Verification of a novel quantitative SARS-CoV-2 IgG immunomethod, and its use in the evaluation of the immune response following vaccination in healthcare workers

A thesis submitted to the University of Manchester for the degree of

Doctor of Clinical Sciences

in the Faculty of Biology, Medicine and Health

2022

Laura E. Cook

School of Health Sciences

Table of Contents

Word Count: 24,837 words

Abstract

Introduction: The novel quantitative SARS-CoV-2 anti-spike IgG method from Abbott was fully evaluated in terms of imprecision, Limit of Detection, Limit of Quantitation, linearity, sensitivity, and specificity. The method was then used in the CALM (COVID-19 antibody longitudinal monitoring) observational cohort study performed at the Norfolk and Norwich University Hospital, which aimed to evaluate the immune response to SARS-CoV-2 vaccination in a real world setting.

Methodology: The Abbott SARS-CoV-2 anti-spike IgG method is an automated two-step chemiluminescent microparticle immunomethod used for the quantitative determination of antibodies to the receptor binding domain of the S1 subunit. 107 healthcare workers were recruited to the CALM study and their antibody concentrations measured at baseline and weekly following vaccination with the SARS-CoV-2 (Pfizer/BioNTech) vaccine.

Results: The SARS-CoV-2 anti-spike IgG method performed well, with excellent imprecision (≤3.9% in the positive range), sensitivity (98.3% [90.6 – 100.0]), and specificity (99.4% [97.1 – 100.0]). It was capable of measuring the immune response to natural infection from a range of SARS-CoV-2 strains, and to vaccination. The CALM study showed 98% of participants developed an antibody response following dose one of the vaccine, and 100% following dose two. For individuals responding to dose one, antibody concentrations peaked three weeks following vaccination, with concentrations remaining detectable ten weeks later prior to the second dose. Antibodies then remained detectable in all participants for six months following both doses. Spearman's correlation showed age had a significant effect on peak SARS-CoV-2 anti-spike IgG concentrations (p=0.015), with participants under 40 years of age having a greater antibody response to both doses of the SARS-CoV-2 (Pfizer/BioNTech) vaccine. Participants with a negative baseline result showed a 10-fold greater response to each vaccine dose than those with a positive baseline result (p=0.015).

Discussion: Use of a quantitative SARS-CoV-2 anti-spike IgG method allows antibody development, peak concentration, and antibody decline over time to be evaluated. The CALM study identified a potential benefit from measuring SARS-CoV-2 anti-spike IgG antibodies three weeks following dose one of the vaccine to offer individuals not responding, an earlier second dose. The results support the UK governments' decision to delay the dose interval, and allow more individuals to be vaccinated with one dose. They also suggest booster vaccines are not required until at least six months post second dose.

Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

Copyright Statement

i. The author of this thesis (including any appendices and/or schedules to this thesis) owns certain copyright or related rights in it (the "Copyright") and has given The University of Manchester certain rights to use such Copyright, including for administrative purposes.

ii. Copies of this thesis, either in full or in extracts and whether in hard or electronic copy, may be made only in accordance with the Copyright, Designs and Patents Act 1988 (as amended) and regulations issued under it or, where appropriate, in accordance with licensing agreements which the University has from time to time. This page must form part of any such copies made.

iii. The ownership of certain Copyright, patents, designs, trademarks and other intellectual property (the "Intellectual Property") and any reproductions of copyright works in the thesis, for example graphs and tables ("Reproductions"), which may be described in this thesis, may not be owned by the author and may be owned by third parties. Such Intellectual Property and Reproductions cannot and must not be made available for use without the prior written permission of the owner(s) of the relevant Intellectual Property and/or Reproductions. iv. Further information on the conditions under which disclosure, publication and commercialisation of this thesis, the Copyright and any Intellectual Property and/or Reproductions described in it may take place is available in the University IP Policy (see http://documents.manchester.ac.uk/DocuInfo.aspx?DocID=24420), in any relevant Thesis restriction declarations deposited in the University Library, The University Library's regulations (see http://www.library.manchester.ac.uk/about/regulations/) and in The University's policy on Presentation of Theses.

Acknowledgements

I would like to thank my current workplace supervisor, Dr Allison Chipchase, for her guidance and encouragement in the final stages of the HSST programme, as well as my Manchester supervisor, Dr Phil Macdonald for his advice over the years. I am also grateful to my previous workplace supervisors, Dr Frances Boa for encouraging me to apply for the HSST programme and believing I could do it, and Mrs Sophie Barnes.

I am extremely grateful to Professor Garry John and the rest of the CALM research team (Dr Isabelle Piec, Dr Emma English, Professor William Fraser, Dr Samir Dervisevic, and Marvin Berman) for the fantastic opportunity to undertake this research, the support in running the project, and our interesting weekly discussions. Special thanks go to Myra Del Rosario, and the rest of the Clinical Biochemistry team at the Norfolk and Norwich Hospital, for their support, and allowing me time on their routine analysers to complete the project.

I would finally like to thank my family, for their support throughout my career.

Chapter 1

Background to the SARS-CoV-2 pandemic, and the United Kingdom's testing and vaccination strategies

1.1 Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a novel coronavirus that causes COVID-19 (coronavirus disease 2019). It was first identified in Wuhan City, Hubei Province of China, during December 2019. Two years later, there have been over 263 million SARS-CoV-2 cases, and over 5.2 million deaths worldwide (Worldometer, 2021a). These figures may be underestimates, with the true number of SARS-CoV-2 cases and deaths possibly even higher, due to under-reporting, and under-testing in some countries. A study in March 2020 (Lau et al., 2021) suggested that France, Italy, the United States, Iran, and Spain had extremely high numbers of undetected SARS-CoV-2 cases, and that these differences were due to testing availability and capacity. *Figure 1.1* shows the rising number of SARS-CoV-2 cases and deaths during the first two years of the pandemic (Worldometer, 2021b).

Large numbers of SARS-CoV-2 cases and deaths are still being recorded each day, and the pandemic is far from over. The identification of SARS-CoV-2 resulted in global collaboration between industry, healthcare providers, and academia, and journals freely shared data to aid the speed at which innovations could occur. Two years following the first cases, there are a number of novel vaccines, treatments, and diagnostic tools available for current SARS-CoV-2 infection, and prior exposure. This project aims to verify a novel quantitative SARS-CoV-2 anti-spike IgG antibody immunomethod that can be used to evaluate the immune response to infection and vaccination. This first chapter details the structure and function of SARS-CoV-2, and the key events that have taken place since December 2019, including the testing and vaccination strategies undertaken in the United Kingdom (UK).

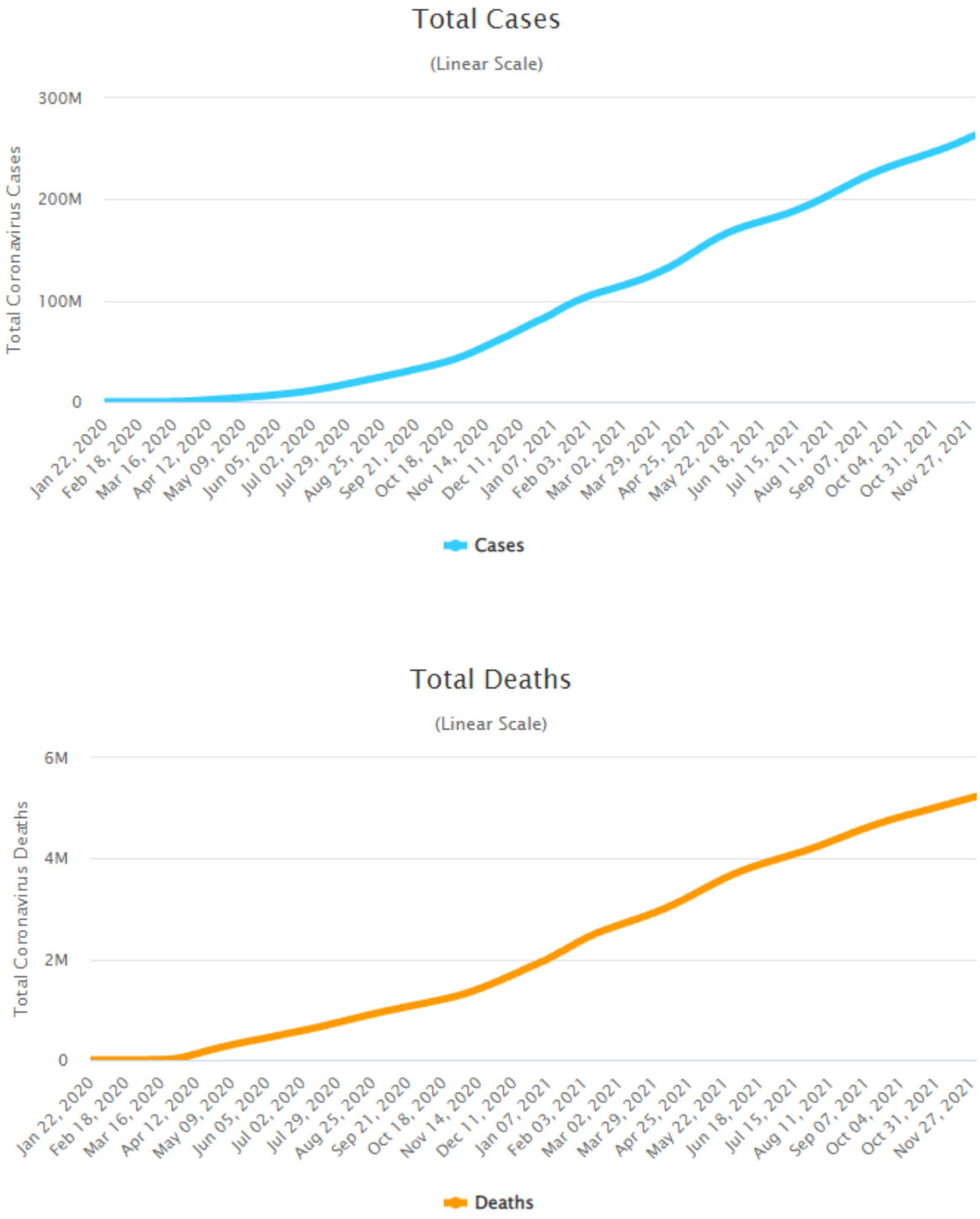


Figure 1.1: Total number of worldwide SARS-CoV-2 cases and deaths during the first two years of the pandemic (Source: Worldometer, 2021b).

1.2 Structure and function of SARS-CoV-2

Like all coronaviruses, SARS-CoV-2 (order *Nidovirales,* family *Coronaviridae,* subfamily *Coronavirinae*) is an enveloped virus with a positive-sense, single-stranded RNA genome (Schoeman and Fielding, 2019). Coronaviruses primarily infect birds and mammals, with impacts on the farming industry (Schoeman and Fielding, 2019). They can also infect humans, but usually cause mild illness (Hu et al., 2021). There have been two previous highly pathogenic coronaviruses that have emerged in humans with zoonotic origin; severe acute respiratory syndrome coronavirus (SARS) in 2002, which led to 774 deaths worldwide, and Middle East respiratory syndrome coronavirus (MERS) in 2012, which led to 858 deaths worldwide (Drosten et al., 2003, Bermingham et al., 2012, Mahase, 2020a).

Phylogenic analysis showed SARS-CoV-2 is closely related to the beta coronaviruses, and early viral sequencing showed the virus shared 80% sequence identity with SARS, 50% sequence identity with MERS, and 96% sequence identity with a bat coronavirus (96%) (Mohamadian et al., 2021, Lu et al., 2020a, Zhou et al., 2020). SARS, MERS, and SARS-CoV-2 all lead to respiratory distress syndromes and are examples of how lethal coronaviruses can be when they cross the species barrier and infect humans (Schoeman and Fielding, 2019). The exact origin of SARS-CoV-2 is unknown, but one theory is it evolved in Rhinolophid bats before passing to humans, likely via an intermediate animal species yet to be identified (Banerjee et al., 2021).

