605		1.7		1.6		0.66		116-130 [10]
116-130 [20]	268	94	1606	129	161	1196	576	655	-2,259		0.1		0.2		0.46		116-130 [20]
126-140 [1]	126	116	101	84	613	290	222	206	-2,613		-0.1		0.1		0.40		126-140 [1]
126-140 [10]	210	143	88	144	1124	1377	514	577	-2,321		0.0		0.2		0.45		126-140 [10]
126-140 [20]	836	31	283	49	28	16	207	324	-2,628		-0.1		0.1		0.39		126-140 [20]
136-150 [1]	1655	202	93	149	112	313	421	610	-2,414		0.0		0.1		0.43		136-150 [1]
136-150 [10]	119	5905	6935	39367	20646	106	12180	15290	9,345	*	4.8	*	4.3	*	0.04	*	136-150 [10]
136-150 [20]	8947	2503	1738	941	27803	144	7013	10660	4,178		2.7	*	2.5	*	0.26		136-150 [20]
146-160 [1]	307	110	72	73	880	299	290	308	-2,545		0.0		0.1		0.41		146-160 [1]
146-160 [10]	942	117	135	121	161	486	327	333	-2,508		0.0		0.1		0.42		146-160 [10]
146-160 [20]	166	382	111	223	451	1304	440	443	-2,395		0.0		0.2		0.44		146-160 [20]
156-170 [1]	702	158	112	366	144	369	309	224	-2,526		0.0		0.1		0.41		156-170 [1]
156-170 [10]	510	274	108	272	366	350	313	133	-2,522		0.0		0.1		0.41		156-170 [10]
156-170 [20]	763	166	114	187	333	16321	2981	6540	146		1.1		1.1		0.96		156-170 [20]
166-180 [1]	75	1592	3001	498	918	11345	2905	4260	70		1.0		1.0		0.98		166-180 [1]
166-180 [10]	1808	136	237	566	2307	4614	1611	1714	-1,224		0.5		0.6		0.69		166-180 [10]
166-180 [20]	726	157	423	333	565	1208	569	369	-2,266		0.1		0.2		0.46		166-180 [20]
176-195 [1]	144	117	179	262	271	427	233	113	-2,602		-0.1		0.1		0.40		176-195 [1]
176-195 [10]	95	141	145	364	293	3151	698	1206	-2,137		0.1		0.2		0.49		176-195 [10]
176-195 [20]	185	335	210	156	2302	192	563	854	-2,272		0.1		0.2		0.46		176-195 [20]
191-210 [1]	1338	192	186	77	392	13154	2557	5212	-278		0.9		0.9		0.93		191-210 [1]
191-210 [10]	150	19671	5177	8308	8427	144	6980	7233	4,145		2.7	*	2.5	*	0.22		191-210 [10]
191-210 [20]	86	9687	3462	18240	504	113	5349	7310	2,514		2.0		1.9		0.46		191-210 [20]
210-229 [1]	2405	742	326	201	129	9684	2248	3741	-587		0.8		0.8		0.85		210-229 [1]
210-229 [10]	270	357	673	146	2575	5068	1515	1961	-1,320		0.5		0.5		0.67		210-229 [10]
210-229 [20]	6557	7843	5381	3189	10997	37170	11856	12671	9,021	*	4.7	*	4.2	*	0.03	*	210-229 [20]
229-248 [1]	175	141	180	124	214	230	177	41	-2,658		-0.1		0.1		0.39		229-248 [1]
229-248 [10]	3589	476	110	172	304	118	795	1376	-2,040		0.2		0.3		0.51		229-248 [10]
229-248 [20]	25131	163	144	675	832	14227	6862	10513	4,027		2.7	*	2.4	*	0.28		229-248 [20]
248-267 [1]	128	13872	219	1323	18145	156	5641	8156	2,806		2.2	*	2.0		0.42		248-267 [1]
248-267 [10]	9230	1032	112	133	448	11656	3769	5237	934		1.4		1.3		0.77		248-267 [10]
248-267 [20]	217	479	226	155	207	1725	502	610	-2,333		0.0		0.2		0.45		248-267 [20]
267-286 [1]	592	305	122	291	173	514	333	186	-2,502		0.0		0.1		0.42		267-286 [1]
267-286 [10]	429	162	231	330	298	188	273	100	-2,562		-0.1		0.1		0.41		267-286 [10]
267-286 [20]	263	225	130	154	329	1672	462	597	-2,373		0.0		0.2		0.44		267-286 [20]
287-306 [1]	96	22771	9124	4930	13364	176	8410	8721	5,575	*	3.3	*	3.0	*	0.12		287-306 [1]
287-306 [10]	1101	190	410	602	271	31155	5622	12513	2,787		2.1	*	2.0		0.48		287-306 [10]
287-306 [20]	474	145	133	86	1435	303	429	513	-2,406		0.0		0.2		0.43		287-306 [20]
307-326 [1]	276	212	113	281	534	675	349	212	-2,486		0.0		0.1		0.42		307-326 [1]
307-326 [10]	518	638	344	165	312	1238	536	382	-2,299		0.1		0.2		0.46		307-326 [10]
307-326 [20]	1045	295	97	89	291	339	359	352	-2,476		0.0		0.1		0.42		307-326 [20]
sAg 10	9862	216	66	332	1527	23570	5929	9428	3,094		2.3	*	2.1		0.39		sAg 10
sAg 100	156	2865	8193	6303	19369	968	6309	7104	3,474		2.4	*	2.2	*	0.31		sAg 100
N	51	101	86	53	49	35	63	25	-2,772		-0.1		0.0		0.37		N
N	1297	210	89	1148	92	1626	744	691	-2,091		0.1		0.3		0.50		N
3H	3300	101	98	485	192	33539	6286	13409	3,451		2.4	*	2.2	*	0.39		3H
3H	149	4881	868	198	15111	196	3567	5944	732		1.3		1.3		0.82		3H
3H	1999	180	74	91	93	6151	1431	2433	-1,404		0.4		0.5		0.65		3H
3H	41	66	39	77	58	49	55	15	-2,780		-0.1		0.0		0.37		3H
	BMC																
N	63	76	27	64	75	135	73	35	-307		0.0		0.2		0.00	*	N
3H	198	351	383	515	404	431	380	105	0		1.0		1.0		1.00		3H
PHA - 1	29259	35673	27013	41740	35954	37138	34463	5411	34,083	*	112.0	*	90.6	*	0.00	*	PHA - 1
PHA - 5	28865	34759	31338	40344	39304	30686	34216	4756	33,836	*	111.2	*	90.0	*	0.00	*	PHA - 5
PHA - 10	39509	29586	29123	39813	34067	29089	33531	5103	33,151	*	109.0	*	88.2	*	0.00	*	PHA - 10
LPS - 1	1170	1206	1069	1461	1296	1018	1203	160	823		3.7	*	3.2	*	0.00	*	LPS - 1
LPS - 5	1420	1435	800	1299	1444	815	1202	310	822		3.7	*	3.2	*	0.00	*	LPS - 5
LPS - 10	970	1071	984	1130	1490	944	1098	204	718		3.3	*	2.9	*	0.00	*	LPS - 10
LPS - 20	699	1174	1288	1286	1606	423	1079	435	699		3.3	*	2.8	*	0.00	*	LPS - 20
LPS - 40	117	483	403	433	384	103	321	167	-60		0.8		0.8		0.47		LPS - 40
	PBMC																
N	67	69	61	57	35	68	60	13	-10		0.0		0.9		0.33		N
3H	60	72	95	48	53	86	69	19	0		1.0		1.0		1.00		3H
PHA - 1	8332	10350	8940	8665	9992	7695	8996	1007	8,927	*	940.6	*	130.4	*	0.00	*	PHA - 1
PHA - 5	12379	14933	9890	9504	9536	11574	11303	2134	11,234	*	1183.5	*	163.8	*	0.00	*	PHA - 5
PHA - 10	6841	9259	6430	8489	9379	7002	7900	1302	7,831	*	825.3	*	114.5	*	0.00	*	PHA - 10
LPS - 1	372	1463	500	395	415	285	572	442	503		53.9	*	8.3	*	0.02	*	LPS - 1
LPS - 5	216	311	223	364	342	264	287	62	218		23.9	*	4.2	*	0.00	*	LPS - 5
LPS - 10	144	287	305	314	282	224	259	65	190		21.0	*	3.8	*	0.00	*	LPS - 10
LPS - 20	92	244	303	333	231	151	226	91	157		17.5	*	3.3	*	0.00	*	LPS - 20
LPS - 40	81	124	125	116	69	48	94	32	25		3.6	*	1.4		0.14		LPS - 40