SARS-CoV-2 is comprised of non-structural proteins (NSPs) and structural proteins (SPs) *(Figure 1.2)*. NSPs are essential for virus replication and assembly processes, and SPs are essential for budding the virus particles released from host cells (Mohamadian et al., 2021). The four SPs are envelope (E), membrane (M), spike (S), and nucleocapsid (N). The N protein is localised inside the virus, in the endoplasmic reticulum-Golgi region, and is structurally bound to nuclei acid material (Astuti and Ysrafil, 2020). It is involved in RNA replication, virion formation, and immune evasion (Mohamadian et al., 2021). The E, M, and S proteins are all located on the surface of the virus. The M protein promotes the assembly and budding of viral particles via interaction with the N protein (Mohamadian et al., 2021). The E protein is a small integral membrane protein. It is involved in life cycle processes such as

assembly, budding, envelope formation, and pathogenesis, and it also interacts with other coronavirus and host cell proteins (Schoeman and Fielding, 2019). The S protein is a transmembrane protein that facilitates host infection. It is composed of the receptor binding (S1), and the cell membrane fusion (S2) subunits.

SARS-CoV-2 has been shown to enter host respiratory tract cells via the angiotensin-converting enzyme 2 (ACE2) receptor, and is primarily transmitted through respiratory droplets (sneeze, cough, and other secretions of infected people) (Zhou et al., 2020). SARS-CoV-2 binds to the ACE2 receptors via the receptor binding domain (RBD) in the S1 subunit of the S protein. The RBD is the most complex component of the genome (Mohamadian et al., 2021). Six RBD amino acids are required to bind to the ACE2 receptor (Mohamadian et al., 2021). Binding of the RBD to the ACE2 receptor results in membrane fusion and host cell infection (Mohamadian et al., 2021). ACE2 receptors are an important component of the renin-angiotensin-aldosterone system, which regulates blood pressure through the maintenance of fluid and electrolyte balance. As well as the respiratory tract, ACE2 receptors are also present in the kidneys, gastrointestinal tract, heart, liver, and blood vessels (Mohamadian et al., 2021).

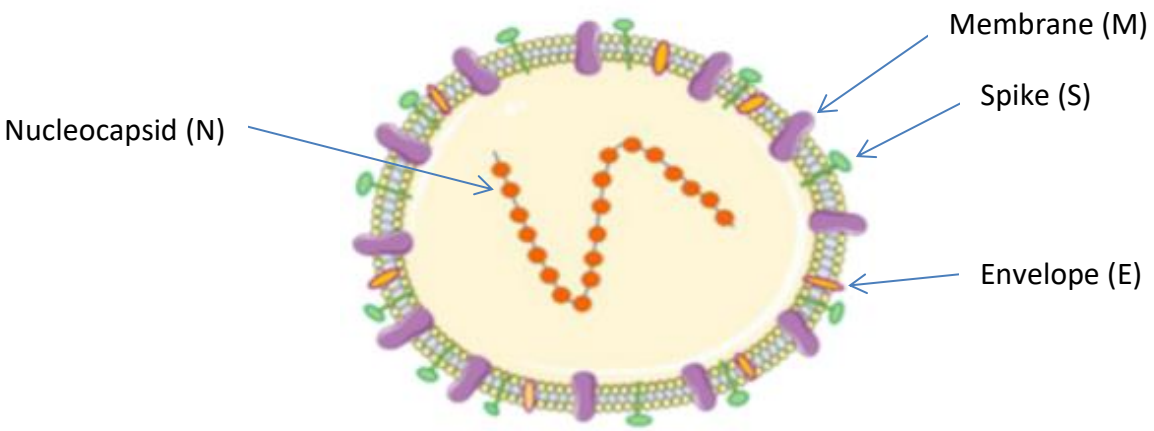


Figure 1.2: Structure of SARS-CoV-2 and the location of the four main structural proteins; Membrane, Envelope, Nucleocapsid, and Spike (Source adapted from: Mohamadian et al., 2021).

1.3 The SARS-CoV-2 pandemic

On the 31st December 2019, a cluster of 27 cases of pneumonia of unknown etiology were reported to the World Health Organisation (WHO) (World Health Organisation, 2020a). A diagnosis of pneumonia of unknown etiology is based on clinical characteristics: a fever (≥38°C), low/normal white cell count or low lymphocyte count, chest imaging (radiographic evidence of pneumonia), and no symptomatic improvement after antimicrobial treatment for three to five days following standard clinical guidelines (Li et al., 2020). The initial cases in Wuhan were identified through a surveillance program conducted by the Chinese Center for Disease Control and Prevention for pneumonia of unknown etiology, which was established in 2004 following the SARS outbreak (Xiang et al., 2013).

On 7th January 2020, a laboratory in Wuhan isolated a novel coronavirus in a patient admitted to the Central Hospital of Wuhan (Wu et al., 2020). The patient was a 41 year old male who worked at the local seafood market with no previous medical history of note. Bronchoalveolar lavage fluid was collected from the patient and deep metatranscriptomic sequencing performed. Its whole genome sequence was assigned GenBank accession number MN908947. At the same time two independent reports by Zhou et al (2020) and Zhu et al (2020) were published with similar findings. The Wuhan Institute of Virology obtained full length genome sequences from five patients admitted to the intensive care unit of Wuhan Jin Yin-Tan Hospital (Zhou et al., 2020). The sequences were 99.9% identical to each other and were reported to GISAID (Global initiative on sharing all influenza data). The report by Zhu et al (2020) identified complete genomes for three hospitalised patients. Their primer sets and standard operating procedures were shared with the WHO to aid surveillance and detection of SARS-CoV-2 globally (Zhu et al., 2020).

The clinical features of 41 initial SARS-CoV-2 patients were reported on 24th January 2020 by Huang et al. The clinical symptoms associated with the novel coronavirus were fever, dry cough, chest tightness, dyspnoea, headache, and pneumonia (Zhou et al., 2020, Wu et al., 2020). Disease onset was then reported to result in progressive respiratory failure due to alveolar damage, and even death (Zhou et al., 2020). These features are similar to other beta-coronaviruses, although 20 – 25% of patients with MERS also reported diarrhoea,

which was not a symptom of SARS-CoV-2 infection (Assiri et al., 2013). Later reports added a loss of taste and smell to the list of main clinical symptoms associated with SARS-CoV-2 (Mullol et al., 2020). The earliest case reported by Huang et al (2020) developed symptoms on 1st December 2019, but it is unclear if this was the first SARS-CoV-2 patient.

The majority of early SARS-CoV-2 cases (55%) were found to have links to the Huanan wholesale fish and live animal market in Wuhan (Li et al, 2020, Zhu et al., 2020). Following an epidemiological alert released by the local health authority on 31st December 2019, the identified market was closed on 1st January 2020 for sanitation and disinfection (Huang et al., 2020, World Health Organisation, 2020a). The large number of bat coronaviruses, together with the culture of eating wild animals in China was previously described as a 'time bomb' (Cheng et al., 2007). Following the identification of SARS-CoV-2, China has now instigated a ban of all trade and consumption of terrestrial wild animals, which should reduce the likelihood of future pandemics originating in this way (Koh et al., 2021).

The early SARS-CoV-2 reports described no large-scale human-to-human transmission, but one of the original papers stated the disease had progressed to be transmitted by human-to-human contact (Zhou et al., 2020). Another report by Huang et al (2020) described a patient whose household contact presented five days later, without any direct links to the seafood market. The first reported case of human-to-human transmission was published in February 2020, which described a family cluster of six individuals from Hong Kong, five of which had travelled to Wuhan, and a sixth individual who hadn't visited China, but still contracted the virus (Chan et al., 2020). The five family members returning from Wuhan had no links to the seafood market, but two individuals had visited a hospital during their visit, which was thought to be the source of infection. An epidemiology study of the first 425 confirmed SARS-CoV-2 cases in Wuhan (Li et al., 2020) found that despite the majority of early cases having links to the seafood market, with zoonotic or environmental exposures as possible infection routes, human to human transmission had also been occurring, and the epidemic was growing.

By mid-January 2020, China had initiated containment measures to try to control the virus. Flights and trains were suspended, and roads blocked to stop movement in and out of

Wuhan, and 15 other cities in Hubei province (Cyranoski, 2020). People were told to stay at home and only venture out for food or medical assistance (Cyranoski, 2020). These restrictions helped to control cases in China, but they were too late to stop the virus spreading to other countries.

The first cases of SARS-CoV-2 in the UK were reported in January 2020. They were a 50 year old female, who had returned from Hubei province in China, and developed symptoms of fever and malaise on 26th January 2020, and a probable transmission to a close household contact (Lillie et al., 2020). Both cases had mild illness and made a full recovery. The first death in the UK from SARS-CoV-2 was reported on 6th March 2020 (Mahase, 2020b). The patient was described as 'older', with underlying health conditions, and they contracted the virus from within the UK (Mahase, 2020b).

On 30th January 2020, as SARS-CoV-2 cases continued to increase across the globe, the WHO declared a Public Health Emergency of International Concern (PHEIC) at an International Health Regulations emergency committee meeting (World Health Organisation, 2020b). The report stated:

'The Committee believes that it is still possible to interrupt virus spread, provided that countries put in place strong measures to detect disease early, isolate and treat cases, trace contacts, and promote social distancing measures commensurate with the risk' (World Health Organisation, 2020b).

The meeting on the 30th January 2020 was the second International Health Regulations emergency committee meeting to take place; the first had been the week prior, on 23rd January 2020, when there were 557 confirmed SARS-CoV-2 cases and 17 deaths, but the committee disagreed as to whether SARS-CoV-2 constituted a PHEIC (World Health Organisation, 2020c). At the time of the second meeting, one week later, there had been a significant increase in cases, with 7,711 confirmed and 12,167 suspected in China, and 170 reported deaths from SARS-CoV-2 (World Health Organisation, 2020b).

During these initial months, the virus was described in a number of different ways (e.g. unidentified viral pneumonia, coronavirus disease 2019, nCoV-2019, 2019-nCoV, WH-Human 1 coronavirus (WHCV), novel coronavirus infected pneumonia (NCIP)). In February 2020, to avoid any confusion the Coronavirus Study Group (CSG) of the International Committee on Taxonomy of Viruses used phylogeny, taxonomy and established practice to designate the virus as SARS-CoV-2 (Coronaviridae Study Group of the International Committee on Taxonomy of Viruses, 2020).

Cases of SARS-CoV-2 continued to increase exponentially, and the SARS-CoV-2 pandemic was formally declared by the WHO on 11th March 2020, with calls for urgent and aggressive action (World Health Organisation, 2020d). A pandemic is defined as 'an epidemic occurring worldwide, or over a very wide area, crossing international boundaries and usually affecting a large number of people' (Last, 2001). In the 20th century, there were three WHO declared pandemics; The Spanish Flu (A(H1N1) virus) in 1918 – 1919, estimated to have resulted in 20-50 million deaths, The Asian Flu (A(H2N2) virus) in 1957 – 1958, and the Hong Kong Flu (A(H3N2) virus) in 1968, both estimated to have resulted in 1 – 4 million deaths (World Health Organisation, 2020e). The first pandemic in the 21st century was also caused by an influenza, (A(H1N1)) in 2009 – 2010, and resulted in 100,000 – 400,000 deaths (World Health Organisation, 2020e).

SARS-CoV-2 is the first pandemic to be classified by the WHO that is caused by a coronavirus, but the increasing number of cases in countries outside China could not be ignored. The SARS outbreak in 2002 – 2004 was not declared a pandemic by the WHO; it was contained within eight months and resulted in 774 deaths worldwide (Lam et al., 2003). In contrast, eight months following the first cases of SARS-CoV-2, on 4th April 2020, there had been over one million confirmed cases, and 70,079 deaths (Worldometer, 2021b). The mortality rate for MERS was 35.5%, compared with 9.6% for SARS, and a reported 6.8% for SARS-CoV-2 (Lu et al., 2020b).

There are a number of factors that may have contributed to the success of SARS-CoV-2. In 2020, China was much more connected to the rest of the world than in 2003 when SARS emerged, enabling SARS-CoV-2 to spread rapidly across the globe (Keshta et al., 2021).

SARS-CoV-2 also emerged during the Chinese New Year celebrations, when many individuals had travelled to Wuhan to visit family and friends. The other important factor is that SARS-CoV-2 is most infectious during the pre-symptomatic and early symptomatic phase of illness, allowing infectious people to socialise and further spread the virus in the community (Keshta et al., 2021). In comparison, SARS was most infectious during the second week of illness, when patients were already likely to be isolating (Keshta et al., 2021).

At the time the SARS-CoV-2 pandemic was declared on 11th March 2020, there had been 118,000 reported cases in 114 countries and 4,291 people had lost their lives (World Health Organisation 2020d). In the UK at this time, there had been 419 reported cases and seven deaths (Worldometer, 2021c). Despite the declaration of a pandemic, and the number of SARS-CoV-2 cases, life in the UK remained unchanged for most of the population, with no formal restrictions in place.

1.4 The UK's response to the SARS-CoV-2 pandemic

A number of measures, including social distancing, self-isolation, and contact tracing, were implemented across the globe, to try to limit the spread of the virus. Each country implemented its own response strategy, varying from zero tolerance to herd immunity. In South Korea as soon as SARS-CoV-2 cases started to emerge a rapidly expanding testing strategy was implemented, with a vigorous contact tracing programme quickly developed (Kang et al., 2020). The public in South Korea were compliant with mask wearing, and the aggressive response allowed the first wave of SARS-CoV-2 cases to be controlled quickly (Kang et al., 2020). This zero tolerance strategy may have been influenced by South Korea's previous experience with MERS in 2015 (Kang et al., 2020). From the start of the pandemic, the Public Health Agency in Sweden embarked on a herd immunity approach, allowing community transmission to occur, with limiting gatherings to fifty people the only restriction implemented (Claeson and Hanson, 2020). This approach allowed SARS-CoV-2 cases to soar in Sweden compared to other Nordic countries that had adopted stricter restrictions (Claeson and Hanson, 2020).

The first UK government advice was from the Foreign Secretary, Dominic Raab, who gave a statement advising against all non-essential travel from 17th March 2020, due to countries closing borders and imposing restrictive measures in response to the pandemic (Foreign & Commonwealth Office, 2020). The first UK national lockdown was announced on 23rd March 2020, nearly two weeks after the pandemic had been declared by the WHO, with the Prime Minister, Boris Johnson, ordering people to stay at home (Prime Minister's Office, 2020a). At this point, there had been 5,145 SARS-CoV-2 cases, and 264 deaths reported in the UK (Worldometer, 2021b). With no effective treatment or vaccinations available at this time, social distancing was enforced to reduce transmission, and prevent hospitals from becoming overwhelmed. The lockdown remained in place for twelve weeks and was lifted gradually, with non-essential shops opening on 15th June 2020, and social distancing rules relaxed on 23rd June 2020. Pubs, restaurants, and hairdressers re-opened on 4th July 2020, and indoor theatres opened on 14th August 2020, almost five months after the lockdown began. Mask wearing was made mandatory in the UK on 24th July 2020, four months after the pandemic had been declared (Department of Health and Social Care, 2020a).

At this time in the pandemic, as soon as restrictions were eased, case numbers began to rise, and hospitals again filled with SARS-CoV-2 patients. An inevitable second UK national lockdown came into force on 5th November 2020 (Prime Minister's Office, 2020b). This lasted for four weeks, and then the country re-entered a three tier system of restrictions. A fourth stricter tier of restrictions advising people to stay at home was introduced on 19th December 2020, to further try to reduce transmission (Prime Minister's Office, 2020c). However, as SARS-CoV-2 cases were still rising, and hospitals were overwhelmed, a third national UK lockdown was unavoidable, which was announced on 6th January 2021 (Prime Minister's Office, 2020d). As with the other lockdowns, restrictions were lifted gradually, with all coronavirus restrictions officially relaxed after six months, on 19th July 2021 (Prime Minister's Office, 2020e). This was the first time some businesses (e.g. nightclubs) had been legally allowed to open since the first lockdown began nearly 16 months earlier.

The relaxation of restrictions following the third lockdown saw SARS-CoV-2 cases again begin to rise. The difference this time was that increases in hospital admissions and deaths from SARS-CoV-2 did not follow. *Figure 1.3* shows the number of reported cases and deaths

from SARS-CoV-2 in the UK during the first two years of the pandemic (Worldometer, 2021c). Note the number of cases reported at the beginning of the pandemic is not representative of the true number of cases in the UK at this time, due to a lack of testing for the general population. The number of deaths in the UK from SARS-Cov-2 has remained relatively stable since July 2021, despite an increase in the number of cases following the relaxation of the restrictions. This can be explained by the UK vaccination programme that began in December 2020 (Public Health England, 2021). By 19th July 2021 when all restrictions were relaxed, 46,349,709 people (68% of the population) had been vaccinated (UK Health Security Agency, 2021a). This high vaccine uptake rate broke the link between SARS-CoV-2 cases and hospital admissions and allowed the UK population to live with the virus for the first time.

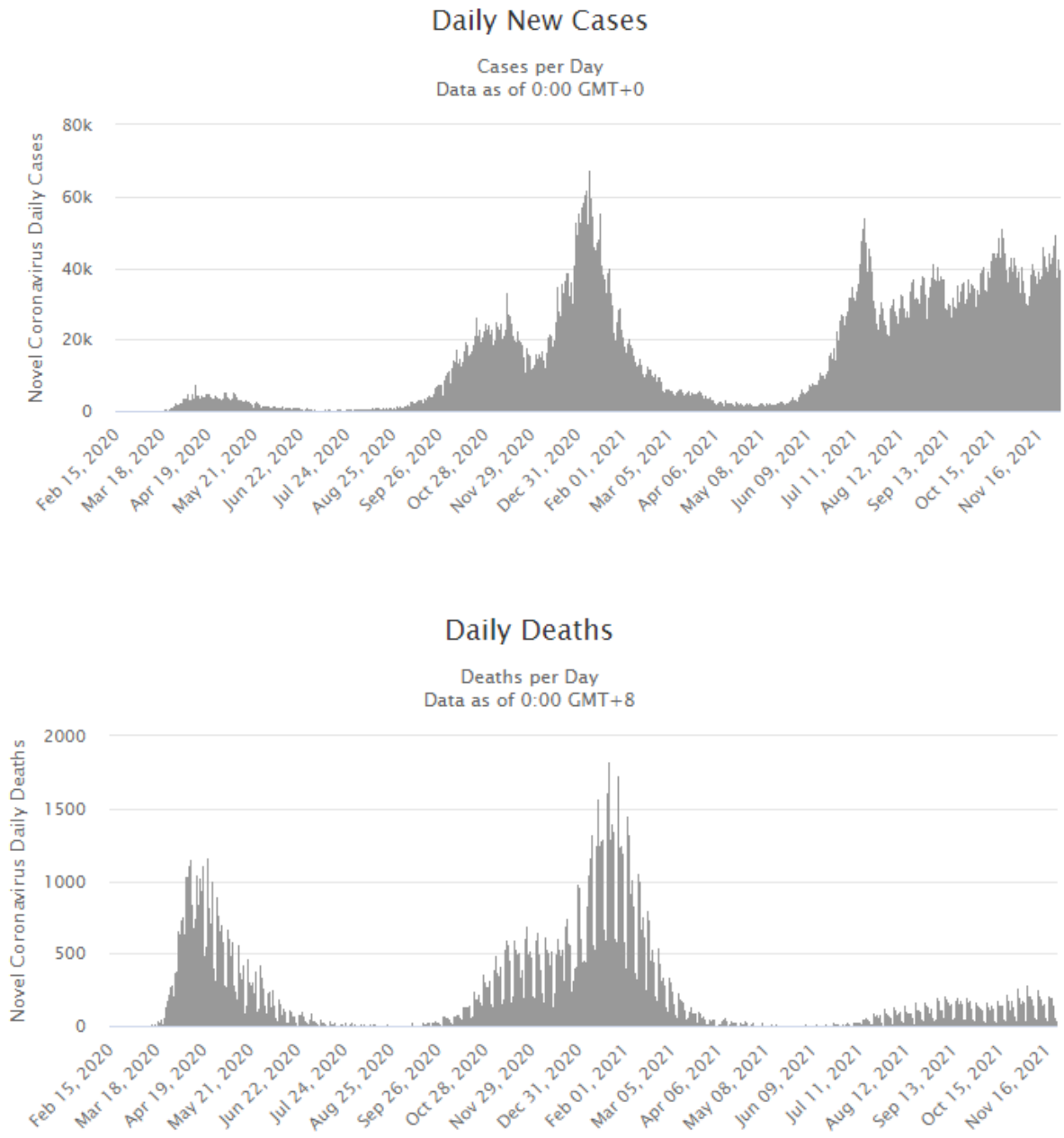


Figure 1.3: Number of daily SARS-CoV-2 new cases and deaths in the UK during the first two years of the pandemic (Source: Worldometer, 2021c).

1.5 SARS-CoV-2 vaccination

The development of SARS-CoV-2 vaccinations gave countries a critical tool in the fight against the virus, and at the time of writing vaccines are being used worldwide to achieve herd immunity. Vaccines work by triggering an immune response. The antibodies developed against the vaccine can then be used to help protect the body from natural infection from the virus. Without vaccinations, as soon as restrictions were eased, the number of cases and deaths began to rise, and restrictions were required to control the virus.

The first SARS-CoV-2 mRNA vaccine to be approved for emergency use by the WHO was the BNT162b2 (Pfizer Inc. [New York] and BioNTech SE [Mainz, Germany]) on 31st December 2020 (World Health Organisation, 2020f). The BNT162b2 vaccine is a nucleoside-modified mRNA vaccine that encodes a pre-fusion stabilised membrane anchored SARS-CoV-2 full length spike protein (Polack et al.,2020). The genetically engineered mRNA provides instructions for cells to produce the S protein, causing the body to produce antibodies against the S protein that can be used to fight SARS-CoV-2 infection (Coccia, 2021). A single 30 μg dose of diluted vaccine solution is administered intramuscularly followed by a second dose 21 days later (Pfizer-BioNTech, 2020).

The Medicines and Healthcare products Regulatory Agency (MHRA) gave temporary authorisation to supply specific batches of the Pfizer/BioNTech vaccine on the 2nd December 2020 (Medicines & healthcare products Regulatory Agency, 2020), and on 8th December 2020, the first individuals in the UK began receiving their SARS-CoV-2 vaccines (Public Health England, 2021). The vaccination programme deviated from the recommended Pfizer-BioNTech protocol by delaying the interval between first and second doses to twelve weeks rather than three (Department of Health and Social Care, 2021a). This decision was based on the high level of protection from SARS-CoV-2 infection afforded by one dose. Vaccinating a larger proportion of the population with one dose aimed to maximise the benefit from limited vaccine supplies, and minimise hospital admissions and deaths. Models considered the optimal targeting of vaccination within the UK, and showed vaccinating older age groups, and the most vulnerable first, was optimal in reducing deaths and quality adjusted life year (QALY) losses (Moore et al., 2021).