Raw data for Thymectomy duck W152

W152	Mean	SD							CPM-3H	S.I.		P/N		t-Test		
Total N	45	18														
Total 3H	634	602														
	R1	R2	R3	R4	R5	R6	Mean	SD	>5000	>2.1		>2.1		<0.05		
1-15 [1]	1428	524	927	381	694	429	731	396	97	1.2		1.2		0.71		1-15 [1]
1-15 [10]	347	183	565	406	153	320	329	151	-305	0.5		0.5		0.23		1-15 [10]
1-15 [20]	1643	1322	1956	339	351	2341	1325	831	691	2.2	*	2.1		0.03	*	1-15 [20]
7-14W-27 [1]	753	192	424	281	630	641	487	223	-147	0.8		0.8		0.57		7-14W-27 [1]
7-14W-27 [10]	789	3652	1089	478	3239	4510	2293	1712	1,659	3.8	*	3.6	*	0.00	*	7-14W-27 [10]
7-14W-27 [20]	3674	1076	350	514	5305	1016	1989	2024	1,355	3.3	*	3.1	*	0.01	*	7-14W-27 [20]
7-14R-27 [1]	2732	1006	853	609	368	378	991	890	357	1.6		1.6		0.25		7-14R-27 [1]
7-14R-27 [10]	487	2776	765	525	931	833	1053	862	419	1.7		1.7		0.17		7-14R-27 [10]
7-14R-27 [20]	1258	1937	392	426	968	766	958	581	324	1.5		1.5		0.25		7-14R-27 [20]
22-41 [1]	476	500	436	203	805	8739	1860	3376	1,226	3.1	*	2.9	*	0.09		22-41 [1]
22-41 [10]	658	651	448	721	1040	1669	865	438	231	1.4		1.4		0.39		22-41 [10]
22-41 [20]	5721	917	1463	849	1627	1317	1982	1857	1,348	3.3	*	3.1	*	0.00	*	22-41 [20]
37-56 [1]	1441	362	1088	550	727	3486	1276	1150	642	2.1		2.0		0.06		37-56 [1]
37-56 [10]	918	276	775	353	348	825	583	286	-51	0.9		0.9		0.84		37-56 [10]
37-56 [20]	255	167	843	361	363	238	371	243	-263	0.6		0.6		0.31		37-56 [20]
54-73 [1]	3301	746	264	1060	392	452	1036	1146	402	1.7		1.6		0.24		54-73 [1]
54-73 [10]	664	552	238	1015	367	505	557	269	-77	0.9		0.9		0.76		54-73 [10]
54-73 [20]	424	409	138	318	305	392	331	106	-303	0.5		0.5		0.24		54-73 [20]
71-90 [1]	503	757	528	67	571	329	459	236	-175	0.7		0.7		0.50		71-90 [1]
71-90 [10]	4447	750	770	664	2492	5285	2401	2045	1,767	4.0	*	3.8	*	0.00	*	71-90 [10]
71-90 [20]	3998	403	326	488	908	531	1109	1430	475	1.8		1.7		0.21		71-90 [20]
87-106 [1]	614	220	167	479	429	112	337	199	-297	0.5		0.5		0.25		87-106 [1]
87-106 [10]	72	350	195	288	200	655	293	201	-341	0.4		0.5		0.19		87-106 [10]
87-106 [20]	534	1848	188	594	311	353	638	611	4	1.0		1.0		0.99		87-106 [20]
101-120 [1]	150	88	434	104	183	144	184	127	-450	0.2		0.3		0.08		101-120 [1]
101-120 [10]	547	365	225	141	74	139	249	177	-385	0.3		0.4		0.14		101-120 [10]
101-120 [20]	936	73	197	313	181	102	300	323	-334	0.4		0.5		0.20		101-120 [20]
116-130 [1]	184	729	1409	658	1202	1276	910	467	276	1.5		1.4		0.31		116-130 [1]
116-130 [10]	598	1070	74	473	807	701	621	336	-13	1.0		1.0		0.96		116-130 [10]
116-130 [20]	382	406	473	570	1962	404	700	622	66	1.1		1.1		0.81		116-130 [20]
126-140 [1]	384	235	211	153	1303	323	435	433	-199	0.7		0.7		0.45		126-140 [1]
126-140 [10]	898	464	250	150	367	553	447	264	-187	0.7		0.7		0.47		126-140 [10]
126-140 [20]	12808	697	360	496	144	779	2547	5032	1,913	4.2	*	4.0	*	0.07		126-140 [20]
136-150 [1]	2098	508	117	909	345	277	709	732	75	1.1		1.1		0.79		136-150 [1]
136-150 [10]	868	252	830	1104	512	2401	995	751	361	1.6		1.6		0.22		136-150 [10]
136-150 [20]	6575	746	718	577	456	493	1594	2443	960	2.6	*	2.5	*	0.08		136-150 [20]
146-160 [1]	558	1118	116	266	482	319	477	351	-157	0.7		0.8		0.55		146-160 [1]
146-160 [10]	217	118	223	183	107	378	204	98	-430	0.3		0.3		0.10		146-160 [10]
146-160 [20]	87	302	149	128	140	559	228	178	-406	0.3		0.4		0.12		146-160 [20]
156-170 [1]	221	444	267	120	590	215	310	174	-324	0.4		0.5		0.21		156-170 [1]
156-170 [10]	504	323	739	251	143	479	407	213	-227	0.6		0.6		0.38		156-170 [10]
156-170 [20]	632	267	409	335	278	364	381	134	-253	0.6		0.6		0.32		156-170 [20]
166-180 [1]	1533	374	906	504	742	590	775	415	141	1.2		1.2		0.59		166-180 [1]
166-180 [10]	145	394	518	816	426	1109	568	342	-66	0.9		0.9		0.80		166-180 [10]
166-180 [20]	839	737	161	321	162	335	426	292	-208	0.6		0.7		0.42		166-180 [20]
176-195 [1]	235	283	471	481	395	192	343	123	-291	0.5		0.5		0.25		176-195 [1]
176-195 [10]	301	254	354	229	421	124	281	103	-353	0.4		0.4		0.17		176-195 [10]
176-195 [20]	177	654	203	174	450	604	377	221	-257	0.6		0.6		0.32		176-195 [20]
191-210 [1]	286	287	247	529	156	184	282	132	-352	0.4		0.4		0.17		191-210 [1]
191-210 [10]	845	77	867	351	436	178	459	332	-175	0.7		0.7		0.50		191-210 [10]
191-210 [20]	3852	1152	1034	286	1084	7369	2463	2698	1,829	4.1	*	3.9	*	0.00	*	191-210 [20]
210-229 [1]	893	827	391	328	616	174	538	288	-96	0.8		0.8		0.71		210-229 [1]
210-229 [10]	987	1093	1829	1457	1715	1561	1440	337	806	2.4	*	2.3	*	0.00	*	210-229 [10]
210-229 [20]	781	2143	4794	1771	3840	3930	2877	1541	2,243	4.8	*	4.5	*	0.00	*	210-229 [20]
229-248 [1]	646	236	285	169	553	485	396	192	-238	0.6		0.6		0.35		229-248 [1]
229-248 [10]	371	128	549	781	258	380	411	229	-223	0.6		0.6		0.39		229-248 [10]
229-248 [20]	1271	517	320	409	783	752	675	345	41	1.1		1.1		0.87		229-248 [20]
248-267 [1]	3771	466	237	898	675	1048	1183	1301	549	1.9		1.9		0.13		248-267 [1]
248-267 [10]	574	172	331	2445	464	323	718	857	84	1.1		1.1		0.78		248-267 [10]
248-267 [20]	281	193	303	385	264	244	278	64	-356	0.4		0.4		0.16		248-267 [20]
267-286 [1]	272	116	342	429	135	433	288	139	-346	0.4		0.5		0.18		267-286 [1]
267-286 [10]	803	314	192	352	173	4365	1033	1648	399	1.7		1.6		0.33		267-286 [10]
267-286 [20]	345	481	157	1119	171	670	491	364	-143	0.8		0.8		0.58		267-286 [20]
287-306 [1]	1516	1137	886	339	1745	610	1039	535	405	1.7		1.6		0.14		287-306 [1]
287-306 [10]	160	319	642	215	532	160	338	204	-296	0.5		0.5		0.25		287-306 [10]
287-306 [20]	753	722	156	446	287	1446	635	462	1	1.0		1.0		1.00		287-306 [20]
307-326 [1]	312	235	345	502	221	717	389	190	-245	0.6		0.6		0.34		307-326 [1]
307-326 [10]	150	121	697	525	473	931	483	313	-151	0.7		0.8		0.56		307-326 [10]
307-326 [20]	687	272	349	640	393	507	475	166	-159	0.7		0.7		0.53		307-326 [20]
sAg 10	1071	271	338	524	796	1890	815	605	181	1.3		1.3		0.52		sAg 10
sAg 100	6228	434	1331	957	451	1814	1869	2200	1,235	3.1	*	2.9	*	0.02	*	sAg 100
N	51	30	42	46	43	41	42	7	-592	0.0		0.1		0.02	*	N
N	98	34	35	45	33	39	47	25	-587	0.0		0.1		0.03	*	N
3H	469	219	590	415	198	166	343	173	-291	0.5		0.5		0.26		3H
3H	528	419	401	2971	429	929	946	1012	312	1.5		1.5		0.33		3H
3H	673	523	569	189	1191	1336	747	434	113	1.2		1.2		0.67		3H
3H	248	530	128	114	1079	899	500	411	-134	0.8		0.8		0.61		3H
	SMC															
N	54	50	38	51	28	41	44	10	-1,179	0.0		0.0		0.00	*	N
3H	2012	942	1230	1100	928	1123	1223	403	0	1.0		1.0		1.00		3H
PHA - 1	39503	43108	1167	33634	21707	23966	27181	15259	25,958	23.0	*	22.2	*	0.00	*	PHA - 1
PHA - 5	31448	30887	32702	16914	18917	24711	25930	6824	24,707	22.0	*	21.2	*	0.00	*	PHA - 5
PHA - 10	17857	24782	23049	13624	15563	21986	19477	4459	18,254	16.5	*	15.9	*	0.00	*	PHA - 10
LPS - 1	1842	1708	1434	1453	1141	1557	1523	243	300	1.3		1.2		0.15		LPS - 1
LPS - 5	1405	1196	1270	998	932	868	1112	211	-111	0.9		0.9		0.56		LPS - 5
LPS - 10	1411	1224	1468	1300	1310	1429	1357	93	135	1.1		1.1		0.44		LPS - 10
LPS - 20	1238	2058	1188	1068	1059	924	1256	408	33	1.0		1.0		0.89		LPS - 20
LPS - 40	981	1209	907	553	709	480	807	277	-416	0.6		0.7		0.06		LPS - 40
	PBMC															
N	49	37	38	22	32	36	36	9	-755	0.0		0.0		0.00	*	N
3H	785	407	762	808	945	1035	790	215	0	1.0		1.0		1.00		3H
PHA - 1	2971	4510	5980	7411	6502	4808	5364	1589	4,573	7.1	*	6.8	*	0.00	*	PHA - 1
PHA - 5	2244	2922	3261	2982	4748	3377	3256	831	2,465	4.3	*	4.1	*	0.00	*	PHA - 5
PHA - 10	1348	2902	3188	3276	3877	1977	2761	930	1,971	3.6	*	3.5	*	0.00	*	PHA - 10
LPS - 1	231	280	173	223	190	220	220	37	-571	0.2		0.3		0.00	*	LPS - 1
LPS - 5	184	193	137	145	197	181	173	25	-618	0.2		0.2		0.00	*	LPS - 5
LPS - 10	158	119	105	112	104	152	125	24	-665	0.1		0.2		0.00	*	LPS - 10
LPS - 20	164	155	86	155	108	128	133	31	-658	0.1		0.2		0.00	*	LPS - 20
LPS - 40	145	141	93	74	82	70	101	34	-690	0.1		0.1		0.00	*	LPS - 40

Raw data for Thymectomy duck W153

W153	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	49	25															
Total 3H	720	747								>5000	>2.1		>2.1		<0.05		
	R1	R2	R3	R4	R5	R6	Mean	SD									
1-15 [1]	517	851	529	395	637	129	510	241	-211		0.7		0.7		0.51		1-15 [1]
1-15 [10]	484	437	1378	2227	1276	53	976	800	256		1.4		1.4		0.47		1-15 [10]
1-15 [20]	632	192	867	525	1474	2364	1009	789	289		1.4		1.4		0.41		1-15 [20]
7-14W-27 [1]	1289	1245	1097	668	631	138	845	447	124		1.2		1.2		0.70		7-14W-27 [1]
7-14W-27 [10]	9273	2179	743	4147	2482	2429	3542	3009	2,822		5.2	*	4.9	*	0.00	*	7-14W-27 [10]
7-14W-27 [20]	113	2325	3391	4074	2065	379	2058	1582	1,338		3.0	*	2.9	*	0.00	*	7-14W-27 [20]
7-14R-27 [1]	66	2357	1833		633	68	991	1050	271		1.4		1.4		0.50		7-14R-27 [1]
7-14R-27 [10]	2365	464	520	69	1425	1492	1056	855	336		1.5		1.5		0.35		7-14R-27 [10]
7-14R-27 [20]	1539	607	1200	1079	267	5073	1628	1747	907		2.4	*	2.3	*	0.06		7-14R-27 [20]
22-41 [1]	175	567	638	352	959	1634	721	521	1		1.0		1.0		1.00		22-41 [1]
22-41 [10]	3422	1704	1037	1494	1450	788	1649	930	929		2.4	*	2.3	*	0.01	*	22-41 [10]
22-41 [20]	864	1241	1357	1415	2223	2060	1527	516	806		2.2	*	2.1	*	0.02	*	22-41 [20]
37-56 [1]	74	6074	156	495	5168	134	2017	2810	1,297		2.9	*	2.8	*	0.05	*	37-56 [1]
37-56 [10]	202	2000	388	615	1109	1899	1036	771	315		1.5		1.4		0.37		37-56 [10]
37-56 [20]	4942	477	550	706	415	295	1231	1823	511		1.8		1.7		0.28		37-56 [20]
54-73 [1]	1378	514	769	1733	399	626	903	532	183		1.3		1.3		0.58		54-73 [1]
54-73 [10]	825	551	773	836	564	91	607	282	-114		0.8		0.8		0.72		54-73 [10]
54-73 [20]	2177	557	187	372	724	716	789	711	69		1.1		1.1		0.84		54-73 [20]
71-90 [1]	1980	266	263	681	210	214	602	698	-118		0.8		0.8		0.73		71-90 [1]
71-90 [10]	106	66	1912	1082	109	86	560	772	-160		0.8		0.8		0.64		71-90 [10]
71-90 [20]	69	18150	909	12923	140	105	5383	8043	4,662		7.9	*	7.5	*	0.01	*	71-90 [20]
87-106 [1]	67	1075	907	84	896	265	549	459	-171		0.7		0.8		0.60		87-106 [1]
87-106 [10]	951	375	191	241	155	392	384	294	-336		0.5		0.5		0.29		87-106 [10]
87-106 [20]	895	1284	87	234	118	774	565	491	-155		0.8		0.8		0.64		87-106 [20]
101-120 [1]	427	547	991	1753	286	862	811	532	91		1.1		1.1		0.78		101-120 [1]
101-120 [10]	7099	860	845	599	209	84	1616	2705	896		2.3	*	2.2	*	0.15		101-120 [10]
101-120 [20]	78	509	1002	1593	335	63	597	598	-124		0.8		0.8		0.71		101-120 [20]
116-130 [1]	85	113	119	92	95	46	92	26	-629		0.1		0.1		0.05		116-130 [1]
116-130 [10]	165	283	529	1024	167	76	374	355	-346		0.5		0.5		0.28		116-130 [10]
116-130 [20]	8709	1231	719	990	1017	65	2122	3252	1,402		3.1	*	2.9	*	0.05		116-130 [20]
126-140 [1]	1858	285	243	599	369	1583	823	711	103		1.2		1.1		0.76		126-140 [1]
126-140 [10]	9787	1083	161	589	599	1837	2343	3692	1,622		3.4	*	3.3	*	0.05	*	126-140 [10]
126-140 [20]	11730	838	608	726	730	702	2556	4495	1,835		3.7	*	3.5	*	0.06		126-140 [20]
136-150 [1]	41	330	513	520	71	46	254	231	-467		0.3		0.4		0.15		136-150 [1]
136-150 [10]	59	92	83	71	73	67	74	12	-646		0.0		0.1		0.05	*	136-150 [10]
136-150 [20]	70	261	854	323	711	12161	2397	4792	1,676		3.5	*	3.3	*	0.10		136-150 [20]
146-160 [1]	1138	2550	690	316	1286	906	1148	768	427		1.6		1.6		0.22		146-160 [1]
146-160 [10]	1099	1926	478	469	354	403	788	621	68		1.1		1.1		0.84		146-160 [10]
146-160 [20]	767	305	264	935	377	1107	626	359	-95		0.9		0.9		0.77		146-160 [20]
156-170 [1]	1173	1785	660	549	408	102	780	605	59		1.1		1.1		0.86		156-170 [1]
156-170 [10]	733	935	528	383	1953	817	892	557	171		1.3		1.2		0.60		156-170 [10]
156-170 [20]	225	651	188	1267	969	61	560	485	-160		0.8		0.8		0.62		156-170 [20]
166-180 [1]	57	98	820	1328	267	70	440	522	-280		0.6		0.6		0.40		166-180 [1]
166-180 [10]	67	247	166	1394	2138	10708	2453	4127	1,733		3.6	*	3.4	*	0.05		166-180 [10]
166-180 [20]	22021	1130	393	180	93	227	4007	8833	3,287		5.9	*	5.6	*	0.07		166-180 [20]
176-195 [1]	129	593	675	591	1107	130	538	369	-183		0.7		0.7		0.57		176-195 [1]
176-195 [10]	104	261	731	198	778	70	357	316	-363		0.5		0.5		0.26		176-195 [10]
176-195 [20]	300	407	1069	382	877	208	541	348	-180		0.7		0.8		0.57		176-195 [20]
191-210 [1]	205	319	791	594	2359	110	730	837	9		1.0		1.0		0.98		191-210 [1]
191-210 [10]	71	109	78	83	93	66	83	16	-637		0.1		0.1		0.05	*	191-210 [10]
191-210 [20]	51	79	2133	2726	110	111	868	1224	148		1.2		1.2		0.71		191-210 [20]
210-229 [1]	416	822	871	2632	1997	997	1289	842	569		1.8		1.8		0.11		210-229 [1]
210-229 [10]	1705	1407	1614	2635	2083		1889	484	1,168		2.7	*	2.6	*	0.00	*	210-229 [10]
210-229 [20]	6593	2147	17872	3927	5631	21653	9637	8077	8,917	*	14.3	*	13.4	*	0.00	*	210-229 [20]
229-248 [1]	767	672	864	650	286	1684	821	466	100		1.1		1.1		0.76		229-248 [1]
229-248 [10]	1040	714	396	345	372	103	495	330	-225		0.7		0.7		0.48		229-248 [10]
229-248 [20]	53	313	109	488	782	65	302	290	-419		0.4		0.4		0.19		229-248 [20]
248-267 [1]	53	8032	580	613	428	71	1630	3146	909		2.4	*	2.3	*	0.19		248-267 [1]
248-267 [10]	98	432	123	148	906	7958	1611	3125	891		2.3	*	2.2	*	0.20		248-267 [10]
248-267 [20]	417	200	692	272	353	181	353	189	-368		0.5		0.5		0.25		248-267 [20]
267-286 [1]	752	528	252	106	95	1139	479	412	-242		0.6		0.7		0.45		267-286 [1]
267-286 [10]	1867	413	968	1143	527	255	862	597	142		1.2		1.2		0.67		267-286 [10]
267-286 [20]	251	103	165	214	154	1423	385	511	-335		0.5		0.5		0.31		267-286 [20]
287-306 [1]	1007	1602	320	71	1599	87	781	720	61		1.1		1.1		0.86		287-306 [1]
287-306 [10]	713	257	711	222	1098	343	557	343	-163		0.8		0.8		0.61		287-306 [10]
287-306 [20]	948	1603	589	590	254	196	697	520	-24		1.0		1.0		0.94		287-306 [20]
307-326 [1]	420	1272	284	1784	2507	342	1102	914	381		1.6		1.5		0.29		307-326 [1]
307-326 [10]	199	461	87	136	199	652	289	220	-431		0.4		0.4		0.18		307-326 [10]
307-326 [20]	148	147	120	79	97	270	144	68	-577		0.1		0.2		0.07		307-326 [20]
sAg 10	757	983	478	484	1152	6402	1709	2314	989		2.5	*	2.4	*	0.08		sAg 10
sAg 100	52	88	354	8604	122	76	1549	3458	829		2.2	*	2.2	*	0.27		sAg 100
N		44	111	72	43	29	60	33	-661		0.0		0.1		0.06		N
N	53	39	38	19	56	40	41	13	-680		0.0		0.1		0.04	*	N
3H	1026	222	970	659	1145	627	775	340	55		1.1		1.1		0.86		3H
3H	75	2031	309	335	103	45	483	768	-237		0.6		0.7		0.49		3H
3H	2626	1454	730	923	107	2007	1308	913	588		1.9		1.8		0.11		3H
3H	67	1504	180	67	46	30	316	585	-405		0.4		0.4		0.23		3H
	SMC																
N	44	34	40	43	108	49	53	27	-606		0.0		0.1		0.00	*	N
3H	413	659	896	485	964	538	659	226	0		1.0		1.0		1.00		3H
PHA - 1	20579	15348	11765	18623	13030	38396	19624	9776	18,964	*	32.3	*	29.8	*	0.00	*	PHA - 1
PHA - 5	26486	36221	32120	16834	40528	37176	31561	8679	30,902	*	52.0	*	47.9	*	0.00	*	PHA - 5
PHA - 10	19455	34182	26483	24866	28107	28418	26919	4828	26,259	*	44.3	*	40.8	*	0.00	*	PHA - 10
LPS - 1	388	861	470	582	521	328	525	188	-134		0.8		0.8		0.29		LPS - 1
LPS - 5	463	531	461	572	349	326	450	97	-209		0.7		0.7		0.06		LPS - 5
LPS - 10	308	448	405	450	336	186	356	101	-304		0.5		0.5		0.01	*	LPS - 10
LPS - 20	223	557	464	432	375	109	360	165	-299		0.5		0.5		0.03	*	LPS - 20
LPS - 40	72	64	138	130	123	106	106	31	-554		0.1		0.2		0.00	*	LPS - 40
	PBMC																
N	52	51	36	21	18	26	34	15	-54		0.0		0.4		0.00	*	N
3H	112	119	81	74	54	88	88	24	0		1.0		1.0		1.00		3H
PHA - 1	1910	5550	3383	4690	6966	10446	5491	2988	5,403	*	101.1	*	62.4	*	0.00	*	PHA - 1
PHA - 5	2438	8631	4061	13866	13194	21652	10640	7110	10,552	*	196.4	*	120.9	*	0.00	*	PHA - 5
PHA - 10	2168	2139	3235	5658	6476	7539	4536	2327	4,448		83.4	*	51.5	*	0.00	*	PHA - 10
LPS - 1	103	553	292	325	212	115	267	167	179		4.3	*	3.0	*	0.03	*	LPS - 1
LPS - 5	68	186	268	300	183	103	185	90	97		2.8	*	2.1		0.03	*	LPS - 5
LPS - 10	76	164	238	249	160	83	162	73	74		2.4	*	1.8		0.04	*	LPS - 10
LPS - 20	61	110	164	173	371	55	156	117	68		2.3	*	1.8		0.19		LPS - 20
LPS - 40	59	48	62	64	69	25	55	16	-34		0.4		0.6		0.02	*	LPS - 40