A second SARS-CoV-2 vaccine, from Oxford-AstraZeneca, was also recommended in the vaccination roll out, again with a twelve week interval between doses. Longer dose intervals (two to three months) had been shown to be effective for the Oxford-AstraZeneca vaccine, and even to improve vaccine efficacy (Voysey et al., 2021). The Oxford-AstraZeneca vaccine is a vector vaccine, where genetic material from SARS-CoV-2 is placed in a modified version of a different virus. When the viral vector enters cells, the genetic material is used to make copies of the S protein, which causes the immune system to produce antibodies ready to fight future SARS-Cov-2 infections (Coccia, 2021).

The first phase of the UK vaccination programme aimed to directly prevent mortality and to support the NHS and social care system. As such, the first priority grouping for vaccination was for older adult care home residents, and their carers. The second priority grouping included those over 80 years of age, and frontline health and social care workers. The subsequent groups were based on decreasing age categories (Department of Health and Social Care, 2021b).

The success of the UK vaccination programme allowed the UK population to live without any formal SARS-CoV-2 restrictions in place. Countries with lower vaccine uptakes rates needed to re-introduce restrictions to prevent further waves of rising hospital admissions. These countries are actively promoting vaccination, with some countries suggesting vaccines will be made mandatory in 2022. However, antibody concentrations were shown to wane after vaccination, and on 14th September 2021, the Joint Committee on Vaccination and Immunisation (JCVI) released a statement advising third booster vaccines were to be rolled out, at least six months after the second vaccine dose was received, to ensure immunity was sustained over winter (Department of Health and Social Care, 2021c). Ensuring the majority of the population is vaccinated will also reduce the risk of more lethal SARS-Cov-2 variants emerging.

1.6 SARS-CoV-2 Variants

The SARS-CoV-2 genome is large and of a low stability, making it prone to mutations (Khateeb et al., 2021). By May 2021, 3,913 variant genomes had been identified since the

original strain of the virus was reported in December 2019 (Khateeb et al., 2021). Not all mutations lead to changes in major proteins, and most have little impact on the structure and function of the virus. Some mutations however, can change how quickly the virus spreads, disease severity, and the performance of vaccines, therapeutic medicines, diagnostic tools, and other public health and social measures (World Health Organisation, 2021).

The SARS-CoV-2 major variant of concerns (VOCs) have shared mutations in the S protein, mostly in the S1 unit, resulting in higher transmissibility rates, affected viral virulence, and worse clinical outcomes (Khateeb et al., 2021). The first reported VOCs with major global health impacts were Alpha (B.1.1.7, UK variant), Beta (B.1.351, South Africa variant), Gamma (P.1, Brazil variant), and Delta (B.1.617.2, India variant) (Khateeb et al., 2021). The S1 mutations significantly increase the ACE2 receptor binding affinity and reduce neutralising antibody binding (Khateeb et al., 2021). The Alpha variant spread rapidly throughout the UK between November 2020 and January 2021 (Volz et al., 2021). It was shown to have a substantial transmission advantage over other lineages, with an approximate 50% increase in transmissibility, especially in younger age groups (Volz et al., 2021). Since August 2021, the Delta variant has been the most dominant strain in the UK, which is 60% more transmissible than the Alpha variant (Shiehzadegan et al., 2021).

On 26th November 2021, a new variant, termed Omicron (B.1.1.529) was reported as a VOC (Callaway, 2021). The Omicron variant is heavily mutated, with 30 changes to the S protein, and scientists are urgently trying to understand whether this variant can evade immune responses trigged by vaccines, and whether it causes a more severe disease (Callaway, 2021). In South Africa, the Omicron variant has resulted in a sharp increase in cases, which has led to countries closing borders, and implementing restrictions to prevent the spread of this new variant. The UK responded on 30th November by re-introducing face masks for public transport and retail spaces, and the requirement for PCR testing for all travellers entering the UK (Department of Health and Social Care, 2021d).

As the pandemic progresses, new variants of SARS-CoV-2 will continue to emerge. Omer et al (2021) stated a low vaccine uptake may promote the emergence of variants and prolong

the social and economic repercussions of the pandemic. The UK government is currently aiming to offer third SARS-CoV-2 booster vaccines to all adults by January 2022, but there are still many countries with poor vaccine uptake rates. Additional booster vaccinations may be required to provide protection against any potential future variants capable of evading current vaccine immunity (Shiehzadegan et al., 2021). Recent studies show the SARS-CoV-2 (Pfizer/BioNTech) mRNA vaccine induces neutralising antibodies against the wild type virus, as well as some variants, such as Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Kappa (B.1.617.1) (Naaber et al., 2021, Liu et al., 2021). Research is currently ongoing to discover how effective vaccines are against the new Omicron (B.1.1.529) variant.

1.7 SARS-CoV-2 testing strategy

As well as vaccinations, the ability to quickly identify infected individuals through testing and tracing their contacts, is critical in the fight against SARS-CoV-2. Each country developed its own SARS-CoV-2 testing strategy in response to the pandemic. Taiwan was the first country outside China to respond to the threat of the virus, with the introduction of airport screening in December 2019 to detect pneumonia-like or flu symptoms in passengers arriving from China (Allam, 2020). The UK did not introduce SARS-CoV-2 testing for international travellers until 15th December 2020 (Department for Transport et al., 2020).

The SARS-CoV-2 genetic sequence was identified in early January 2020, and soon after the Wuhan Institute of Virology developed a qPCR based SARS-CoV-2 detection method based on the RBD of the S gene (Zhou et al., 2020). As the genome was freely shared, many other laboratories and companies began developing their own methods. In the UK, Public Health England (PHE) developed a highly sensitive test to detect SARS-CoV-2 viral RNA in early January 2020, which was rolled out to their regional laboratories (NHS, 2020). At this time, PHE were able to test approximately 1,500 samples a day, with tests reserved for symptomatic individuals with a recent travel history to China, and hospital in-patients showing symptoms (NHS, 2020). This meant many symptomatic individuals in the community were not tested or identified at this time.

On 11th March 2020, the NHS announced it was undertaking a significant expansion of SARS-CoV-2 testing to help the health service carry out 10,000 tests daily (NHS, 2020). The roll out of testing to additional labs was phased in slowly, with an extra ten NHS microbiology services included in the first phase, followed by twenty-nine NHS pathology networks (NHS, 2020). The speed of the roll-out may have been related to reagent supply issues, machine availability, and staff training requirements. However, the centralisation of testing in the early stage of the pandemic dramatically limited the number of tests that could be processed, as well as delayed results due to transporting samples.

The importance of testing has been emphasized by public health experts throughout the pandemic (Giri et al., 2021). On 21st March 2020, the WHO released 'Laboratory testing strategy recommendations for COVID-19, interim guidance', which acknowledged the shortages of molecular testing reagents globally for COVID-19 testing (World Health Organisation, 2020g). The document stated good laboratory practices that produce accurate results were key to assuring laboratory testing benefits the public health response.

The Department of Health and Social Care (DHSC) then released 'Coronavirus (COVID-19) Scaling up our testing programmes' on 4th April 2020 (Department of Health and Social Care, 2020b). The document praised PHE for their work to date with testing and recognised the UK was undertaking the highest number of tests in Europe, after Italy, Spain, and Germany. It also acknowledged the lack of a major diagnostics manufacturing industry in the UK, and the huge international demand for testing materials. The document set out their ultimate goal of offering a test to anyone that needs one. The strategy was to have a phased approach of offering tests to NHS workers and their families, then other critical key workers, and finally expanding to the wider community over time.

Testing in the UK rose from 10,000 samples per day at the end of March 2020, to 25,000 samples per day by the end of April 2020, as aimed for by DHSC (Department of Health and Social Care, 2020b). By November 2021, the UK was processing over one million samples a day for anyone who requested a SARS-CoV-2 test (UK Health Security Agency, 2021a).

There are several SARS-CoV-2 testing methods available. Detecting SARS-CoV-2 RNA is the standard approach for diagnosing SARS-CoV-2, with reverse transcription polymerase chain reaction (RT-PCR) considered the gold standard for detecting SARS-CoV-2 nucleic acid in respiratory samples (Cheng et al., 2020). Reverse transcriptase-loop-mediated isothermal amplification (RT-LAMP) is a cheaper alternative that has recently been used to detect viral RNA (Keaney et al., 2021). Testing wastewater for direct detection of viral RNA was also suggested as a possibility for community surveillance of SARS-CoV-2 (Ahmed et al., 2020).

Rapid antigen tests can also be used to detect viral antigens to SARS-CoV-2. These lateral flow tests are inexpensive, easy to use, and can quickly screen a population (Keaney et al., 2021). However, they show poor sensitivity and have a high false positive rate; hence positive SARS-CoV-2 lateral flow tests require an RT-PCR test for confirmation.

The other type of SARS-CoV-2 testing currently available is serology testing. SARS-CoV-2 antibody methods are not as specific for SARS-CoV-2 as tests that recognise RNA sequences, but there is a need for reliable and rapid serological diagnostic tests to screen asymptomatic individuals (Giri et al., 2021). Zhang et al (2020) showed serology tests improved positive detection rates and suggested they could have a use in future.

Figure 1.4 shows the time kinetics of the viral load and antibody response to SARS-CoV-2 infection. All SARS-CoV-2 detection methods are unsuitable during the initial five day incubation period (Ghaffari et al., 2020). Viral RNA and antigen methods can then be used in the initial weeks of infection when the viral load is sufficiently high. In the convalescence stage of infection, antibody methods are required to detect prior infection (Ghaffari et al., 2020).

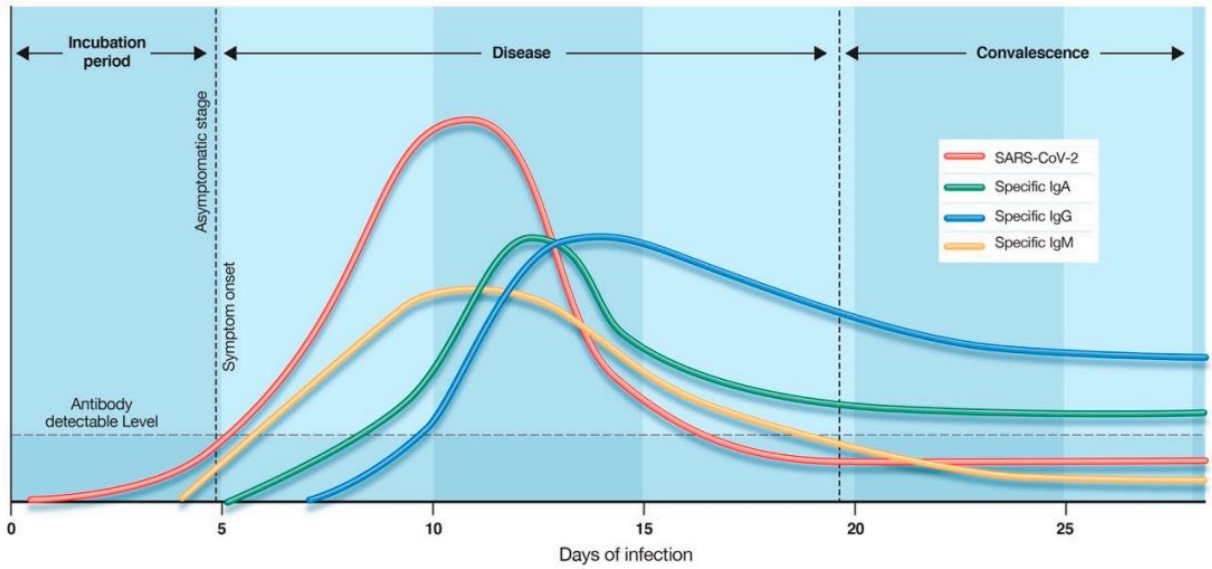


Figure 1.4: Time kinetics of viral load and antibody response in SARS-CoV-2 infection. (Source: Ghaffari et al., 2020).

1.8 SARS-CoV-2 antibody testing

SARS-CoV-2 antibody methods play an important role in research and surveillance but are not recommended by the WHO for case detection (World Health Organization, 2020g). Antibodies can be measured by enzyme linked immunosorbent assays (ELISA), or chemiluminescence immunoassays (CIA). Immunossays use the antigen-antibody reaction to detect and quantify the analyte. Immunomethods are cheap, automatable and can offer high throughput sample processing. However, they can suffer from cross-reactivity e.g. from non-specific IgM binding.

There are five types of antibodies (IgM, IgG, IgA, IgE, IgD). Each antibody is produced by the B cells, and is composed of two identical heavy chains and two identical light chains. IgM is produced in the primary response to infection, and concentrations decline soon after infection (Watson et al., 2020). IgG and IgA are produced in the secondary immune response, with concentrations persisting to reflect longer term immunity (Watson et al., 2020). IgG is generally more specific for an antigen and affinity increases with continued exposure to the antigen (Janeway, 2001). IgG antibodies can be measured post vaccination to evaluate the quality of the immune response after vaccination (e.g. polio, pneumococcal polysaccharide, diphtheria toxoid, and tetanus toxoid), or to evaluate the production of antibodies following infection (e.g. measles and varicella-zoster) (Justiz Vaillant et al., 2021).

There are a large number of commercially available serology methods for measurement of SARS-CoV-2 IgM, IgA, and IgG concentrations. The methods available vary in terms of throughput capability, batching ability, required infrastructure, analytical performance, and turnaround times (Giri et al., 2021). There is also a lack of standardisation or harmonisation between methods, with results reported in different units, different positivity cut-offs, and methods detecting different parts of the virus. Some serology methods measure IgG only (Abbott method) and others measure IgG and IgM combined (Roche method). Some methods are based on the spike protein (Abbott (quantitative IgG) method), whereas others are based on the nucelocapsid protein (Abbott (qualitative IgG) method). SARS-CoV-2 antibodies can also be measured by lateral flow tests using finger prick blood samples.

On 25th May 2020, pathology laboratories received letters from NHS England (NHSE) and NHS Improvement (NHSI) asking them to ramp up capacity and to offer antibody testing at short notice for all NHS staff (NHS England and NHS Improvement, 2020). The antibody results were reported to DHSC, with the aim of providing information on the prevalence of SARS-CoV-2 in different regions of the country, allowing a better understanding of how the virus was spreading. The results do not appear to have been published. The letter from NHSE/NHSI acknowledged the science behind antibody testing was uncertain, and that at the time there was no clear evidence to show patients cannot be re-infected, or pass the virus on to others. The letter also stated they were waiting for advice from DHSC as to whether this would be a rolling programme.

In response to this demand from NHSE/NHSI, a letter was published in the BMJ on 24th June 2020 expressing concerns over establishing SARS-CoV-2 antibody testing so quickly in England (Andersson et al., 2020). The authors described a lack of clinical indication for the test with no evidence a positive antibody result infers immunity, the waste of resources in performing unnecessary tests, and the lack of performance verifications that are usually completed prior to implementation of a novel test. The speed the SARS-CoV-2 antibody test was required, and a lack of positive sample material, meant many methods were introduced without rigorous evaluations. As of December 2021, laboratories have not been asked to recheck SARS-CoV-2 antibody status of NHS staff, and relatively few requests are received to check for prior exposure.

On 15th September 2021, the UK government rolled out a coronavirus testing programme that included home testing to identify coronavirus antibodies (UK Health Security Agency, 2021b). The test is voluntary and available to anyone ordering an RT-PCR test on the government website, with the aim of understanding the level of immunity within the community.

As well as research, and detecting prior exposure, a new use for SARS-CoV-2 serology tests was identified in September 2021, when DHSC issued an Interim Clinical Commissioning Policy introducing a new therapy, casirivimab and imdevimab, for the treatment of patients hospitalised due to SARS-CoV-2 (Department of Health and Social Care, 2021e). This

neutralising monoclonal antibody (nMAB) combination therapy binds specifically to two different sites on the spike protein of SARS-CoV-2, blocking its entry into the host cell, and inhibiting replication. The policy allowed the routine commissioning of nMAB for those who met certain criteria; one of which was a negative baseline serum antibody test against SARS-CoV-2. The document specifically stated a SARS-CoV-2 anti-spike IgG method was required.

1.9 Aims and Objectives

This project aims to verify a novel quantitative SARS-CoV-2 anti-spike IgG method that allows antibody development, peak concentration, and antibody decline to be evaluated. It then aims to use this method to evaluate the immune response to natural SARS-CoV-2 infection and following vaccination to assess effectiveness in a group of Health Care Workers (HCW). The HCW will be followed up for nine months to determine how long SARS-CoV-2 antibodies remain detectable following vaccination.

The following paper has been published from the work completed in this thesis:

English, E., Cook, L.E., et al. (2021). 'Performance of the Abbott SARS-CoV-2 IgG II Quantitative antibody method, including the new Variants of Concern, VOC 202012/V1 (United Kingdom) and VOC 202012/V2 (South Africa), and first steps towards global harmonization of COVID-19 antibody methods', *Journal of Clinical Microbiology,* 59(9), e00288-21.

The project has been completed for module C2 of the University of Manchester qualification for the Doctorate of Clinical Sciences degree. Details of the other components (Modules A, B, and C1) are included in Appendixes A, B, and C. Appendix D details publications and other achievements during the programme.

Chapter 2

Verification of a robust quantitative SARS-CoV-2 anti-spike IgG method

2.1 Introduction

The Clinical Biochemistry laboratory at the Norfolk and Norwich University Hospital (NNUH) routinely uses a previously-evaluated qualitative test for SARS-CoV-2 IgG antibodies (Piec et al., 2021). This method reports the presence or absence of antibodies. It indicates previous exposure to SARS-CoV-2, but does not provide a quantitative antibody result. To allow the immune response to be evaluated fully following infection or vaccination, a quantitative SARS-CoV-2 IgG method is required. Using a quantitative SARS-CoV-2 IgG method would provide valuable insight into how individuals react to the virus or vaccine, in terms of their antibody development, peak concentration, and antibody decline over time. A quantitative method may also allow vaccine effectiveness to be assessed, in a similar way to measurement of antibodies following the Hepatitis B vaccination programme.

The first part of the project was to complete a comprehensive method evaluation of the novel Abbott SARS-CoV-2 anti-spike IgG (quantitative) method using the Abbott Alinity i system immunomethod. This evaluation was performed in collaboration with Abbott whilst the method was still in development. The results were used to optimise the final product and ensure it was suitable for commercial release.

2.2 Methodology

The novel quantitative SARS-CoV-2 anti-spike IgG method from Abbott was fully evaluated to assess its utility as a routine serology method capable of detecting IgG antibodies against SARS-CoV-2. The imprecision, limit of quantitation (LOQ), limit of detection (LOD), linearity, cross reactivity, accuracy, sensitivity, specificity and concordance with other SARS-CoV-2 serology methods were assessed.

2.2.1 Sample collection 143 positive serum samples were collected from confirmed SARS-CoV-2 RT-PCR positive inpatients at the NNUH, using the AusDiagnostics (Chesham, UK), Panther (Manchester, UK) or Altona (Hamburg, Germany) platforms. Negative samples were collected and pooled from routine GP samples analysed the same day at the NNUH Clinical Biochemistry laboratory. All samples were anonymised, aliquoted, and frozen at –80°C until analysis.

2.2.2 Abbott SARS-CoV-2 IgG (quantitative) method The Abbott SARS-CoV-2 anti-spike IgG (quantitative) method is an automated two-step chemiluminescent microparticle immunomethod (CMIA). It is used for the quantitative determination of IgG antibodies, including neutralising antibodies, to the receptor binding domain (RBD) of the S1 subunit of the spike protein of SARS-CoV-2 in human serum and plasma on the Abbott Alinity i system (Abbott Park, Chicago, IL, USA). The analytical measuring interval is 21.0 – 40,000.0 Arbitrary Units per mL (AU/mL). Samples above 40,000.0 AU/mL are diluted by the Abbott Alinity i system, with Abbott Alinity i multi method manual diluent, and samples above 80,000.0 AU/mL are diluted manually. The positivity cut-off stated by Abbott is 50.0 AU/mL.

The method works by combining sample with SARS-CoV-2 antigen coated paramagnetic microparticles and assay diluent in a reaction cuvette *(Figure 2.0)*. The mixture is then vortexed and incubated to allow any SARS-CoV-2 IgG antibodies in the sample to bind to the SARS-CoV-2 antigen coated microparticles. This forms an immune complex that is attracted by a magnet to the wall of the cuvette, and any unbound material is washed away. Anti-human IgG acridinium-labelled conjugate is then added, which binds to the immune complex. Following an incubation period there is a second wash step. Hydrogen peroxide is then added to the reaction mixture. This creates an acidic environment to prevent early release of light emission, prevents clumping of microparticles and separates the acridinium dye from the conjugate. Sodium hydroxide is then added to create an alkaline environment that, with the exposure to the peroxide added previously, causes the acridinium dye to undergo an oxidative reaction. A chemiluminescent reaction occurs in which light energy is released as N-methylacridone returns to its ground state. The measured relative light units (RLU) have a direct relationship with the amount of SARS-CoV-2 IgG antibodies in the sample.