Raw data for Thymectomy duck W156

W156	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	76	55															
Total 3H	267	406								>5000		>2.1		>2.1		<0.05	
	R1	R2	R3	R4	R5	R6	Mean	SD									
1-15 [1]	109	239	205	280	193	182	201	58	-66		0.7		0.8		0.70		1-15 [1]
1-15 [10]	314	203	243	334	322	87	251	95	-17		0.9		0.9		0.92		1-15 [10]
1-15 [20]	209	278	687	482	207	67	322	224	54		1.3		1.2		0.76		1-15 [20]
7-14W-27 [1]	464	206	421	460	212	55	303	170	36		1.2		1.1		0.84		7-14W-27 [1]
7-14W-27 [10]	47	258	995	294	324	32	325	351	58		1.3		1.2		0.75		7-14W-27 [10]
7-14W-27 [20]	61	361	343	481	393	65	284	178	17		1.1		1.1		0.92		7-14W-27 [20]
7-14R-27 [1]	50	67	112	112	126	115	97	31	-170		0.1		0.4		0.32		7-14R-27 [1]
7-14R-27 [10]	37	519	650	252	864	68	398	333	131		1.7		1.5		0.47		7-14R-27 [10]
7-14R-27 [20]	77	279	442	519	1559	81	493	553	226		2.2	*	1.8		0.27		7-14R-27 [20]
22-41 [1]	471	759	386	276	338	112	390	217	123		1.6		1.5		0.48		22-41 [1]
22-41 [10]	404	265	345	725	1469	156	561	485	293		2.5	*	2.1		0.14		22-41 [10]
22-41 [20]	78	756	694	1113	3825	200	1111	1383	844		5.4	*	4.2	*	0.01	*	22-41 [20]
37-56 [1]	154	81	85	113	94	112	107	27	-161		0.2		0.4		0.35		37-56 [1]
37-56 [10]	64	258	428	461	723	82	336	252	69		1.4		1.3		0.70		37-56 [10]
37-56 [20]	485	615	519	443	471	281	469	110	202		2.1		1.8		0.24		37-56 [20]
54-73 [1]	337	192	214	195	323	1117	396	359	129		1.7		1.5		0.48		54-73 [1]
54-73 [10]	364	246	310	403	480	411	369	82	102		1.5		1.4		0.55		54-73 [10]
54-73 [20]	213	543	1377	135	380	1058	618	496	350		2.8	*	2.3	*	0.08		54-73 [20]
71-90 [1]	130	239	234	177	422	499	284	145	16		1.1		1.1		0.92		71-90 [1]
71-90 [10]	104	76	76	142	116	116	105	26	-162		0.2		0.4		0.34		71-90 [10]
71-90 [20]	121	59	184	70	116	114	111	44	-157		0.2		0.4		0.36		71-90 [20]
87-106 [1]	100	318	304	249	393	99	244	121	-23		0.9		0.9		0.89		87-106 [1]
87-106 [10]	440	594	501	281	429	69	386	186	118		1.6		1.4		0.50		87-106 [10]
87-106 [20]	255	430	361	1119	503	369	506	311	239		2.2	*	1.9		0.19		87-106 [20]
101-120 [1]	203	273	414	1003	423	133	408	313	141		1.7		1.5		0.44		101-120 [1]
101-120 [10]	117	534	863	595	599	66	462	309	195		2.0		1.7		0.28		101-120 [10]
101-120 [20]	53	915	1125	459	844	95	582	449	315		2.6	*	2.2	*	0.11		101-120 [20]
116-130 [1]	70	108	131	99	88	96	99	20	-169		0.1		0.4		0.32		116-130 [1]
116-130 [10]	79	1011	740	4577	2009	69	1414	1707	1,147		7.0	*	5.3	*	0.00	*	116-130 [10]
116-130 [20]	2535	753	975	956	828	111	1026	804	759		5.0	*	3.8	*	0.00	*	116-130 [20]
126-140 [1]	379	220	304	476	494	486	393	113	126		1.7		1.5		0.46		126-140 [1]
126-140 [10]	1594	378	587	1463	612	1406	1007	537	739		4.9	*	3.8	*	0.00	*	126-140 [10]
126-140 [20]	1041	596	294	249	196	98	412	351	145		1.8		1.5		0.43		126-140 [20]
136-150 [1]	99	279	320	199	427	93	236	131	-31		0.8		0.9		0.86		136-150 [1]
136-150 [10]	79	118	147	100	100	87	105	24	-162		0.2		0.4		0.34		136-150 [10]
136-150 [20]	78	106	133	796	124	173	235	277	-32		0.8		0.9		0.86		136-150 [20]
146-160 [1]	93	151	158	208	320	72	167	89	-100		0.5		0.6		0.56		146-160 [1]
146-160 [10]	483	276	228	379	580	750	449	196	182		2.0		1.7		0.30		146-160 [10]
146-160 [20]	238	441	772	482	343	432	451	180	184		2.0		1.7		0.29		146-160 [20]
156-170 [1]	290	280	164	216	418	272	273	85	6		1.0		1.0		0.97		156-170 [1]
156-170 [10]	894	377	164	262	561	690	491	275	224		2.2	*	1.8		0.21		156-170 [10]
156-170 [20]	61	303	976	443	675	79	423	356	156		1.8		1.6		0.40		156-170 [20]
166-180 [1]	180	80	84	107	83	83	103	39	-164		0.1		0.4		0.34		166-180 [1]
166-180 [10]	64	1066	802	251	329	259	462	385	195		2.0		1.7		0.30		166-180 [10]
166-180 [20]	120	550	418	778	2249	856	829	744	561		3.9	*	3.1	*	0.02	*	166-180 [20]
176-195 [1]	197	218	222	178	350	315	247	69	-21		0.9		0.9		0.90		176-195 [1]
176-195 [10]	204	269	274	218	677	358	333	177	66		1.3		1.2		0.70		176-195 [10]
176-195 [20]	99	241	242	260	910	415	361	287	94		1.5		1.4		0.60		176-195 [20]
191-210 [1]	79	193	235	161	169	48	148	71	-120		0.4		0.6		0.48		191-210 [1]
191-210 [10]	139	107	82	74	63	61	88	30	-180		0.1		0.3		0.30		191-210 [10]
191-210 [20]	110	97	115	137	168	162	132	29	-136		0.3		0.5		0.43		191-210 [20]
210-229 [1]	154	473	202	525	249	77	280	180	13		1.1		1.0		0.94		210-229 [1]
210-229 [10]	543	668	455	1804	2373	104	991	889	724		4.8	*	3.7	*	0.01	*	210-229 [10]
210-229 [20]	5088	806	1206	531	2111	223	1661	1802	1,394		8.3	*	6.2	*	0.00	*	210-229 [20]
229-248 [1]	421	737	291	169	179	189	331	221	64		1.3		1.2		0.72		229-248 [1]
229-248 [10]	93	485	240	769	758	81	404	314	137		1.7		1.5		0.45		229-248 [10]
229-248 [20]	85	509	204	158	530	78	261	206	-7		1.0		1.0		0.97		229-248 [20]
248-267 [1]	94	123	114	314	196	138	163	82	-104		0.5		0.6		0.54		248-267 [1]
248-267 [10]	67	585	517	1282	161	63	446	468	179		1.9		1.7		0.36		248-267 [10]
248-267 [20]	290	503	881	245	793	84	466	318	199		2.0		1.7		0.28		248-267 [20]
267-286 [1]	427	222	365	321	81	885	384	274	116		1.6		1.4		0.51		267-286 [1]
267-286 [10]	435	334	370	420	436	374	395	42	128		1.7		1.5		0.45		267-286 [10]
267-286 [20]	609	543	465	552	504	545	536	49	269		2.4	*	2.0		0.12		267-286 [20]
287-306 [1]	97	88	93	116	105	79	96	13	-171		0.1		0.4		0.32		287-306 [1]
287-306 [10]	188	273	1153	330	533	98	429	384	162		1.8		1.6		0.39		287-306 [10]
287-306 [20]	680	513	194	362	458	89	383	216	115		1.6		1.4		0.51		287-306 [20]
307-326 [1]	146	287	226	184	317	106	211	82	-56		0.7		0.8		0.74		307-326 [1]
307-326 [10]	347	269	431	686	443	75	375	203	108		1.6		1.4		0.54		307-326 [10]
307-326 [20]	437	325	345	442	911	278	456	232	189		2.0		1.7		0.29		307-326 [20]
sAg 10	66	410	593	361	491	81	334	216	66		1.3		1.2		0.70		sAg 10
sAg 100	79	61	59	87	74	136	83	28	-185		0.0		0.3		0.28		sAg 100
N	70	51	86	238	90	54	98	70	-169		0.1		0.4		0.32		N
N	36	80	26	45	82	48	53	23	-214		-0.1		0.2		0.21		N
3H	75	233	162	202	243	59	162	79	-105		0.5		0.6		0.54		3H
3H	92	133	115	79	96	96	102	19	-165		0.1		0.4		0.33		3H
3H	49	322	153	302	1664	47	423	620	156		1.8		1.6		0.46		3H
3H	77	74	1344	665	74	57	382	528	115		1.6		1.4		0.56		3H
	SMC																
N	64	78	94	60	68	80	74	13	-87		0.0		0.5		0.08		N
3H	67	133	211	162	344	49	161	108	0		1.0		1.0		1.00		3H
PHA - 1	1223	6804	3055	5929	6895	1598	4251	2606	4,090		48.0	*	26.4	*	0.00	*	PHA - 1
PHA - 5	4722	4781	34879	31610	33271	10029	19882	14810	19,721	*	227.7	*	123.5	*	0.01	*	PHA - 5
PHA - 10	4025	9222	17734	14756	25522	13063	14054	7355	13,893	*	160.7	*	87.3	*	0.00	*	PHA - 10
LPS - 1	247	244	359	366	223	166	274	89	113		2.3	*	1.7		0.08		LPS - 1
LPS - 5	203	227	275	210	247	112	212	56	51		1.6		1.3		0.32		LPS - 5
LPS - 10	118	270	299	301	259	96	224	92	63		1.7		1.4		0.30		LPS - 10
LPS - 20	79	240	206	205	261	61	175	84	14		1.2		1.1		0.80		LPS - 20
LPS - 40	96	98	127	83	89	114	101	16	-60		0.3		0.6		0.21		LPS - 40
	PBMC																
N	24	40	66	47	50	60	48	15	-82		0.0		0.4		0.00	*	N
3H	66	100	177	120	164	150	130	42	0		1.0		1.0		1.00		3H
PHA - 1	949	2575	1928	1912	2706	1827	1983	628	1,853		23.7	*	15.3	*	0.00	*	PHA - 1
PHA - 5	1590	3581	3324	4875	3867	3777	3502	1076	3,373		42.3	*	27.0	*	0.00	*	PHA - 5
PHA - 10	962	2441	2355	2056	1938	2487	2040	571	1,910		24.4	*	15.8	*	0.00	*	PHA - 10
LPS - 1	66	338	343	407	323	90	261	145	132		2.6	*	2.0		0.06		LPS - 1
LPS - 5	50	262	278	256	251	78	196	103	66		1.8		1.5		0.17		LPS - 5
LPS - 10	46	171	221	374	228	75	186	119	56		1.7		1.4		0.30		LPS - 10
LPS - 20	47	56	88	129	132	83	89	36	-40		0.5		0.7		0.10		LPS - 20
LPS - 40	132	43	62	77	94	118	88	34	-42		0.5		0.7		0.09		LPS - 40