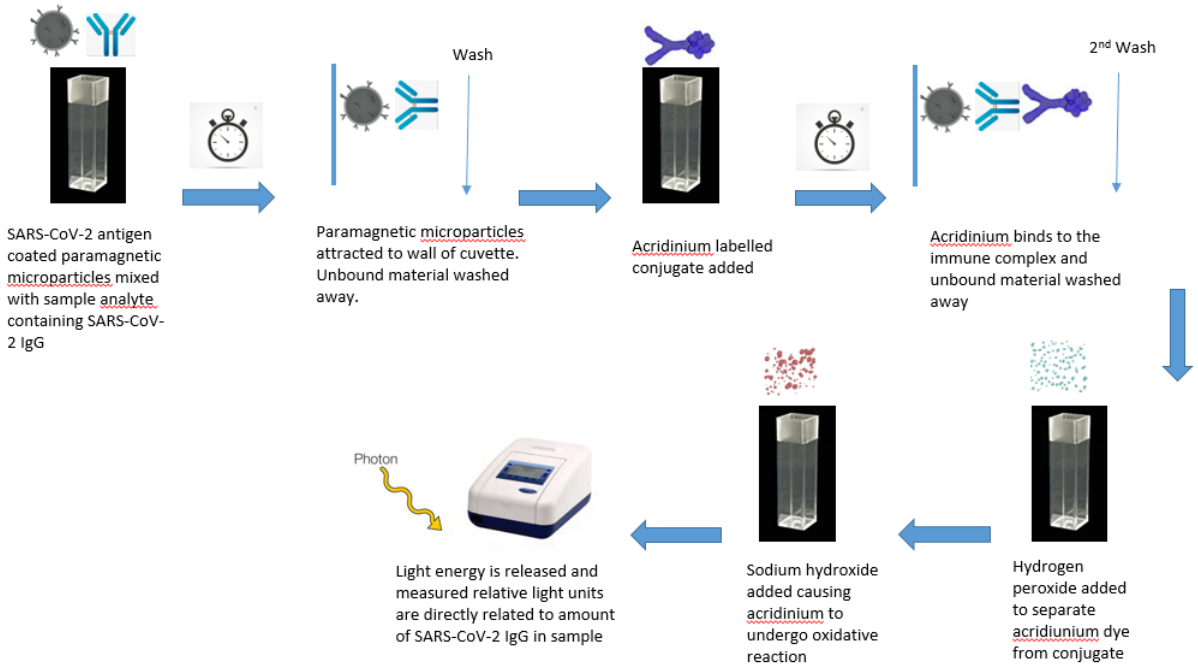


Figure 2.0: Schematic of the quantitative Abbott SARS-CoV-2 anti-spike IgG immunoassay. Sample is combined with SARS-CoV-2 antigen coated paramagnetic microparticles and assay diluent in a reaction cuvette. The mixture is vortexed and incubated to allow any SARS-CoV-2 IgG antibodies in the sample to bind to the SARS-CoV-2 antigen coated microparticles. This forms an immune complex that is attracted by a magnet to the wall of the cuvette, and any unbound material is washed away. Anti-human IgG acridinium-labelled conjugate is added, which binds to the immune complex. Following an incubation period and a second wash step, hydrogen peroxide is added. This creates an acidic environment to prevent early release of light emission, prevents clumping of microparticles and separates the acridinium dye from the conjugate. Sodium hydroxide is added to create an alkaline environment that causes the acridinium dye to undergo an oxidative reaction. A chemiluminescent reaction occurs in which light energy is released as N-methylacridone returns to its ground state. The measured relative light units (RLU) have a direct relationship with the amount of SARS-CoV-2 IgG antibodies in the sample.

The quantitative Abbott SARS-CoV-2 anti-spike IgG immunoassay was evaluated using the Clinical & Laboratory Standards Institute (CLSI) global standards. These are internationally recognised standards based on consensus opinion from expert industry, government and healthcare professions. There are individual standards for impression, LOD, Linearity and accuracy, which are described in the following sections.

2.2.3 Imprecision CLSI EP-15 (CLSI, 2014a) and EP-5 (CLSI, 2014b) based protocols were followed to evaluate imprecision. EP-15 describes imprecision from samples analysed in quintuplet, twice a day for five days. For this study, three different Abbott Internal Quality Control (IQC) samples were analysed (one negative and two positive). EP-5 describes a more robust imprecision study where samples are analysed in duplicate, twice a day for twenty days. For this study serum samples were combined to make four patient pools (one negative and three positive). Data from both imprecision studies were analysed using EP evaluator (Data Innovations, Build 11.3.0.23) to generate imprecision data for between-run and total standard deviations.

2.2.4 LOQ and LOD CLSI EP17 (CLSI, 2012) defines the LOD as LOD = LOB + 1.645 x $SD_{low\ conc.\ sample}$ (Armbruster & Pry, 2008). The limit of the blank (LOB) was determined from five replicates of the Abbott Alinity i multi method manual diluent (09P15). The LOB was used together with the standard deviation (SD) of a low-concentration sample to calculate the LOD. Five low-concentration patient serum pools were prepared and each patient pool was analysed in quintuplet, twice a day, for two days. The LOQ was estimated as the lowest concentration with a 20% coefficient of variation (CV) (Armbruster & Pry, 2008).

2.2.5 Linearity CLSI EP-6 (CLSI, 2020) based protocols were followed to evaluate the linearity of the method. A series of dilutions were performed on a high concentration patient sample (38,365 AU/mL) using Abbott Alinity i multi method manual diluent (09P15). The dilutions covered 95% of the analytical range and all samples were measured in triplicate for SARS-CoV-2 anti-spike IgG using the Abbott Alinity i system.

A panel of six heat-inactivated SARS-CoV-2 reference materials (Technopath, Tipperary, Ireland) was also evaluated in triplicate according to CLSI EP-06 (CLSI, 2020). The samples

were derived from human plasma and included a series of five positive vials and one negative vial, pre-diluted from positive stock. The manufacturer's information did not describe the primary material, diluent, or heat inactivation process.

2.2.6 Cross-reactivity 191 samples were analysed to assess cross-reactivity, which included:

- 65 negative control samples (N) collected pre-pandemic in 2018 from healthy volunteers with no recorded history of infection or immune disorders.
- 97 pre-pandemic samples (CR) from patients with a confirmed range of respiratory infections (including influenza A and B and seasonal coronaviruses).
- 29 samples (TSI) from patients positive for thyroid stimulating immunoglobulin.

Details of these samples and how they were collected have been described previously (Piec et al., 2021).

2.2.7 Sensitivity and specificity 143 RT-PCR positive SARS-CoV-2 samples and 191 control samples (as described for cross-reactivity) were analysed to determine the sensitivity and specificity of the Abbott SARS-CoV-2 anti-spike IgG (quantitative) method. Samples were collected at different time points following a positive SARS-CoV-2 RT-PCR result. Analysis was performed for all samples, and then for only those samples collected more than 14 days post positive RT-PCR result (n=57).

2.2.8 Accuracy Due to the lack of available reference material or a reference method, it was deemed inappropriate to evaluate trueness using CLSI EP9 (CLSI, 2013). The following candidate standard materials were analysed from the National Institute for Biological Standards and Controls (NIBSC):

1) CE-marked verification panel of 37 convalescent plasma samples (NIBSC code 20/B770), consisting of 23 anti-SARS-CoV-2 positive and 14 anti-SARS-CoV-2 negative samples.
2) CE-marked 'working standard' (NIBSC code 20/162) intended as a diagnostic calibrant with an assigned arbitrary unit of 1000 U, consisting of convalescent plasma positive for anti-SARS-CoV-2 antibodies pooled from three donors.

3) CE-marked ‘quality control 1’ (NIBSC code 20/B764) consisting of plasma positive for anti-SARS-CoV-2 antibodies, derived from two donors, and diluted in defibrinated convalescent plasma.

2.2.9 Concordance The results from the 143 RT-PCR positive and 191 cross-reactivity samples were compared with results obtained from three other SARS-CoV-2 IgG methods:

1) Abbott Diagnostics (Abbott Park, Chicago, IL, USA) qualitative method based on the nucleocapsid protein, using the Alinity i systems immunomethod.
2) Epitope Diagnostics Inc. (EDI, San Diego, CA, USA) qualitative method based on the nucleocapsid protein, using an automated Agility enzyme-linked immunosorbent method (ELISA) (Dynex Technologies, Chantilly, VA, USA).
3) DiaSorin (Dartford, UK) based on the spike protein, on the Liaison XL analyser. This is a semi-quantitative assay and results are expressed as AU/mL.

Concordance between the methods was determined using Cohen’s Kappa test. The NIBSC verification panel and the Technopath linearity panel were also analysed by these three additional SARS-CoV-2 IgG methods.

2.2.10 Variants of concern Three different strains of SARS-CoV-2 were analysed by the Abbott SARS-CoV-2 anti-spike IgG method to assess method performance with different variants. Three SARS-CoV-2 strains prevalent in the UK at the time were tested; the Spanish lineage B.1.117, the UK variant of concern (VOC) B1.1.7 (VOC 202012/V1 [UK]), and the South African VOC 202012/V2.

2.2.11 Statistics Microsoft Excel (2010) was used for all calculations and graph production. Imprecision data for between-run and total standard deviations were analysed using EP evaluator (Data Innovations, Build 11.3.0.23). Correlation graphs with linear regression and r^2 values determined linearity. A four-parameter logistic curve fit calculated the LOQ. Cohen’s Kappa concordance analysis measured the agreement of results between four SARS-CoV-2 IgG methods.

2.3 Results

The novel quantitative SARS-CoV-2 anti-spike IgG method was fully evaluated to assess its utility as a routine serology method capable of detecting antibodies against SARS-CoV-2. The imprecision, LOQ, LOD, linearity, cross reactivity, sensitivity, specificity, accuracy, and concordance with other SARS-CoV-2 serology methods were assessed.

2.3.1 Imprecision The performance of three Abbott IQC materials (one negative and two positive) were analysed using the CLSI EP-15 (CLSI, 2014a) based protocol *(Table 2.1)*. The IQC samples were analysed in quintuplet, twice a day, for five days. The imprecision of the two positive materials was ≤3.3% (3.0% at a mean of 162.9 AU/mL, and 3.3% at a mean of 604.2 AU/mL). The negative IQC showed a total imprecision of 20.5%, although the mean concentration for this material was 3.4 AU/mL, which is below the analytical measuring interval quoted by Abbott (21.0 – 40,000.0 AU/mL). The clinical cut-off for the method is 50.0 AU/mL and all negative IQC replicates were reported as negative, with 5.7 AU/mL the highest concentration measured.

Imprecision was also evaluated using four patient serum pools (one negative and three positive) using the CLSI EP-5 (CLSI, 2014b) based protocol. Pools were analysed in duplicate, twice a day, for twenty days. The imprecision data *(Table 2.2)* shows a total %CV of ≤3.4% for the three positive patient pools (2.9% at a mean of 71.1 AU/mL, 3.3% at a mean of 282.5 AU/mL, and 3.4% at a mean of 2429.3 AU/mL). The imprecision of the negative patient pool was 64.0%, although the mean antibody concentration of this pool was 2.7 AU/mL, which is below the analytical measuring interval quoted by Abbott (21.0 – 40,000 AU/mL).

Table 2.1: CLSI EP-15 study using three Abbott IQC samples (one negative and two positive). Samples were analysed in quintuplet for SARS-CoV-2 IgG on the Abbott Alinity i system, twice a day, for five days. IQC targets and ranges were provided by Abbott. Data were analysed using EP evaluator (Data Innovations, Build 11.3.0.23) to generate imprecision data for between-run and total standard deviations.

	Negative IQC Target (N/A) Range (<44.5 AU/mL)		**Positive 1 IQC** Target (154.6 AU/ml) Range (92.8 – 201.0 AU/ml)		**Positive 2 IQC** Target (766.0 AU/ml) Range (459.6 – 995.8 AU/ml)	
	Mean 3.4 AU/mL		Mean 162.9 AU/mL		Mean 604.2 AU/mL	
	%CV	SD	%CV	SD	%CV	SD
Between Run	20.1	0.7	2.7	4.3	2.6	15.6
Between Day	4.3	0.1	1.4	2.3	2.0	12.1
Total	**20.5**	**0.7**	**3.0**	**4.9**	**3.3**	**19.7**

Table 2.2: CLSI EP-5 study using patient serum pools (one negative and three positive). Serum pools were analysed in duplicate for SARS-CoV-2 IgG on the Abbott Alinity i system, twice a day, for twenty days. Data were analysed using EP evaluator (Data Innovations, Build 11.3.0.23) to generate total standard deviations.

	Negative patient pool		**Positive patient pool 1**		**Positive patient pool 2**		**Positive patient pool 3**	
	Mean 2.7 AU/mL		Mean 71.1 AU/mL		Mean 282.5 AU/mL		Mean 2429.3 AU/mL	
	%CV	SD	%CV	SD	%CV	SD	%CV	SD
Total	**64.0**	**1.7**	**2.9**	**2.1**	**3.3**	**9.3**	**3.4**	**83.4**

2.3.2 LOQ Five low-concentration patient serum pools were analysed in quintuplet, twice a day for two days. The mean, SD, and %CV for the replicates are shown in *Table 2.3* and a precision profile graphical representation of the data in *Figure 2.1*. The LOQ was estimated as the lowest concentration with a 20% CV, and a four-parameter logistic curve fit calculated the LOQ as 15.4 AU/mL.