Raw data for Thymectomy duck W157

W157	Mean	SD														
Total N	58	28														
Total 3H	609	1015							CPM-3H		S.I.		P/N		t-Test	
	R1	R2	R3	R4	R5	R6	Mean	SD	>5000		>2.1		>2.1		<0.05	
1-15 [1]	234	240	105	102	118	1025	304	359	-305		0.4		0.5		0.48	1-15 [1]
1-15 [10]	689	838	138	347	84	1118	536	413	-73		0.9		0.9		0.87	1-15 [10]
1-15 [20]	3236	390	297	304	212	8121	2093	3178	1,484		3.7	*	3.4	*	0.06	1-15 [20]
7-14W-27 [1]	267	113	99	219	197	1587	414	578	-195		0.6		0.7		0.66	7-14W-27 [1]
7-14W-27 [10]	1187	293	302	1218	409	231	607	465	-2		1.0		1.0		1.00	7-14W-27 [10]
7-14W-27 [20]	72	3961	2183	605	765	59	1274	1528	665		2.2	*	2.1		0.21	7-14W-27 [20]
7-14R-27 [1]	63	569	743	2391	109	131	668	889	59		1.1		1.1		0.90	7-14R-27 [1]
7-14R-27 [10]	451	79	145	96	194	83	175	142	-434		0.2		0.3		0.31	7-14R-27 [10]
7-14R-27 [20]	349	347	110	103	95	5895	1150	2328	541		2.0		1.9		0.39	7-14R-27 [20]
22-41 [1]	127	85	97	482	313	3806	818	1472	209		1.4		1.3		0.68	22-41 [1]
22-41 [10]	127	220	144	73	103	2012	447	769	-162		0.7		0.7		0.72	22-41 [10]
22-41 [20]	614	98	199	202	231	4265	935	1641	326		1.6		1.5		0.54	22-41 [20]
37-56 [1]	109	3534	685	563	1329	107	1055	1296	446		1.8		1.7		0.37	37-56 [1]
37-56 [10]	1669	93	77	96	96	776	468	649	-141		0.7		0.8		0.75	37-56 [10]
37-56 [20]	338	60	113	95	86	745	240	268	-369		0.3		0.4		0.39	37-56 [20]
54-73 [1]	1204	506	74	223	147	241	399	421	-210		0.6		0.7		0.63	54-73 [1]
54-73 [10]	1534	111	72	117	76	103	336	587	-273		0.5		0.6		0.53	54-73 [10]
54-73 [20]	1436	79	138	171	93	245	360	530	-249		0.5		0.6		0.57	54-73 [20]
71-90 [1]	431	678	505	174	67	198	342	233	-267		0.5		0.6		0.53	71-90 [1]
71-90 [10]	95	89	707	266	933	141	372	360	-237		0.6		0.6		0.58	71-90 [10]
71-90 [20]	94	274	2552	2659	1086	88	1126	1204	517		1.9		1.8		0.29	71-90 [20]
87-106 [1]	523	174	182	222	154	5864	1187	2296	578		2.0		1.9		0.35	87-106 [1]
87-106 [10]	466	506	86	106	73	2671	651	1009	42		1.1		1.1		0.93	87-106 [10]
87-106 [20]	542	155	92	99	107	143	190	174	-419		0.2		0.3		0.33	87-106 [20]
101-120 [1]	2202	648	94	414	163	4143	1277	1603	668		2.2	*	2.1		0.21	101-120 [1]
101-120 [10]	758	397	96	122	279	1851	584	666	-25		1.0		1.0		0.95	101-120 [10]
101-120 [20]	148	282	273	218	283	484	281	112	-328		0.4		0.5		0.44	101-120 [20]
116-130 [1]	79	734	232	1020	570	100	456	381	-153		0.7		0.7		0.72	116-130 [1]
116-130 [10]	2887	393	261	398	763	529	872	1002	263		1.5		1.4		0.57	116-130 [10]
116-130 [20]	426	412	207	192	370	1158	461	356	-148		0.7		0.8		0.73	116-130 [20]
126-140 [1]	263	390	481	429	704	373	440	148	-169		0.7		0.7		0.69	126-140 [1]
126-140 [10]	289	103	101	352	137	679	277	223	-332		0.4		0.5		0.44	126-140 [10]
126-140 [20]	2116	319	172	142	118	3785	1109	1523	500		1.9		1.8		0.34	126-140 [20]
136-150 [1]	75	99	199	131	1997	69	428	770	-181		0.7		0.7		0.69	136-150 [1]
136-150 [10]	74	91	90	132	106	67	93	23	-516		0.1		0.2		0.23	136-150 [10]
136-150 [20]	76	130	1887	4080	2223	95	1415	1623	806		2.5	*	2.3	*	0.14	136-150 [20]
146-160 [1]	2307	208	151	109	179	247	534	870	-75		0.9		0.9		0.87	146-160 [1]
146-160 [10]	475	176	80	134	498	321	281	179	-328		0.4		0.5		0.44	146-160 [10]
146-160 [20]	654	97	485	106	565	386	382	235	-227		0.6		0.6		0.60	146-160 [20]
156-170 [1]	391	262	103	101	214	242	219	109	-390		0.3		0.4		0.36	156-170 [1]
156-170 [10]	347	142	85	168	117	1581	407	583	-202		0.6		0.7		0.65	156-170 [10]
156-170 [20]	65	102	264	166	132	788	253	271	-356		0.4		0.4		0.41	156-170 [20]
166-180 [1]	82	174	156	331	960	97	300	335	-309		0.4		0.5		0.47	166-180 [1]
166-180 [10]	2801	153	174	279	194	1902	917	1148	308		1.6		1.5		0.52	166-180 [10]
166-180 [20]	1214	256	357	115	458	709	518	395	-91		0.8		0.9		0.83	166-180 [20]
176-195 [1]	4687	93	81	359	407	2390	1336	1859	727		2.3	*	2.2	*	0.20	176-195 [1]
176-195 [10]	770	67	102	83	226	313	260	267	-349		0.4		0.4		0.42	176-195 [10]
176-195 [20]	719	72	73	540	282	223	318	261	-291		0.5		0.5		0.50	176-195 [20]
191-210 [1]	76	304	83	101	229	67	143	99	-466		0.2		0.2		0.28	191-210 [1]
191-210 [10]	79	86	134	317	116	83	136	91	-473		0.1		0.2		0.27	191-210 [10]
191-210 [20]	104	2500	4358	477	355	79	1312	1748	703		2.3	*	2.2	*	0.20	191-210 [20]
210-229 [1]	609	544	273	113	418	5143	1183	1948	574		2.0		1.9		0.32	210-229 [1]
210-229 [10]	415	235	493	83	362	3797	898	1428	289		1.5		1.5		0.57	210-229 [10]
210-229 [20]	2869	332	156	370	1663	3078	1411	1326	802		2.5	*	2.3	*	0.11	210-229 [20]
229-248 [1]	3243	173	310	228	559	1526	1007	1205	398		1.7		1.7		0.41	229-248 [1]
229-248 [10]	2161	350	122	135	226	4035	1172	1608	563		2.0		1.9		0.29	229-248 [10]
229-248 [20]	3070	218	388	336	497	720	872	1090	263		1.5		1.4		0.58	229-248 [20]
248-267 [1]	67	230	2984	11087	7907	95	3728	4712	3,119		6.7	*	6.1	*	0.00	* 248-267 [1]
248-267 [10]	149	317	805	264	470	227	372	238	-237		0.6		0.6		0.58	248-267 [10]
248-267 [20]	1873	183	99	460	160	13809	2764	5452	2,155		4.9	*	4.5	*	0.07	248-267 [20]
267-286 [1]	326	130	90	105	74	3169	649	1238	40		1.1		1.1		0.93	267-286 [1]
267-286 [10]	637	69	80	211	742	2396	689	884	80		1.1		1.1		0.86	267-286 [10]
267-286 [20]	1324	2961	289	374	95	5196	1707	2014	1,098		3.0	*	2.8	*	0.07	267-286 [20]
287-306 [1]	67	77	1166	874	75	95	392	495	-217		0.6		0.6		0.62	287-306 [1]
287-306 [10]	1587	289	210	357	155	82	447	567	-162		0.7		0.7		0.71	287-306 [10]
287-306 [20]	868	93	76	176	414	645	379	324	-230		0.6		0.6		0.59	287-306 [20]
307-326 [1]	317	114	143	111	385	3127	700	1195	91		1.2		1.1		0.85	307-326 [1]
307-326 [10]	333	276	107	100	27	107	158	119	-451		0.2		0.3		0.29	307-326 [10]
307-326 [20]	561	118	141	131	643	584	363	257	-246		0.6		0.6		0.57	307-326 [20]
sAg 10	70	192	69	66	107	72	96	49	-513		0.1		0.2		0.23	sAg 10
sAg 100	52	72	70	81	60	57	65	11	-544		0.0		0.1		0.21	sAg 100
N	57	78	80	134	48	51	75	32	-534		0.0		0.1		0.21	N
N	33	34	48	49	44	37	41	7	-568		0.0		0.1		0.19	N
3H	2129	194	281	81	739	289	619	773	10		1.0		1.0		0.98	3H
3H	69	205	341	264	72	115	178	111	-431		0.2		0.3		0.31	3H
3H	241	89	69	271	821	278	295	273	-314		0.4		0.5		0.46	3H
3H	118	128	607	3306	3854	54	1345	1751	736		2.3	*	2.2	*	0.18	3H
	SMC															
N	87	71	44	45	52	50	58	17	-461		0.0		0.1		0.00	* N
3H	480	950	674	385	276	352	520	251	0		1.0		1.0		1.00	3H
PHA - 1	29795	20104	29278	35903	40502	35344	31821	7103	31,302	*	68.9	*	61.3	*	0.00	* PHA - 1
PHA - 5	64641	75801	48083	53280	48436	48113	56392	11448	55,873	*	122.1	*	108.6	*	0.00	* PHA - 5
PHA - 10	66171	80664	59620	59688	49048	51172	61061	11458	60,541	*	132.2	*	117.5	*	0.00	* PHA - 10
LPS - 1	539	839	986	994	1258	501	853	291	333		1.7		1.6		0.06	LPS - 1
LPS - 5	555	1495	1310	1229	1499	321	1068	505	549		2.2	*	2.1		0.04	* LPS - 5
LPS - 10	283	835	1155	1482	1384	200	890	550	370		1.8		1.7		0.16	LPS - 10
LPS - 20	142	1419	1206	1333	1160	95	893	607	373		1.8		1.7		0.19	LPS - 20
LPS - 40	145	254	247	221	119	70	176	76	-344		0.3		0.3		0.01	* LPS - 40
	PBMC															
N	26	77	48	39	55	80	54	21	-13		0.0		0.8		0.33	N
3H	68	107	72	54	46	54	67	22	0		1.0		1.0		1.00	3H
PHA - 1	481	280	184	128	176	147	233	133	166		14.1	*	3.5	*	0.01	* PHA - 1
PHA - 5	539	764	445	387	492	416	507	137	440		35.8	*	7.6	*	0.00	* PHA - 5
PHA - 10	722	521	554	681	387	597	577	120	510		41.3	*	8.6	*	0.00	* PHA - 10
LPS - 1	184	381	222	327	449	233	299	103	233		19.4	*	4.5	*	0.00	* LPS - 1
LPS - 5	154	149	151	215	397	238	217	96	151		12.9	*	3.3	*	0.00	* LPS - 5
LPS - 10	100	72	120	94	123	110	103	19	36		3.9	*	1.5		0.01	* LPS - 10
LPS - 20	96	51	78	90	102	143	93	30	27		3.1	*	1.4		0.11	LPS - 20
LPS - 40	155	86	119	160	107	193	137	40	70		6.5	*	2.0		0.00	* LPS - 40

Raw data for Thymectomy duck W160

W160	Mean	SD														
Total N	65	40														
Total 3H	3163	5766							CPM-3H		S.I.		P/N		t-Test	
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1		<0.05
1-15 [1]	811	239	177	131	203	3400	827	1286	-2,336		0.2		0.3		0.34		1-15 [1]
1-15 [10]	232	451	153	432	302	475	341	132	-2,822		0.1		0.1		0.25		1-15 [10]
1-15 [20]	262	353	144	404	445	298	318	108	-2,845		0.1		0.1		0.24		1-15 [20]
7-14W-27 [1]	380	553	200	165	695	180	362	222	-2,800		0.1		0.1		0.25		7-14W-27 [1]
7-14W-27 [10]	1278	575	127	522	575	7792	1812	2953	-1,351		0.6		0.6		0.59		7-14W-27 [10]
7-14W-27 [20]	100	309	293	540	905	7007	1526	2699	-1,637		0.5		0.5		0.51		7-14W-27 [20]
7-14R-27 [1]	160	30896	5851	10983	6090	112	9015	11479	5,853	*	2.9	*	2.9	*	0.08		7-14R-27 [1]
7-14R-27 [10]	438	548	312	698	570	121	448	206	-2,715		0.1		0.1		0.26		7-14R-27 [10]
7-14R-27 [20]	1141	231	499	112	541	[redacted]	505	399	-2,658		0.1		0.2		0.32		7-14R-27 [20]
22-41 [1]	282	246	375	159	217	1115	399	358	-2,764		0.1		0.1		0.26		22-41 [1]
22-41 [10]	150	332	225	132	509	602	325	194	-2,838		0.1		0.1		0.24		22-41 [10]
22-41 [20]	4972	489	450	376	692	1439	1403	1791	-1,760		0.4		0.4		0.47		22-41 [20]
37-56 [1]	90	993	381	557	4447	116	1097	1674	-2,065		0.3		0.3		0.40		37-56 [1]
37-56 [10]	1796	305	353	124	1011	8023	1935	3046	-1,227		0.6		0.6		0.62		37-56 [10]
37-56 [20]	259	163	194	59	326	117	186	96	-2,976		0.0		0.1		0.22		37-56 [20]
54-73 [1]	127	434	112	256	169	124	204	125	-2,959		0.0		0.1		0.23		54-73 [1]
54-73 [10]	263	284	119	133	251	157	201	73	-2,961		0.0		0.1		0.22		54-73 [10]
54-73 [20]	182	150	305	151	350	231	228	84	-2,934		0.1		0.1		0.23		54-73 [20]
71-90 [1]	20339	175	268	126	426	142	3579	8211	417		1.1		1.1		0.89		71-90 [1]
71-90 [10]	102	7709	7660	41336	12883	131	11637	15364	8,474	*	3.7	*	3.7	*	0.03	*	71-90 [10]
71-90 [20]	63	7995	16867	2666	293	142	4671	6704	1,508		1.5		1.5		0.58		71-90 [20]
87-106 [1]	134	282	138	177	155	2026	485	757	-2,677		0.1		0.2		0.27		87-106 [1]
87-106 [10]	62	303	191	118	243	278	199	94	-2,963		0.0		0.1		0.22		87-106 [10]
87-106 [20]	181	346	240	243	123	156	215	80	-2,948		0.0		0.1		0.23		87-106 [20]
101-120 [1]	73	197	239	128	169	445	209	129	-2,954		0.0		0.1		0.23		101-120 [1]
101-120 [10]	2733	269	180	246	313	7208	1825	2818	-1,338		0.6		0.6		0.59		101-120 [10]
101-120 [20]	4034	75	330	194	381	123	856	1561	-2,306		0.3		0.3		0.35		101-120 [20]
116-130 [1]	85	214	4551	2912	162	59	1331	1931	-1,832		0.4		0.4		0.45		116-130 [1]
116-130 [10]	6174	109	134	74	73	9037	2600	3981	-562		0.8		0.8		0.82		116-130 [10]
116-130 [20]	163	1054	167	106	161	2675	721	1024	-2,442		0.2		0.2		0.32		116-130 [20]
126-140 [1]	108	303	387	167	216	178	227	102	-2,936		0.1		0.1		0.23		126-140 [1]
126-140 [10]	111	438	197	563	348	260	320	165	-2,843		0.1		0.1		0.24		126-140 [10]
126-140 [20]	426	602	338	274	314	389	391	117	-2,772		0.1		0.1		0.25		126-140 [20]
136-150 [1]	286	105	122	243	116	3674	758	1431	-2,405		0.2		0.2		0.33		136-150 [1]
136-150 [10]	113	1637	1416	363	102	97	621	712	-2,541		0.2		0.2		0.30		136-150 [10]
136-150 [20]	83	19770	586	138	10477	81	5189	8239	2,027		1.7		1.6		0.49		136-150 [20]
146-160 [1]	95	122	98	118	575	172	197	187	-2,966		0.0		0.1		0.22		146-160 [1]
146-160 [10]	84	770	197	430	80	139	283	271	-2,879		0.1		0.1		0.24		146-160 [10]
146-160 [20]	118	208	422	185	155	133	204	112	-2,959		0.0		0.1		0.23		146-160 [20]
156-170 [1]	69	79	917	154	186	313	286	321	-2,876		0.1		0.1		0.24		156-170 [1]
156-170 [10]	234	454	144	312	283	147	262	116	-2,900		0.1		0.1		0.23		156-170 [10]
156-170 [20]	4840	171	473	53	73	475	1014	1884	-2,148		0.3		0.3		0.38		156-170 [20]
166-180 [1]	158	218	11901	23651	9545	225	7614	9425	4,454		2.4	*	2.4	*	0.15		166-180 [1]
166-180 [10]	8119	103	535	147	291	6195	2565	3612	-598		0.8		0.8		0.81		166-180 [10]
166-180 [20]	9480	367	501	440	288	330	1901	3714	-1,262		0.6		0.6		0.62		166-180 [20]
176-195 [1]	106	116	98	233	156	772	247	262	-2,916		0.1		0.1		0.23		176-195 [1]
176-195 [10]	108	124	374	161	1087	227	347	375	-2,816		0.1		0.1		0.25		176-195 [10]
176-195 [20]	94	435	166	346	496	128	278	171	-2,885		0.1		0.1		0.24		176-195 [20]
191-210 [1]	21051	689	610	203	174	18669	6899	10070	3,737		2.2	*	2.2	*	0.23		191-210 [1]
191-210 [10]	59	6705	2610	5962	7995	64	3899	3465	737		1.2		1.2		0.77		191-210 [10]
191-210 [20]	50	23847	2830	7216	13952	103	8000	9375	4,837		2.6	*	2.5	*	0.12		191-210 [20]
210-229 [1]	136	193	134	151	161	1937	452	728	-2,711		0.1		0.1		0.27		210-229 [1]
210-229 [10]	187	426	1868	304	779	903	745	616	-2,418		0.2		0.2		0.32		210-229 [10]
210-229 [20]	6324	1259	1123	1578	1379	27162	6471	10332	3,308		2.1		2.0		0.30		210-229 [20]
229-248 [1]	175	749	384	249	191	240	331	217	-2,831		0.1		0.1		0.25		229-248 [1]
229-248 [10]	96	109	132	119	655	879	332	345	-2,831		0.1		0.1		0.25		229-248 [10]
229-248 [20]	4885	70	275	183	82	14216	3285	5680	123		1.0		1.0		0.96		229-248 [20]
248-267 [1]	85	465	2153	7552	18603	176	4839	7314	1,676		1.5		1.5		0.55		248-267 [1]
248-267 [10]	327	124	192	157	135	15485	2737	6246	-426		0.9		0.9		0.87		248-267 [10]
248-267 [20]	192	134	266	530	184	354	277	146	-2,886		0.1		0.1		0.24		248-267 [20]
267-286 [1]	395	206	208	702	167	140	303	215	-2,860		0.1		0.1		0.24		267-286 [1]
267-286 [10]	769	348	939	645	253	130	514	318	-2,649		0.1		0.2		0.28		267-286 [10]
267-286 [20]	202	277	401	508	338	381	351	106	-2,811		0.1		0.1		0.25		267-286 [20]
287-306 [1]	75	2218	443	271	5653	58	1453	2213	-1,710		0.4		0.5		0.49		287-306 [1]
287-306 [10]	760	112	217	176	133	[redacted]	280	272	-2,883		0.1		0.1		0.28		287-306 [10]
287-306 [20]	94	343	188	291	229	18157	3217	7320	54		1.0		1.0		0.98		287-306 [20]
307-326 [1]	393	435	518	181	173	246	324	144	-2,838		0.1		0.1		0.24		307-326 [1]
307-326 [10]	110	328	522	146	2288	120	586	849	-2,577		0.2		0.2		0.29		307-326 [10]
307-326 [20]	318	222	114	305	64	227	208	102	-2,954		0.0		0.1		0.23		307-326 [20]
sAg 10	5225	113	189	70	159	18411	4028	7335	865		1.3		1.3		0.76		sAg 10
sAg 100	95	324	12403	10447	3148	38	4409	5592	1,247		1.4		1.4		0.64		sAg 100
N	53	84	39	34	143	140	82	49	-3,080		0.0		0.0		0.21		N
N	48	44	30	26	62	76	48	19	-3,115		0.0		0.0		0.20		N
3H	1895	278	735	137	77	715	640	677	-2,523		0.2		0.2		0.30		3H
3H	86	3888	1658	1776	14808	147	3727	5603	565		1.2		1.2		0.83		3H
3H	13881	263	720	664	107	11468	4517	6369	1,355		1.4		1.4		0.62		3H
3H	76	20811	410	1011	190	101	3767	8357	604		1.2		1.2		0.84		3H
	SMC															
N	53	30	55	43	35	33	42	11	-651		0.0		0.1		0.00	*	N
3H	343	952	722	910	753	475	693	240	0		1.0		1.0		1.00		3H
PHA - 1	25637	42805	37861	40225	35189	38664	36730	5993	36,038	*	56.4	*	53.0	*	0.00	*	PHA - 1
PHA - 5	35498	33195	33071	34428	30510	36603	33884	2136	33,192	*	52.0	*	48.9	*	0.00	*	PHA - 5
PHA - 10	25802	29379	30178	38346	28970	32984	30943	4297	30,251	*	47.5	*	44.7	*	0.00	*	PHA - 10
LPS - 1	853	1585	1559	1390	1520	1388	1383	273	690		2.1		2.0		0.00	*	LPS - 1
LPS - 5	1054	1437	1595	1190	1471	891	1273	272	581		1.9		1.8		0.00	*	LPS - 5
LPS - 10	540	1156	1105	1193	975	840	968	247	276		1.4		1.4		0.08		LPS - 10
LPS - 20	164	848	930	941	1079	670	772	327	80		1.1		1.1		0.64		LPS - 20
LPS - 40	94	163	284	346	219	84	198	105	-494		0.2		0.3		0.00	*	LPS - 40
	PBMC															
N	19	43	36	36	46	75	43	18	-49		0.0		0.5		0.00	*	N
3H	125	80	65	104	65	107	91	25	0		1.0		1.0		1.00		3H
PHA - 1	3194	4702	4951	3318	6209	9020	5232	2167	5,141	*	107.0	*	57.5	*	0.00	*	PHA - 1
PHA - 5	7400	13235	14	9926	21183	28076	13306	10032	13,215	*	273.5	*	146.2	*	0.01	*	PHA - 5
PHA - 10	5612	5228	7177	6245	9730	21002	9166	6017	9,075	*	188.1	*	100.7	*	0.00	*	PHA - 10
LPS - 1	340	390	6767	190	169	238	1349	2656	1,258		26.9	*	14.8	*	0.27		LPS - 1
LPS - 5	302	263	190	163	218	216	225	50	134		3.8	*	2.5	*	0.00	*	LPS - 5
LPS - 10	186	177	427	177	184	146	216	104	125		3.6	*	2.4	*	0.02	*	LPS - 10
LPS - 20	154	254	239	161	179	84	179	62	88		2.8	*	2.0		0.01	*	LPS - 20
LPS - 40	48	86	130	102	114	37	86	37	-5		0.9		0.9		0.80		LPS - 40