Patient pool number 5 had a mean concentration of 52.2 AU/mL and a %CV of 3.9%, suggesting good imprecision at the positivity cut-off (50.0 AU/mL). With a target %CV <5% (taken from the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) (Kaza et al., 2019)), the lowest concentration pool with good imprecision was 30.4 AU/mL (patient pool number 3)(%CV = 5.4%). The lower end of the analytical measuring range quoted by Abbott is 21.0 AU/mL, and at a mean concentration of 18.1 AU/mL (patient pool 2) the %CV was 13.6%. The method was found not to be precise at low concentrations, with a %CV of 59.3% at a mean concentration of 3.8 AU/mL (patient pool number 1), although this concentration was below the analytical measuring range of the method.

2.3.3 LOD Abbott Alinity i multi method manual diluent (09P15), normal saline, and deionised water were analysed five times to calculate the LOB *(Table 2.4).* The LOB = $mean_{blank} + 1.645(SD_{blank})$ (Armbruster and Pry, 2008). For the Abbott diluent, the LOB was calculated to be 0.2807 from LOB = 0.06 + 1.645(0.134). The LOD = LOB + $1.645(SD_{low\ concentration\ sample})$ (Armbruster and Pry, 2008). For the Abbott diluent, the LOD = 0.2807 + 1.645(2.5), using the SD from patient pool number two, with a mean concentration of 18.1 AU/mL. The LOD was determined to be 4.3 AU/mL.

Table 2.3: Mean SARS-CoV-2 IgG concentration (AU/mL), SD, and %CV for five low-concentration patient serum pools analysed in quintuplet on the Abbott Alinity i system, twice a day, for two days.

	Patient Pool Number				
	1	**2**	**3**	**4**	**5**
Mean SARS-CoV-2 IgG (AU/mL)	3.8	18.1	30.4	36.9	52.2
SD	2.2	2.5	1.6	1.3	2.1
%CV	**59.3**	**13.6**	**5.4**	**3.4**	**3.9**

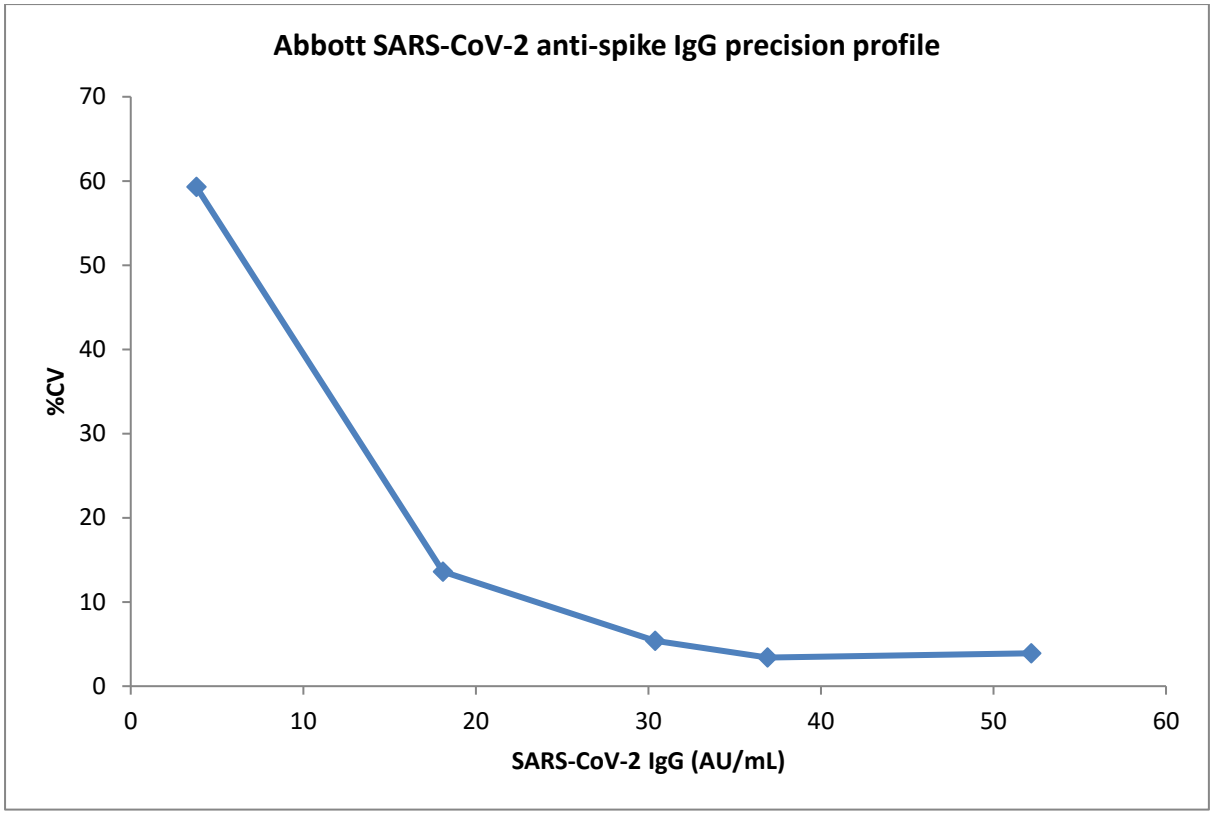


Figure 2.1: Precision profile for five low-concentration patient serum pools analysed in quintuplet for SARS-CoV-2 IgG on the Abbott Alinity i system, twice a day, for two days.

Table 2.4: SARS-CoV-2 IgG concentration for the Abbott Alinity i multi method manual diluent (09P15), normal saline, and deionised water, measured in quintuplet on the Abbott Alinity i system. Mean and standard deviation for each is shown.

Replicate	Matrix		
	Diluent	Saline	Water
1	0.0	0.0	0.0
2	0.0	0.0	0.0
3	0.3	0.0	0.0
4	0.0	0.0	0.0
5	0.0	0.0	0.0
Mean SARS-CoV-2 IgG (AU/mL)	**0.060**	**0.0**	**0.0**
SD	**0.134**	**0.0**	**0.0**

2.3.4 Linearity CLSI EP-6 (CLSI, 2020) based protocols were followed to evaluate the linearity of the Abbott SARS-CoV-2 anti-spike IgG method. A series of dilutions were performed on a high concentration patient sample using Abbott Alinity i multi method manual diluent (09P15). The dilutions covered 95% of the analytical range and all samples were measured in triplicate. The method was found to be linear up to 38,365 AU/mL, with a slope of 1.004 and an R^2 of 0.9992 (*Figure 2.2)*. Abbott state the method is linear up to 40,000.0 AU/mL, but this was not tested in this study. The highest concentration measured without dilution was 38,365 AU/mL. This sample was deemed acceptable for assessing linearity as it was within 95% of the upper limit.

The percentage difference between the average measured SARS-CoV-2 IgG concentrations, compared with the expected results is shown in *Table 2.5*. The average percentage difference was 7% between measured IgG and expected IgG concentrations. The largest percentage difference was 23%, and this did result in an expected negative result of 41 AU/mL being reported as positive, with a measured antibody concentration of 50 AU/mL.

A Technopath panel of six heat-inactivated SARS-CoV-2 reference materials was also evaluated according to CLSI EP-06 (CLSI, 2020). The samples included a series of five positive vials and one negative vial, pre-diluted from positive stock, and all samples were measured in triplicate. Analysis of the Technopath linearity panel gave a linear response, with a regression equation of $y = 41x – 48.672$ and an R^2 of 0.9984 *(Figure 2.3)*. The values of measured IgG were 5.6 AU/mL for the negative sample, and ranged from 147.5 AU/mL to 4,098.3 AU/mL for the positive samples.

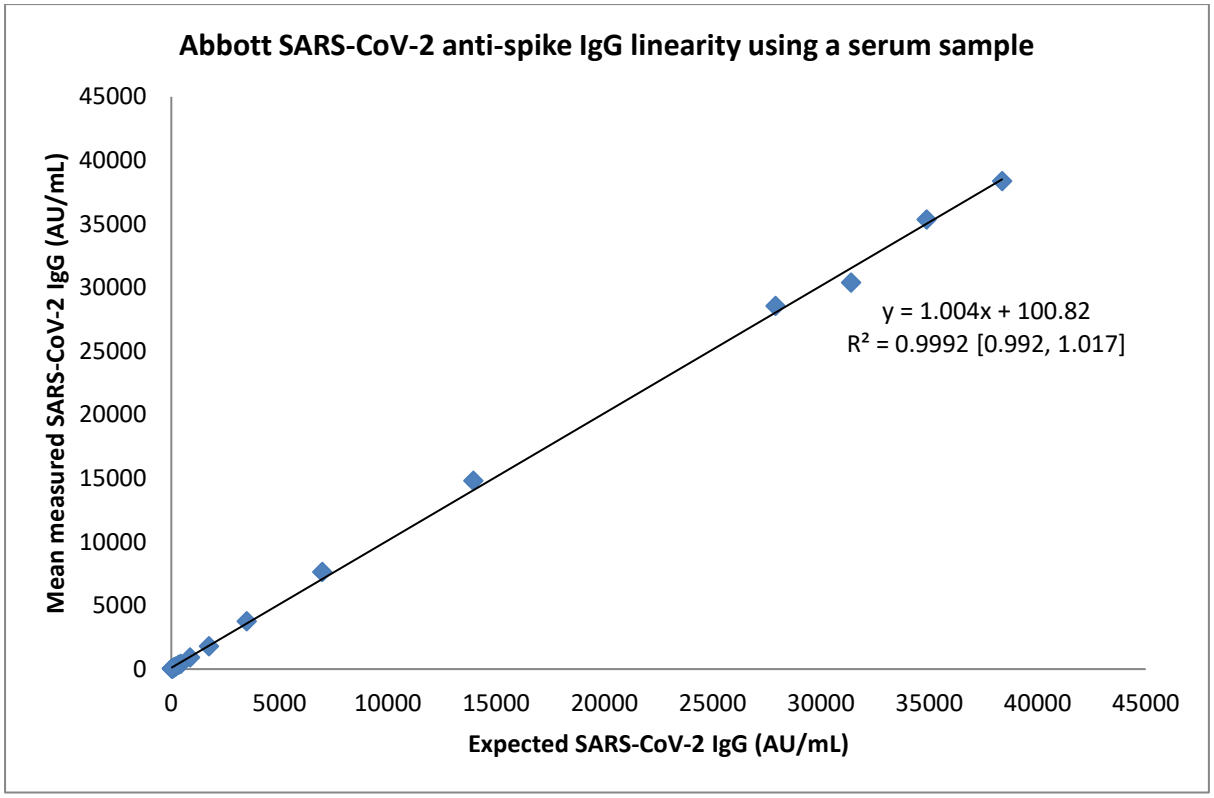


Figure 2.2: Linearity of the Abbott SARS-CoV-2 anti-spike IgG (quantitative) method, using a patient serum sample with a measured antibody concentration of 38,365 AU/mL, and a range of dilutions in the Abbott Alinity i multi method manual diluent (09P15). Results are presented as the mean of triplicate measurements from the Abbott Alinity i system.

Table 2.5: Expected, measured, and percentage difference values for the assessment of linearity of the Abbott SARS-CoV-2 IgG (quantitative) method on the Alinity i system using a range of dilutions of a high positive patient sample (mean 38,365 AU/mL) in Abbott Alinity i multi method manual diluent (09P15).