Raw data for Thymectomy duck W167

W167	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	52	23															
Total 3H	708	1004															
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1		<0.05	
1-15 [1]	894	260	168	244	91	676	389	320	-319		0.5		0.5		0.45		1-15 [1]
1-15 [10]	416	121	145	206	86	2391	561	904	-147		0.8		0.8		0.75		1-15 [10]
1-15 [20]	394	592	239	182	296	488	365	156	-343		0.5		0.5		0.42		1-15 [20]
7-14W-27 [1]	258	146	106	507	179	375	262	153	-446		0.3		0.4		0.29		7-14W-27 [1]
7-14W-27 [10]	716	297	258	156	349	1521	550	513	-159		0.8		0.8		0.71		7-14W-27 [10]
7-14W-27 [20]	1569	951	404	585	807	687	834	406	126		1.2		1.2		0.77		7-14W-27 [20]
7-14R-27 [1]	66	114	322	187	320	129	190	109	-519		0.2		0.3		0.22		7-14R-27 [1]
7-14R-27 [10]	556	221	160	779	370	1000	514	329	-194		0.7		0.7		0.65		7-14R-27 [10]
7-14R-27 [20]	584	566	300	100	560	231	390	207	-318		0.5		0.6		0.45		7-14R-27 [20]
22-41 [1]	226	177	97	134	455	421	252	151	-457		0.3		0.4		0.28		22-41 [1]
22-41 [10]	906	585	80	587	176	628	494	309	-215		0.7		0.7		0.61		22-41 [10]
22-41 [20]	1578	1151	382	845	609	1411	996	466	288		1.4		1.4		0.50		22-41 [20]
37-56 [1]	75	474	576	439	253	62	313	216	-395		0.4		0.4		0.35		37-56 [1]
37-56 [10]	453	83	607	275	206	309	322	185	-386		0.4		0.5		0.36		37-56 [10]
37-56 [20]	358	184	402	137	139	366	264	124	-444		0.3		0.4		0.30		37-56 [20]
54-73 [1]	426	228	106	136	241	288	238	115	-471		0.3		0.3		0.27		54-73 [1]
54-73 [10]	221	93	136	94	109	302	159	85	-549		0.2		0.2		0.20		54-73 [10]
54-73 [20]	572	157	170	259	178	118	242	168	-466		0.3		0.3		0.27		54-73 [20]
71-90 [1]	457	367	187	90	118	797	336	268	-372		0.4		0.5		0.38		71-90 [1]
71-90 [10]	71	221	285	688	637	43	324	278	-384		0.4		0.5		0.37		71-90 [10]
71-90 [20]	74	112	337	1873	2015	76	748	933	40		1.1		1.1		0.93		71-90 [20]
87-106 [1]	264	122	149	691	381	224	305	210	-403		0.4		0.4		0.34		87-106 [1]
87-106 [10]	290	528	60	453	273	340	324	162	-384		0.4		0.5		0.36		87-106 [10]
87-106 [20]	359	150	128	141	98	337	202	115	-506		0.2		0.3		0.23		87-106 [20]
101-120 [1]	523	197	110	98	103	169	200	163	-508		0.2		0.3		0.23		101-120 [1]
101-120 [10]	307	116	70	99	226	162	163	89	-545		0.2		0.2		0.20		101-120 [10]
101-120 [20]	206	122	96	94	348	73	157	105	-552		0.2		0.2		0.20		101-120 [20]
116-130 [1]	182	91	350	458	67	66	202	165	-506		0.2		0.3		0.23		116-130 [1]
116-130 [10]	234	85	303	152	181	895	308	297	-400		0.4		0.4		0.35		116-130 [10]
116-130 [20]	893	502	156	160	143	424	380	295	-329		0.5		0.5		0.44		116-130 [20]
126-140 [1]	185	317	158	262	329	762	336	220	-373		0.4		0.5		0.38		126-140 [1]
126-140 [10]	261	161	179	207	118	410	223	103	-486		0.3		0.3		0.25		126-140 [10]
126-140 [20]	464	181	130	125	149	240	215	129	-493		0.2		0.3		0.25		126-140 [20]
136-150 [1]	1125	1419	272	111	298	193	570	556	-139		0.8		0.8		0.75		136-150 [1]
136-150 [10]	56	1200	598	237	1846	67	667	721	-41		0.9		0.9		0.93		136-150 [10]
136-150 [20]	42	955	573	1122	1613	168	746	599	37		1.1		1.1		0.93		136-150 [20]
146-160 [1]	588	197	93	152	580	306	319	217	-389		0.4		0.5		0.36		146-160 [1]
146-160 [10]	781	157	67	71	644	655	396	331	-312		0.5		0.6		0.46		146-160 [10]
146-160 [20]	946	81	134	125	571	345	367	338	-341		0.5		0.5		0.42		146-160 [20]
156-170 [1]	444	131	138	552	555	396	369	192	-339		0.5		0.5		0.42		156-170 [1]
156-170 [10]	144	113	257	105	306	507	239	155	-470		0.3		0.3		0.27		156-170 [10]
156-170 [20]	340	263	220	417	550	257	341	124	-367		0.4		0.5		0.39		156-170 [20]
166-180 [1]	157	420	700	184	614	105	363	253	-345		0.5		0.5		0.42		166-180 [1]
166-180 [10]	740	107	85	109	130	658	305	307	-403		0.4		0.4		0.34		166-180 [10]
166-180 [20]	241	198	101	172	109	159	163	53	-545		0.2		0.2		0.20		166-180 [20]
176-195 [1]	269	132	104	132	566	832	339	297	-369		0.4		0.5		0.39		176-195 [1]
176-195 [10]	190	89	104	101	118	413	169	125	-539		0.2		0.2		0.21		176-195 [10]
176-195 [20]	178	318	127	128	141	268	193	81	-515		0.2		0.3		0.23		176-195 [20]
191-210 [1]	162	142	202	232	464	962	361	317	-348		0.5		0.5		0.41		191-210 [1]
191-210 [10]	94	349	392	148	172	65	203	136	-505		0.2		0.3		0.24		191-210 [10]
191-210 [20]	95	360	387	471	939	85	390	313	-319		0.5		0.5		0.45		191-210 [20]
210-229 [1]	127	253	230	544	902	684	457	303	-252		0.6		0.6		0.55		210-229 [1]
210-229 [10]	770	994	326	816	1294	589	798	332	90		1.1		1.1		0.83		210-229 [10]
210-229 [20]	1431	502	926	1130	1087	2002	1180	505	471		1.7		1.7		0.28		210-229 [20]
229-248 [1]	718	323	1056	1000	580	277	659	329	-49		0.9		0.9		0.91		229-248 [1]
229-248 [10]	408	140	508	203	172	370	300	149	-408		0.4		0.4		0.34		229-248 [10]
229-248 [20]	514	112	285	1655	96	7641	1717	2960	1,009		2.5	*	2.4	*	0.16		229-248 [20]
248-267 [1]	70	510	1391	1324	2387	104	964	904	256		1.4		1.4		0.57		248-267 [1]
248-267 [10]	295	205	129	343	254	4163	898	1601	190		1.3		1.3		0.72		248-267 [10]
248-267 [20]	712	99	216	431	1155	367	497	384	-212		0.7		0.7		0.62		248-267 [20]
267-286 [1]	423	206	187	293	459	475	341	129	-368		0.4		0.5		0.38		267-286 [1]
267-286 [10]	336	320	148	192	307	298	267	77	-441		0.3		0.4		0.30		267-286 [10]
267-286 [20]	135	131	467	415	342	2030	587	721	-122		0.8		0.8		0.78		267-286 [20]
287-306 [1]	121	1359	1088	1675	720	103	844	648	136		1.2		1.2		0.76		287-306 [1]
287-306 [10]	590	132	106	141	2062	384	569	755	-139		0.8		0.8		0.75		287-306 [10]
287-306 [20]	453	270	906	293	389	498	468	232	-240		0.6		0.7		0.57		287-306 [20]
307-326 [1]	211	167	95	103	671	320	261	217	-447		0.3		0.4		0.29		307-326 [1]
307-326 [10]	149	286	239	163	212	1159	368	391	-340		0.5		0.5		0.43		307-326 [10]
307-326 [20]	510	313	215	521	483	695	456	169	-252		0.6		0.6		0.55		307-326 [20]
sAg 10	3596	183	518	150	232	752	905	1339	197		1.3		1.3		0.69		sAg 10
sAg 100	56	50	82	792	244	62	214	292	-494		0.2		0.3		0.25		sAg 100
N	38	42	69	94	74	46	61	22	-648		0.0		0.1		0.13		N
N	43	29	28	87	28	41	43	23	-666		0.0		0.1		0.12		N
3H	749	1756	101	320	473	451	642	585	-67		0.9		0.9		0.88		3H
3H	100	4851	582	158	614	132	1073	1865	365		1.6		1.5		0.51		3H
3H	1773	579	78	120	422	975	658	637	-50		0.9		0.9		0.91		3H
3H	167	1014	248	952	296	86	461	411	-248		0.6		0.7		0.56		3H
	SMC																
N	24	50	60	63	42	35	46	15	-1,763		0.0		0.0		0.00	*	N
3H	1406	1874	1997	1986	2472	1114	1808	481	0		1.0		1.0		1.00		3H
PHA - 1	78238	68482	65977	51485	50574	51820	61096	11501	59,288	*	34.6	*	33.8	*	0.00	*	PHA - 1
PHA - 5	70631	87928	78231	63226	58863	53080	68660	12923	66,852	*	38.9	*	38.0	*	0.00	*	PHA - 5
PHA - 10	69548	71849	66733	63192	52208	34250	59630	14213	57,822	*	33.8	*	33.0	*	0.00	*	PHA - 10
LPS - 1	1545	1366	1618	1801	1421	1434	1531	161	-277		0.8		0.8		0.21		LPS - 1
LPS - 5	1534	2201	2240	1516	1700	1028	1703	459	-105		0.9		0.9		0.71		LPS - 5
LPS - 10	1541	1952	2310	2206	1961	1265	1873	399	64		1.0		1.0		0.81		LPS - 10
LPS - 20	1442	2156	1817	3226	1733	1580	1992	651	184		1.1		1.1		0.59		LPS - 20
LPS - 40	219	785	1828	2199	1362	701	1182	748	-626		0.6		0.7		0.12		LPS - 40
	PBMC																
N	62	47	79	83	45	58	62	16	-18		0.0		0.8		0.06		N
3H	91	80	84	96	69	60	80	14	0		1.0		1.0		1.00		3H
PHA - 1	51398	24314	24802	50423	16467	40946	34725	14855	34,645	*	1962.0	*	434.1	*	0.00	*	PHA - 1
PHA - 5	49362	49591	48529	55684	49722	38950	48640	5406	48,560	*	2749.7	*	608.0	*	0.00	*	PHA - 5
PHA - 10	35013	41932	39991	59059	46022	36476	43082	8758	43,002	*	2435.1	*	538.5	*	0.00	*	PHA - 10
LPS - 1	860	945	1182	1281	800	799	978	206	898		51.8	*	12.2	*	0.00	*	LPS - 1
LPS - 5	1106	1101	910	1477	1176	1237	1168	187	1,088		62.6	*	14.6	*	0.00	*	LPS - 5
LPS - 10	727	1005	887	1335	1773	867	1099	389	1,019		58.7	*	13.7	*	0.00	*	LPS - 10
LPS - 20	1211	1763	1219	1616	1486	660	1329	397	1,249		71.7	*	16.6	*	0.00	*	LPS - 20
LPS - 40	294	1208	853	1131	1149	218	809	446	729		42.3	*	10.1	*	0.00	*	LPS - 40

Raw data for Thymectomy duck W168

W168	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	84	51															
Total 3H	144	110								>5000		>2.1		>2.1		<0.05	
	R1	R2	R3	R4	R5	R6	Mean	SD									
1-15 [1]	191	212	265	124	349	298	240	81	96		2.6	*	1.7		0.06		1-15 [1]
1-15 [10]	722	703	196	1411	192	398	604	459	460		8.7	*	4.2	*	0.00	*	1-15 [10]
1-15 [20]	2593	955	394	1592	2162	1521	1536	794	1,393		24.3	*	10.7	*	0.00	*	1-15 [20]
7-14W-27 [1]	312	238	501	254	334	336	329	94	186		4.1	*	2.3	*	0.00	*	7-14W-27 [1]
7-14W-27 [10]	6045	1962	3436	7454	460	2540	3650	2627	3,506		59.6	*	25.4	*	0.00	*	7-14W-27 [10]
7-14W-27 [20]	2008	8972	6028	14073	2030	16112	8204	5980	8,060	*	135.6	*	57.1	*	0.00	*	7-14W-27 [20]
7-14R-27 [1]	148	810	326	5936	1321	862	1567	2180	1,424		24.8	*	10.9	*	0.00	*	7-14R-27 [1]
7-14R-27 [10]	1299	1932	1100	1584	4901		2163	1562	2,020		34.7	*	15.1	*	0.00	*	7-14R-27 [10]
7-14R-27 [20]	1301	5330	30906	7062	39007	46729	21723	19540	21,579	*	361.4	*	151.2	*	0.00	*	7-14R-27 [20]
22-41 [1]	243	551	409	333	228	344	351	119	208		4.5	*	2.4	*	0.00	*	22-41 [1]
22-41 [10]	398	368	1356	593	289	638	607	391	463		8.7	*	4.2	*	0.00	*	22-41 [10]
22-41 [20]	943	659	587	1209	725	3411	1256	1080	1,112		19.6	*	8.7	*	0.00	*	22-41 [20]
37-56 [1]	118	246	853	253	185	829	414	334	270		5.5	*	2.9	*	0.00	*	37-56 [1]
37-56 [10]	926	209	157	475	345	333	408	277	264		5.4	*	2.8	*	0.00	*	37-56 [10]
37-56 [20]	963	417	396	890	377	226	545	304	401		7.7	*	3.8	*	0.00	*	37-56 [20]
54-73 [1]	264	213	240	208	126	183	206	48	62		2.0		1.4		0.19		54-73 [1]
54-73 [10]	3997	458	177	172	573	133	918	1519	775		13.9	*	6.4	*	0.01	*	54-73 [10]
54-73 [20]	358	823	194	374	670	163	430	264	287		5.8	*	3.0	*	0.00	*	54-73 [20]
71-90 [1]	572	136	105	77	140	149	197	186	53		1.9		1.4		0.37		71-90 [1]
71-90 [10]	3891	204	170	191	2945	647	1341	1646	1,198		21.0	*	9.3	*	0.00	*	71-90 [10]
71-90 [20]	153	225	234	294	1724	244	479	612	335		6.6	*	3.3	*	0.01	*	71-90 [20]
87-106 [1]	333	152	129	131	148	775	278	256	134		3.2	*	1.9		0.06		87-106 [1]
87-106 [10]	337	585	188	297	546	277	372	158	228		4.8	*	2.6	*	0.00	*	87-106 [10]
87-106 [20]	393	316	493	370	248	345	361	82	217		4.6	*	2.5	*	0.00	*	87-106 [20]
101-120 [1]	246	125	131	127	326	283	206	90	63		2.0		1.4		0.21		101-120 [1]
101-120 [10]	702	170	121	124	180	171	245	225	101		2.7	*	1.7		0.12		101-120 [10]
101-120 [20]	87	111	182	290	111	222	167	79	24		1.4		1.2		0.63		101-120 [20]
116-130 [1]	88	130	526	286	175	2283	581	848	438		8.3	*	4.0	*	0.02	*	116-130 [1]
116-130 [10]	90	197	2588	1341	113	75	734	1032	590		10.9	*	5.1	*	0.01	*	116-130 [10]
116-130 [20]	116	406	207	334	374	320	293	110	149		3.5	*	2.0		0.01	*	116-130 [20]
126-140 [1]	302	63	177	166	209	414	222	122	78		2.3	*	1.5		0.14		126-140 [1]
126-140 [10]	256	433	299	650	117	250	334	185	191		4.2	*	2.3	*	0.00	*	126-140 [10]
126-140 [20]	164	196	224	201	146	408	223	95	80		2.3	*	1.6		0.12		126-140 [20]
136-150 [1]	519	181	704	145	235	158	324	233	180		4.0	*	2.3	*	0.01	*	136-150 [1]
136-150 [10]	113	2310	1583	237	608	3118	1328	1218	1,185		20.8	*	9.2	*	0.00	*	136-150 [10]
136-150 [20]	240	433	173	201	469	1168	447	374	304		6.1	*	3.1	*	0.00	*	136-150 [20]
146-160 [1]	584	296	294	278	322	567	390	144	247		5.1	*	2.7	*	0.00	*	146-160 [1]
146-160 [10]	237	431	541	268	140	392	335	146	191		4.2	*	2.3	*	0.00	*	146-160 [10]
146-160 [20]	256	150	375	188	140	416	254	118	111		2.8	*	1.8		0.04	*	146-160 [20]
156-170 [1]	532	166	415	279	545	897	472	254	329		6.5	*	3.3	*	0.00	*	156-170 [1]
156-170 [10]	547	923	460	173	209	314	438	278	294		5.9	*	3.0	*	0.00	*	156-170 [10]
156-170 [20]	223	527	390	114	349	389	332	144	188		4.1	*	2.3	*	0.00	*	156-170 [20]
166-180 [1]	754	672	280	361	152	210	405	250	261		5.4	*	2.8	*	0.00	*	166-180 [1]
166-180 [10]	384	210	156	183	91	188	202	98	58		2.0		1.4		0.25		166-180 [10]
166-180 [20]	264	207	187	215	100	451	237	118	94		2.6	*	1.7		0.08		166-180 [20]
176-195 [1]	210	289	132	187	101	170	182	66	38		1.6		1.3		0.43		176-195 [1]
176-195 [10]	756	130	168	202	162	148	261	244	117		3.0	*	1.8		0.08		176-195 [10]
176-195 [20]	109	86	123	210	173	173	146	47	2		1.0		1.0		0.97		176-195 [20]
191-210 [1]	126	94	88	167	93	50	103	40	-41		0.3		0.7		0.39		191-210 [1]
191-210 [10]	4121	557	1986	184	499	2659	1668	1543	1,524		26.5	*	11.6	*	0.00	*	191-210 [10]
191-210 [20]	8044	201	74	134	143	36151	7458	14408	7,314	*	123.2	*	51.9	*	0.01	*	191-210 [20]
210-229 [1]	149	85	140	113	221	181	148	48	5		1.1		1.0		0.92		210-229 [1]
210-229 [10]	255	1201	212	151	130	936	481	465	337		6.6	*	3.3	*	0.00	*	210-229 [10]
210-229 [20]	2291	19722	836	17381	2842	1703	7463	8647	7,319	*	123.2	*	52.0	*	0.00	*	210-229 [20]
229-248 [1]	114	155	100	104	139	356	161	98	18		1.3		1.1		0.72		229-248 [1]
229-248 [10]	98	156	189	509	257	316	254	146	111		2.8	*	1.8		0.05	*	229-248 [10]
229-248 [20]	1013	790	916	223	216	780	656	349	513		9.6	*	4.6	*	0.00	*	229-248 [20]
248-267 [1]	5199	159	124	147	228	1740	1266	2027	1,123		19.7	*	8.8	*	0.01	*	248-267 [1]
248-267 [10]	352	161	229	254	160	506	277	133	133		3.2	*	1.9		0.02	*	248-267 [10]
248-267 [20]	128	267	314	133	155	171	195	77	51		1.9		1.4		0.30		248-267 [20]
267-286 [1]	323	219	172	94	135	136	180	82	36		1.6		1.3		0.46		267-286 [1]
267-286 [10]	231	267	166	243	151	287	224	55	81		2.3	*	1.6		0.10		267-286 [10]
267-286 [20]	506	278	165	413	526	182	345	159	201		4.4	*	2.4	*	0.00	*	267-286 [20]
287-306 [1]	474	214	204	86	327	571	313	182	169		3.8	*	2.2	*	0.01	*	287-306 [1]
287-306 [10]	210	143	120	128	102	379	180	104	37		1.6		1.3		0.47		287-306 [10]
287-306 [20]	229	330	300	257	261	477	309	90	165		3.8	*	2.2	*	0.00	*	287-306 [20]
307-326 [1]	204	332	258	256	197	267	252	49	109		2.8	*	1.8		0.03	*	307-326 [1]
307-326 [10]	238	281	1134	458	1165	467	624	418	480		9.0	*	4.3	*	0.00	*	307-326 [10]
307-326 [20]	841	589	793	1259	911	1519	985	341	842		15.1	*	6.9	*	0.00	*	307-326 [20]
sAg 10	258	379	335	327	338	379	336	44	192		4.2	*	2.3	*	0.00	*	sAg 10
sAg 100	94	391	276	311	275	1206	426	395	282		5.7	*	3.0	*	0.00	*	sAg 100
N	37	43	58	45	54	45	47	8	-97		-0.6		0.3		0.04	*	N
N	96	95	66	104	180	182	121	49	-23		0.6		0.8		0.62		N
3H	135	247	184	73	70	253	160	81	17		1.3		1.1		0.73		3H
3H	216	171	117	67	235	154	160	62	16		1.3		1.1		0.73		3H
3H	264	153	66	105	92	530	202	175	58		2.0		1.4		0.32		3H
3H	50	44	70	62	39	50	53	12	-91		-0.5		0.4		0.06		3H
	SMC																
N	39	19	48	51	34	68	43	17	-731		0.0		0.1		0.00	*	N
3H	997	597	1049	617	507	880	775	230	0		1.0		1.0		1.00		3H
PHA - 1	49328	54388	56232	46110	40269	23849	45029	11866	44,255	*	61.5	*	58.1	*	0.00	*	PHA - 1
PHA - 5	42891	65246	66628	52624	43427	34502	50886	13000	50,112	*	69.5	*	65.7	*	0.00	*	PHA - 5
PHA - 10	41770	49096	19	53032	33080	33770	35128	18966	34,353	*	48.0	*	45.4	*	0.00	*	PHA - 10
LPS - 1	4439	9800	6576	6191	4868	2853	5788	2373	5,013	*	7.9	*	7.5	*	0.00	*	LPS - 1
LPS - 5	5397	9649	56909	5492	5686	4123	14543	20839	13,768	*	19.8	*	18.8	*	0.14		LPS - 5
LPS - 10	4790	5949	8252	7100	6898	3640	6105	1677	5,330	*	8.3	*	7.9	*	0.00	*	LPS - 10
LPS - 20	4588	5946	6643	6586	5738	4177	5613	1024	4,839		7.6	*	7.2	*	0.00	*	LPS - 20
LPS - 40	2452	7470	8177	6050	4717	1301	5028	2740	4,253		6.8	*	6.5	*	0.00	*	LPS - 40
	PBMC																
N	40	54	54	36	66	51	50	11	-612		0.0		0.1		0.00	*	N
3H	590	527	731	870	544	711	662	132	0		1.0		1.0		1.00		3H
PHA - 1	91996	96915	60380	49673	65837	63712	71419	16755	70,757	*	116.6	*	107.9	*	0.00	*	PHA - 1
PHA - 5	56531	72210	39661	66802	46872	40837	53819	13648	53,157	*	87.9	*	81.3	*	0.00	*	PHA - 5
PHA - 10	53691	60813	41515	50432	36065	39351	46978	9545	46,316	*	76.7	*	70.9	*	0.00	*	PHA - 10
LPS - 1	1011	1258	1065	1242	607	681	977	277	315		1.5		1.5		0.03	*	LPS - 1
LPS - 5	944	1668	1007	1115	545	520	967	423	304		1.5		1.5		0.12		LPS - 5
LPS - 10	615	1283	1089	1241	737	386	892	366	230		1.4		1.3		0.18		LPS - 10
LPS - 20	534	1056	888	839	738	369	737	250	75		1.1		1.1		0.53		LPS - 20
LPS - 40	131	327	269	415	382	58	264	142	-399		0.3		0.4		0.00	*	LPS - 40

Raw data for Thymectomy duck W170

W170	Mean	SD							CPM-3H		S.I.		P/N		t-Test		
Total N	52	41															
Total 3H	328	498															
	R1	R2	R3	R4	R5	R6	Mean	SD		>5000		>2.1		>2.1		<0.05	
1-15 [1]	124	262	176	476	126	211	229	132	-98		0.6		0.7		0.64		1-15 [1]
1-15 [10]	166	157	271	220	416	120	225	107	-103		0.6		0.7		0.62		1-15 [10]
1-15 [20]	217	370	569	1511	209	226	517	506	190		1.7		1.6		0.41		1-15 [20]
7-14W-27 [1]	136	148	302	420	127	129	210	123	-117		0.6		0.6		0.58		7-14W-27 [1]
7-14W-27 [10]	329	247	225	461	264	176	284	100	-44		0.8		0.9		0.83		7-14W-27 [10]
7-14W-27 [20]	573	129	7653	293	295	119	1510	3014	1,183		5.3	*	4.6	*	0.07		7-14W-27 [20]
7-14R-27 [1]	79	202	158	239	468	98	207	141	-120		0.6		0.6		0.57		7-14R-27 [1]
7-14R-27 [10]	66	136	179	76	195	295	158	85	-170		0.4		0.5		0.42		7-14R-27 [10]
7-14R-27 [20]	263	264	292	666	499	282	378	168	50		1.2		1.2		0.81		7-14R-27 [20]
22-41 [1]	117	207	248	168	132	284	193	66	-135		0.5		0.6		0.52		22-41 [1]
22-41 [10]	156	314	431	284	387	111	281	126	-47		0.8		0.9		0.82		22-41 [10]
22-41 [20]	540	939	1764	544	547	2222	1093	729	765		3.8	*	3.3	*	0.00	*	22-41 [20]
37-56 [1]	143	389	372	111	116	335	244	134	-83		0.7		0.7		0.69		37-56 [1]
37-56 [10]	59	143	109	127	58	79	96	36	-232		0.2		0.3		0.27		37-56 [10]
37-56 [20]	120	240	378	1350	339	58	414	475	87		1.3		1.3		0.70		37-56 [20]
54-73 [1]	135	126	257	208	80	184	165	64	-163		0.4		0.5		0.44		54-73 [1]
54-73 [10]	123	155	407	480	173	191	255	150	-73		0.7		0.8		0.73		54-73 [10]
54-73 [20]	140	622	106	414	182	140	267	206	-60		0.8		0.8		0.78		54-73 [20]
71-90 [1]	83	83	108	950	75	407	284	350	-43		0.8		0.9		0.84		71-90 [1]
71-90 [10]	235	276	139	367	93	123	206	106	-122		0.6		0.6		0.56		71-90 [10]
71-90 [20]	111	160	151	119	247	139	155	49	-173		0.4		0.5		0.41		71-90 [20]
87-106 [1]	137	393	219	1076	287	426	423	337	96		1.3		1.3		0.66		87-106 [1]
87-106 [10]	145	356	638	705	380	188	402	229	75		1.3		1.2		0.73		87-106 [10]
87-106 [20]	253	322	202	350	244	282	276	54	-52		0.8		0.8		0.80		87-106 [20]
101-120 [1]	243	353	193	201	188	114	215	79	-112		0.6		0.7		0.59		101-120 [1]
101-120 [10]	96	258	151	219	210	234	195	60	-133		0.5		0.6		0.52		101-120 [10]
101-120 [20]	321	121	440	223	151	154	235	123	-93		0.7		0.7		0.66		101-120 [20]
116-130 [1]	96	101	148	122	271	171	152	65	-176		0.4		0.5		0.40		116-130 [1]
116-130 [10]	102	144	276	171	96	94	147	70	-180		0.3		0.4		0.39		116-130 [10]
116-130 [20]	228	378	854	835	75	128	416	347	89		1.3		1.3		0.68		116-130 [20]
126-140 [1]	530	307	239	209	163	94	257	152	-71		0.7		0.8		0.74		126-140 [1]
126-140 [10]	481	152	274	283	178	223	265	118	-62		0.8		0.8		0.77		126-140 [10]
126-140 [20]	114	180	299	309	101	241	207	90	-120		0.6		0.6		0.57		126-140 [20]
136-150 [1]	76	67	247	236	103	87	136	83	-192		0.3		0.4		0.36		136-150 [1]
136-150 [10]	123	205	408	140	107	98	180	118	-147		0.5		0.6		0.48		136-150 [10]
136-150 [20]	94	109	63	66	141	122	99	31	-228		0.2		0.3		0.28		136-150 [20]
146-160 [1]	110	97	240	155	140	129	145	51	-182		0.3		0.4		0.38		146-160 [1]
146-160 [10]	137	207	209	133	222	158	178	40	-150		0.5		0.5		0.47		146-160 [10]
146-160 [20]	130	115	272	118	206	91	155	69	-172		0.4		0.5		0.41		146-160 [20]
156-170 [1]	148	179	89	73	212	125	138	53	-190		0.3		0.4		0.37		156-170 [1]
156-170 [10]	925	1206	187	79	170	102	445	491	117		1.4		1.4		0.61		156-170 [10]
156-170 [20]	241	235	287	218	140	61	197	82	-131		0.5		0.6		0.53		156-170 [20]
166-180 [1]	110	129	230	155	119	89	139	50	-189		0.3		0.4		0.37		166-180 [1]
166-180 [10]	187	68	117	500	121	118	185	159	-142		0.5		0.6		0.50		166-180 [10]
166-180 [20]	115	228	304	747	405	7985	1631	3120	1,303		5.7	*	5.0	*	0.05	*	166-180 [20]
176-195 [1]	128	177	148	887	206	101	275	302	-53		0.8		0.8		0.81		176-195 [1]
176-195 [10]	96	99	200	155	137	158	141	39	-187		0.3		0.4		0.37		176-195 [10]
176-195 [20]	111	194	167	73	149	112	134	44	-193		0.3		0.4		0.36		176-195 [20]
191-210 [1]	77	159	112	132	104	105	115	28	-213		0.2		0.4		0.31		191-210 [1]
191-210 [10]	210	105	128	220	122	318	184	81	-144		0.5		0.6		0.49		191-210 [10]
191-210 [20]	58	124	165	167	318	108	157	89	-171		0.4		0.5		0.42		191-210 [20]
210-229 [1]	95	445	107	233	92	119	182	139	-146		0.5		0.6		0.49		210-229 [1]
210-229 [10]	203	268	555	751	1169	321	545	368	217		1.8		1.7		0.33		210-229 [10]
210-229 [20]	2437	10845	2275	666	2821	5766	4135	3682	3,808		14.8	*	12.6	*	0.00	*	210-229 [20]
229-248 [1]	211	126	844	1279	172	153	464	484	137		1.5		1.4		0.55		229-248 [1]
229-248 [10]	71	1975	120	126	281	164	456	747	129		1.5		1.4		0.61		229-248 [10]
229-248 [20]	162	188	2173	886	112	170	615	817	288		2.0		1.9		0.28		229-248 [20]
248-267 [1]	429	340	757	1465	1357	136	747	553	420		2.5	*	2.3	*	0.08		248-267 [1]
248-267 [10]	422	1048	2386	1070	704	1630	1210	705	883		4.2	*	3.7	*	0.00	*	248-267 [10]
248-267 [20]	498	794	946	1843	9715	5402	3200	3669	2,872		11.4	*	9.8	*	0.00	*	248-267 [20]
267-286 [1]	471	513	719	951	286	771	619	240	291		2.1		1.9		0.18		267-286 [1]
267-286 [10]	197	534	814	788	660	1756	792	523	464		2.7	*	2.4	*	0.05		267-286 [10]
267-286 [20]	416	210	293	773	485	646	471	212	143		1.5		1.4		0.50		267-286 [20]
287-306 [1]	112	269	1202	172	107	1094	493	512	165		1.6		1.5		0.48		287-306 [1]
287-306 [10]	115	135	319	635	1126	275	434	387	107		1.4		1.3		0.63		287-306 [10]
287-306 [20]	167	257	140	330	1065	347	384	344	57		1.2		1.2		0.79		287-306 [20]
307-326 [1]	172	640	265	260	159	185	280	182	-47		0.8		0.9		0.82		307-326 [1]
307-326 [10]	324	210	420	1065	380	554	492	303	165		1.6		1.5		0.45		307-326 [10]
307-326 [20]	555	241	1129	479	835	448	615	317	287		2.0		1.9		0.19		307-326 [20]
sAg 10	227	475	251	65	93	183	216	147	-112		0.6		0.7		0.59		sAg 10
sAg 100	104	93	820	1001	178	159	393	407	65		1.2		1.2		0.77		sAg 100
N	53	41	67	91	91	148	82	38	-246		0.1		0.2		0.24		N
N	30	16	16	18	22	30	22	7	-306		-0.1		0.1		0.15		N
3H	175	264	94	141	105	299	180	85	-148		0.5		0.5		0.48		3H
3H	1462	392	132	155	143	2093	730	842	402		2.5	*	2.2	*	0.14		3H
3H	88	116	119	142	1067	82	269	392	-59		0.8		0.8		0.79		3H
3H	146	81	120	181	106	157	132	36	-196		0.3		0.4		0.35		3H
	SMC																
N	33	52	59	44	22	19	38	16	-858		0.0		0.0		0.00	*	N
3H	1375	762	913	904	825	596	896	262	0		1.0		1.0		1.00		3H
PHA - 1	38666	35989	39082	40690	34436	20510	34896	7398	34,000	*	40.6	*	39.0	*	0.00	*	PHA - 1
PHA - 5	21411	25773	30975	33671	23769	17163	25460	6100	24,565	*	29.6	*	28.4	*	0.00	*	PHA - 5
PHA - 10	17915	20508	24102	29246	21325	11754	20808	5878	19,913	*	24.2	*	23.2	*	0.00	*	PHA - 10
LPS - 1	2607	3331	3660	3623	2358	1370	2825	891	1,929		3.2	*	3.2	*	0.00	*	LPS - 1
LPS - 5	2766	2710	4454	3065	2223	1369	2765	1019	1,869		3.2	*	3.1	*	0.00	*	LPS - 5
LPS - 10	2611	3776	2996	4820	3848	2341	3399	924	2,503		3.9	*	3.8	*	0.00	*	LPS - 10
LPS - 20	2717	5242	3742	4015	4477	2547	3790	1032	2,894		4.4	*	4.2	*	0.00	*	LPS - 20
LPS - 40	2722	4781	2899	4282	4448	1800	3489	1184	2,593		4.0	*	3.9	*	0.00	*	LPS - 40
	PBMC																
N	29	31	24	54	45	54	40	13	-25		0.0		0.6		0.06		N
3H	47	45	37	100	72	88	65	26	0		1.0		1.0		1.00		3H
PHA - 1	1673	1975	4836	4966	2657	3110	3203	1409	3,138		124.9	*	49.4	*	0.00	*	PHA - 1
PHA - 5	15891	4001	13291	35544	18839	7958	15921	11009	15,856	*	626.9	*	245.6	*	0.01	*	PHA - 5
PHA - 10	18233	3900	17534	30900	22979	14676	18037	8959	17,972	*	710.4	*	278.2	*	0.00	*	PHA - 10
LPS - 1	368	239	650	980	600	316	526	275	461		19.2	*	8.1	*	0.00	*	LPS - 1
LPS - 5	397	235	496	733	400	232	416	187	351		14.8	*	6.4	*	0.00	*	LPS - 5
LPS - 10	202	136	348	404	256	207	259	100	194		8.7	*	4.0	*	0.00	*	LPS - 10
LPS - 20	201	157	287	412	214	129	233	103	169		7.7	*	3.6	*	0.00	*	LPS - 20
LPS - 40	196	135	156	172	118	80	143	41	78		4.1	*	2.2	*	0.00	*	LPS - 40

11.10. MANIPULATION OF IMMUNE MECHANISMS

		Liver		Spleen		Thymus	
Group	Duck	Inflamation	Notes	Follicles	Periarterialar Lymphocytes	Present	Description
Negative Control	1A	Slight		Normal			
	1B	Slight		Normal			
	1C	Slight	One Intralobular	Normal			
	1D	Slight	Several lymphoid aggregates	Normal			
	1E	Slight		Normal			
	1F	Slight	Several lymphoid aggregates	Normal			
	1G	Slight	Moderate lymphoid aggregates	Normal			
	1H	Slight	Mostly lymphoid aggregates	Normal			
	1I	Slight		Normal			
	1J	Slight		Normal			
	1K	Slight		Normal			
	1L	No Liver		Normal			
	2A	Slight	Moderate Fat infiltration				
	2B	Slight	Moderate Fat infiltration				
	2C	Slight	One lymphoid aggregate				
	2D	Normal					
	2E	Slight					
	2F	Normal					
	2G	Slight		Reduced			
	2H	Slight		Normal			
	2I	Slight		Normal			
	P24P53	Mild	Focal moderate and a few lymphoid aggregates	Reduced			
	V2T	Slight		Reduced			
	V2U	Slight		Normal			
Protein Vaccinated group	G51	Slight	Some Intralobular and 1 Lymphoid Aggregate	Reduced			
	G53	Slight	Some Intralobular	Reduced			
	G63	Slight-Moderate	A few lymphoid aggregates	Reduced	Increased		
	G99	Mild	Focal moderate	Reduced	Increased		
	P63G81	Slight		Reduced			
	W45	Slight	One lymphoid aggregate	Reduced	Increased		
	V2J	Slight		Reduced			
	V2K	Slight		Reduced	Increased		
	V2L	Normal		Reduced			
	V2M	Slight		Reduced			
	V2N	Slight		Normal			
	V2O	Normal	Moderate Fat infiltration	Normal			
	V2P	Slight	Moderate Fat infiltration	Normal	Increased		
	V2Q	Normal		Normal			
	V2S	Mild		Reduced			
Positive control group	G511	Normal		Reduced	Increased		
	G531	Slight		?????	Increased		
	G58	Slight		Normal	Normal		
	G72	Mild		Normal	Normal		
	G86	Normal		Reduced	Increased		
	G89	Mild		Normal	Normal		
	G991			Reduced	Normal		
	P531	Slight	Some neutrophils	Reduced	Increased		
	P57	Slight		Reduced	Increased		
	P631	Slight		Normal	Normal		
	W34	Normal					
	W451	Mild		Reduced	Increased		
	P72W48	Mild	Focal moderate	Normal	Increased		
	W103	Slight		No Follicles			
	W105	Slight		Normal		No	Adipose tissue
	W106	Slight		Normal		No	Vascular Adipose Tissue and Nerves
	W107		No Liver	Normal		No	Adipose tissue and nerves
	W111	Mild		Normal		No	Adipose tissue and nerves
	W139	Slight		Reduced			
Bursectomised group	W101	Mild		Normal			
	W104	Mild		Reduced			
	W109	Mild		Reduced			
	W110	Normal		Reduced			
	W121	Mild	Fat infiltration	Normal			
	W132	Mild	Some eosinophils	Normal			
	W131	Slight	Fat infiltration	Normal			
	W140	Slight	Fat infiltration	Reduced			
	W145	Slight	Fat infiltration	Reduced	Normal		
Thymectomy group	W122	Slight		Normal		No	Adipose tissue
	W125	Slight		Normal		Yes	Adipose tissue, with one small focus of lymphoid tissue.
	W126	Mild		Normal		No	Vascular Adipose Tissue and Nerves
	W147	Slight		No Spleen		No	Adipose tissue and nerves
	W151	Slight	No Fat	Reduced	Increased	No	Fibroadipose tissue former large vessels
	W152	Mild	No Fat	Normal		No	Adipose tissue and nerves
	W153	Slight	No Fat	Reduced	Increased	No	Adipose tissue large vessels and nerves
	W156	Slight	No Fat	Normal	Increased	No	Fibroadipose tissue
	W157	Slight	No Fat	Normal	Increased	No	Adipose tissue
	W160	Slight	No Fat	Normal		No	Adipose tissue
	W167	Slight	No Fat	Reduced	Increased	No	Fibroadipose tissue ??? And nerves
	W168	Slight	No Fat	Normal	Increased	No	Adipose tissue and nerve
	W170	Slight	No Fat	Reduced		No	Adipose tissue

Table 85. *Histopathology results for individual ducks.*

	WBC Ave x10⁷	SD	PBMC Ave x10⁷	SD	SMC Ave x10⁷	SD	Heterophil WBC-PBMC	%
Negative control	3.3	2.1	1.8	1.0	6.8	3.9	1.5	53.4
Positive control	8.1	7.6	1.0	0.5	3.8	3.6	7.0	13.0
Bursectomised	17.9	3.4	1.1	0.6	5.1	4.1	16.8	6.3
Thymectomised	2.1	1.4	1.6	0.5	11.4	3.8	0.5	77.2
Protein Vaccinated	6.2	2.6	2.0	0.7	7.0	3.6	4.2	31.7

Group	Duck	WBC 1	2	3	Ave x10⁷	PBMC 1	2	3	Ave x10⁷	SMC 1	2	3	Ave x10⁷
Negative Control	1A	4	6	3	4.3	440	405		4.2	128	92	102	10.7
	1B	6	4	4	4.7	142	139	171	1.5	63	35	53	5.0
	1C	2	2	2	2.0	155	134	138	1.4	23	19	25	2.2
	1D	1	3	6	3.3	95	110	122	1.1	74	68	70	7.1
	1E	2	2	2	2.0	55	52	48	0.5	48	72	67	6.2
	1F	3	3	2	2.7	80	71	113	0.9	40	36	64	4.7
	1G	0	4	1	1.7	89	102	90	0.9	86	50	60	6.5
	1H	4	3	0	2.3	89	112	74	0.9	54	50	79	6.1
	1I	2	1	1	1.3	119	184	202	1.7	62	66	84	7.1
	1J	2	3	4	3.0	158	165	244	1.9	59	53	54	5.5
	1K	5	3	0	2.7	89	108	138	1.1	138	143	137	13.9
	1L	2	4	3	3.0	92	128	141	1.2	43	48	61	5.1
	2A									54	65	45	5.5
	2B									43	43	39	4.2
	2C									39	29	36	3.5
	2D									73	72	89	7.8
	2E									71	61	58	6.3
	2F									32	39	36	3.6
	2G					339	397	348	3.6	65	61	72	6.6
	2H					299	285	293	2.9	33	42	30	3.5
	2I					230	211	219	2.2	41	44	46	4.4
	P24P53	8	12	9	9.7	127	131	148	1.4	166	232	213	20.4
	V2T					217	197	186	2.0	108	92	109	10.3
	V2U					196	225	207	2.1	69	60	65	6.5
Positive control group	G58					100	98	113	1.0	29	18	35	2.7
	G72									16	26	14	1.9
	G86					56	74	88	0.7	7	9	3	0.6
	G89									28	12	24	2.1
	G511					143	172	160	1.6	4	1	4	0.3
	G531					138	142	140	1.4				
	G631									14	22	18	1.8
	G991					142	166	174	1.6	75	95	75	8.2
	P17	7	9		8.0								
	P54					22	27	26	0.3	7	7	7	0.7
	P57					83	86	91	0.9	19	18	19	1.9
	P72W48	7	5	9	7.0	93	83	88	0.9	32	40	43	3.8
	P531					70	67	61	0.7	7	10	7	0.8
	P631					122	154	158	1.4				
	V2R									69	62	66	6.6
	W34					56	53	67	0.6	6	5	9	0.7
	W43					86	95	118	1.0				
	W48					60	74	60	0.6	32	39	60	4.4
	W103	23	32	18	24.3	29	33	26	0.3	18	11	27	1.9
	W105	3	5	3	3.7	137	155	174	1.6	64	73	77	7.1
	W106	4	3	2	3.0	115	149	133	1.3	102	97	115	10.5
	W107	3	3	3	3.0	204	199	182	2.0	62	72	60	6.5
	W111	1	3	2	2.0	117	159	130	1.4	115	163	111	13.0
	W139	15	16	10	13.7	107	107		1.1	10	9		1.0
	W451					67	84	76	0.8	13	16	7	1.2
Bursectomised group	W101	17	23	22	20.7	22	23		0.2	7	12	9	0.9
	W104	17	21	24	20.7	140	167		1.5	18	22	28	2.3
	W109	14	21	15	16.7	126	136	146	1.4	20	34	24	2.6
	W110	19	25	22	22.0	124	158	151	1.4	60	56		5.8
	W121	14	11	13	12.7	42	46	44	0.4	46	49	54	5.0
	W130	21	28	19	22.7	99	100	108	1.0	102	116	117	11.2
	W131	15	17	17	16.3	74	88		0.8	100	125		11.3
	W132	8	23	14	15.0	220	267	235	2.4	99	87	85	9.0
	W140	16	18	11	15.0	124	170	140	1.4	14	22	18	1.8
	W145	17	17	19	17.7	52	65	87	0.7	15	9	6	1.0
Thymectomy group	W122	4	3	2	3.0	97	110	86	1.0	105	144	121	12.3
	W125	3	6	3	4.0	250	245	228	2.4	60	71	91	7.4
	W126	6	5	5	5.3	140	186	205	1.8	69	60	52	6.0
	W147	3	0	4	2.3	191	200	218	2.0	46	29	57	4.4
	W151	0	1	1	0.7	100	195	100	1.3	162	149	128	14.6
	W152	2	1	3	2.0	86	101	86	0.9	126	91	106	10.8
	W153	2	2	2	2.0	157	198	163	1.7	118	116	103	11.2
	W156	1	2	1	1.3	83	88	82	0.8	166	195	159	17.3
	W157	1	0	1	0.7	154	139	149	1.5	120	135	136	13.0
	W160	3	2	1	2.0	189	178	176	1.8	105	116	123	11.5
	W167	1	2	0	1.0	136	141	110	1.3	96	96	112	10.1
	W168	1	2	2	1.7	227	257	229	2.4	136	141	152	14.3
	W170	2	0	0	0.7	164	180	150	1.6	159	159	149	15.6
Protein Vaccinated group	G51	6	5	3	4.7	291	254		2.7	139	126		13.3
	G53	2	5	11	6.0	196	187		1.9	64	52	63	6.0
	G63	9	7	2	6.0	164	121	147	1.4	71	93	96	8.7
	G99	6	8	13	9.0	135	122	151	1.4	136	108	108	11.7
	P63	2	2	3	2.3	265	297	311	2.9	74	57	73	6.8
	W45	10	2	15	9.0	136	140		1.4	137	139		13.8
	V2J									47	49	39	4.5
	V2K									63	72	68	6.8
	V2L									28	32	26	2.9
	V2M									37	26	27	3.0
	V2N									89	72	76	7.9
	V2O									82	69	77	7.6
	V2P									39	26	32	3.2
	V2Q									41	54	39	4.5
	V2S									34	43	49	4.2

Table 86. *Cell counts for individual ducks.*

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