Expected IgG (AU/mL)	Measured IgG (AU/mL)	Percentage Difference (%)
38365	38365	0
34877	35343	1
31389	30392	-3
27902	28553	2
13951	14810	6
6975	7646	10
3488	3770	8
1744	1795	3
872	926	6
436	422	-3
327	326	0
218	223	2
163	164	1
109	122	12
82	90	10
68	74	9
41	50	23
34	40	19
27	32	18

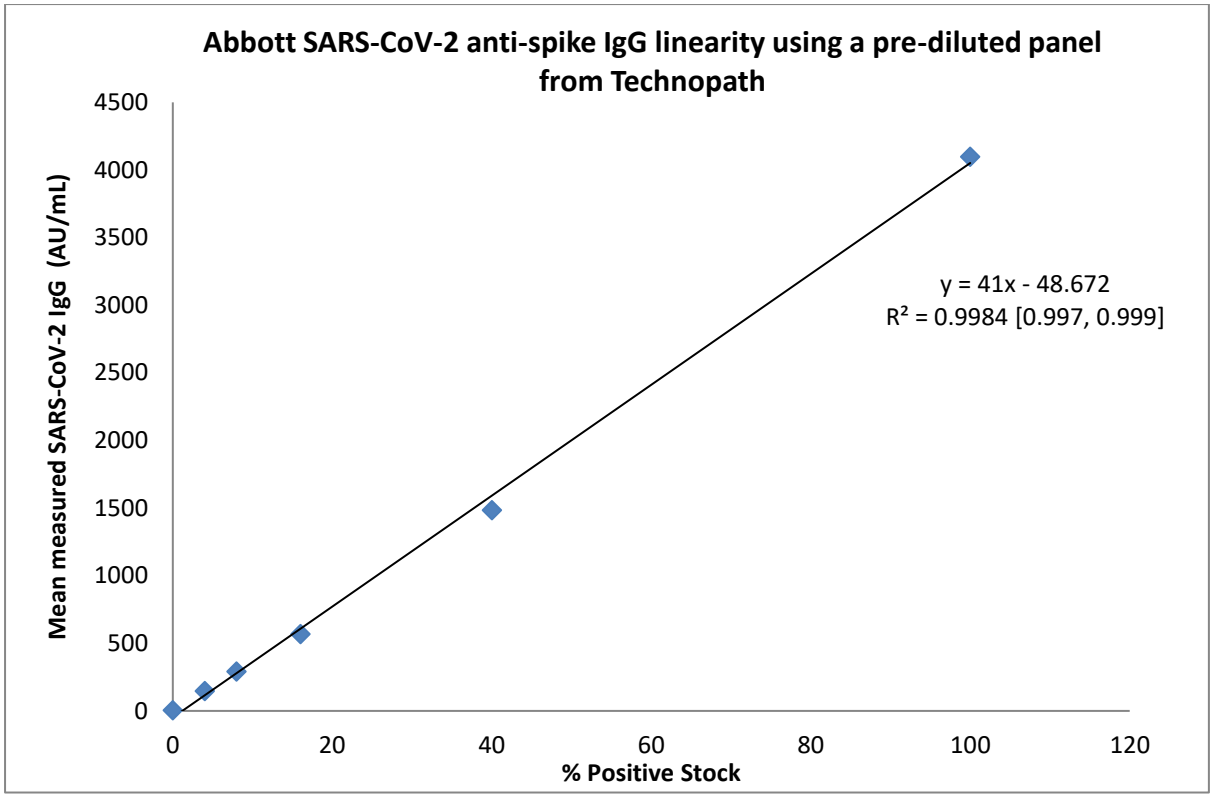


Figure 2.3: Linearity of Abbott SARS-CoV-2 anti-spike IgG (quantitative) method using a pre-diluted linearity panel from Technopath. Results are presented as the mean of triplicate measurements from the Abbott Alinity i system.

2.3.5 Trueness A NIBSC verification panel of 37 convalescent plasma samples (23 anti-SARS-CoV-2 positive and 14 anti-SARS-CoV-2 negative) gave the expected positive or negative outcome *(Table 2.6)*. There were no assigned values to the samples and the panel could not truly be used to assess accuracy. There was a clear separation in measured IgG between the two groups however, with the 23 positive results ranging from 210.1 to 9709.5 AU/mL, and the 14 negative results ranging from 0.7 to 4.5 AU/mL.

The results for the NIBSC calibrant (assigned an arbitrary unit of 1000 U) and the NIBSC quality control (QC) are shown in *Table 2.7*. Samples were analysed in triplicate on two different reagent lot numbers. The average measured IgG was 14,560.2 AU/mL for the NIBSC calibrant. This concentration could be used to convert all AU/mL results to standardised units (U), although no volume unit was supplied. The mean SARS-CoV-2 anti-spike IgG measured for the NIBSC QC was 300.6 AU/mL, but there was no assigned value for this material.

Table 2.6: NIBSC verification panel of 37 convalescent plasma samples (23 anti-SARS-CoV-2 positive and 14 anti-SARS-CoV-2 negative). Samples were measured for SARS-CoV-2 IgG in duplicate on the Abbott Alinity i system.

NIBSC Sample ID	SARS-CoV-2 IgG (AU/mL); positivity cut-off = 50.0 AU/mL			
	Replicate 1	Replicate 2	Mean of two replicates	Outcome
1	211.0	209.1	210.1	Positive
2	348.4	339.6	344.0	Positive
3	8707.3	8559.6	8633.5	Positive
4	6260.3	6384.1	6322.2	Positive
5	9710.5	9708.5	9709.5	Positive
6	4225.2	3974.8	4100.0	Positive
7	2304.3	2147.7	2226.0	Positive
8	4428.8	4011.8	4220.3	Positive
9	6429.8	6383.6	6406.7	Positive
10	6674.9	7115.1	6895.0	Positive
11	1172.3	1186.2	1179.3	Positive
12	1167.6	1127.9	1147.8	Positive
13	1395.3	1382.3	1388.8	Positive
14	961.6	999.0	980.3	Positive
15	1590.9	1685.6	1638.3	Positive
16	787.6	747.4	767.5	Positive
17	2617.7	2541.6	2579.7	Positive
18	6062.8	6065.6	6064.2	Positive
19	2329.8	2355.0	2342.4	Positive
20	1920.4	1954.4	1937.4	Positive
21	3111.3	3117.9	3114.6	Positive
22	3517.7	3447.6	3482.7	Positive
23	2290.0	2314.6	2302.3	Positive
24	4.0	2.0	3.0	Negative
25	4.7	4.2	4.5	Negative
26	1.9	3.8	2.9	Negative
27	3.4	2.4	2.9	Negative
28	0.8	1.0	0.9	Negative
29	2.0	1.3	1.7	Negative
30	5.6	3.2	4.4	Negative
31	1.6	1.3	1.5	Negative
32	0.7	3.0	1.9	Negative
33	0.6	3.1	1.9	Negative
34	1.6	1.5	1.6	Negative
35	0.9	0.4	0.7	Negative
36	2.3	2.3	2.3	Negative
37	5.1	0.6	2.9	Negative

Table 2.7: Mean SARS-CoV-2 IgG concentration for the NIBSC calibrant (assigned arbitrary unit of 1000 U) and NIBSC Quality Control (no assigned value). Samples were measured in triplicate on the Abbott Alinity i system, using two reagent lot numbers.

	Reagent Lot Number	**Replicate**	**SARS-CoV-2 IgG (AU/mL)**	**Mean (AU/mL)**
NIBSC Calibrant	23266FN00	1	15759.1	15053.2
		2	14619.3	
		3	14781.2	
	22001F900	1	13660.0	14067.2
		2	14599.7	
		3	13941.9	
NIBSC QC	23266FN00	1	307.5	304.6
		2	300.2	
		3	306.0	
	22001F900	1	326.4	296.6
		2	279.3	
		3	284.2	

2.3.6 Sensitivity and specificity *Table 2.8* shows the number of SARS-CoV-2 IgG positive (≥50.0 AU/mL) and negative (<50.0 AU/mL) results for all SARS-CoV-2 RT-PCR positive samples (n=143), and then for only samples collected at least 14 days after a positive RT-PCR result (n=57). The Abbott SARS-CoV-2 anti-spike IgG method had a sensitivity of 91.6% [85.8 – 95.6] when all samples were considered. The sensitivity increased to 98.3% [90.6 – 100.0] when only samples collected at least 14 days after an RT-PCR result were considered. One of the 57 samples collected over 14 days after a positive RT-PCR result, tested negative for SARS-CoV-2 IgG antibodies.

Table 2.8 also shows the number of SARS-CoV-2 IgG positive (≥50.0 AU/mL) and negative (<50.0 AU/mL) results for the healthy negative controls collected in 2018 (n=65), other coronavirus/influenza controls collected in 2019 pre pandemic (n=97), and TSI control samples (n=29). The specificity of the Abbott SARS-CoV-2 anti-spike IgG (quantitative) method was 99.4% [97.1 – 100.0]. One sample in the control group (n=191) tested positive for SARS-CoV-2 IgG antibodies with a measured concentration of 140.5 AU/mL. This sample had been collected in 2019 (pre-pandemic), and at that time tested positive on a combined method for four seasonal coronaviruses. The other samples in the control group had concentrations ranging from 1.1 AU/mL to 48.3 AU/mL, with a mean IgG concentration of 6.9 AU/mL. All samples collected from healthy volunteers before the pandemic in 2018 were negative (n=65), but one sample was close to the positivity cut-off (50.0 AU/mL), with a measured concentration of 48.3 AU/mL. All 29 TSI samples were negative for SARS-CoV-2 IgG antibodies, with concentrations ranging from 0.0 to 29.0 AU/mL (n=29).

Table 2.8: Sensitivity and specificity of the Abbott SARS-CoV-2 anti-spike IgG (quantitative) method. Number of SARS-CoV-2 IgG positive and negative results for RT-PCR positive patients (all samples, and those collected at least 14 days post RT-PCR), pre pandemic control samples collected in 2018 (N), other respiratory infections samples collected in 2019 (pre-pandemic)(CR), and samples with known thyroid stimulation immunoglobulins (TSI).

Group	Number of samples			% Agreement [95% CI]
	Total	SARS-CoV-2 IgG Positive (≥50.0 AU/mL)	SARS-CoV-2 IgG Negative (< 50.0 AU/mL)	
SARS-CoV-2 RT-PCR positive samples (All time points)	143	131	12	91.6 [85.8 – 95.6]
SARS-CoV-2 RT-PCR positive samples (>14 days post RT-PCR)	57	56	1	98.3 [90.6 – 100.0]
Pre pandemic controls (N)	65	0	65	100.0 [94.5 – 100.0]
Other respiratory infections (CR)	97	1	96	99.0 [94.5 – 100.0]
Thyroid stimulation immunoglobulin (TSI)	29	0	29	100.0 [88.1 – 100.0]
All controls (N, CR, TSI)	191	1	190	99.4 [97.1 – 100.0]

2.3.7 Concordance Cohen's Kappa concordance analysis was used to measure agreement between four SARS-CoV-2 IgG methods. *Figure 2.4* shows the value of Kappa, with the standard error (calculated by dividing the standard deviation by the square root of the number of samples), as well as the percentage agreement of results, and the number of samples included in the comparison. The percentage agreement includes those samples that agree by chance, whereas the Kappa value removes this chance agreement and provides a more realistic measure of agreement. For example, comparing the positive or negative antibody outcomes from the two Abbott methods gave a percentage agreement of 98.4%, but removing the agreement due to chance gave an agreement of 96.6% (cohens kappa 0.966). The DiaSorin and EDI SARS-CoV-2 IgG methods showed the poorest agreement between results (Cohen's Kappa 0.899), and the Abbott anti-nucleocapsid (qualitative) and Abbott anti-spike (quantitative) methods showed the closest agreement (Cohen's Kappa 0.966).

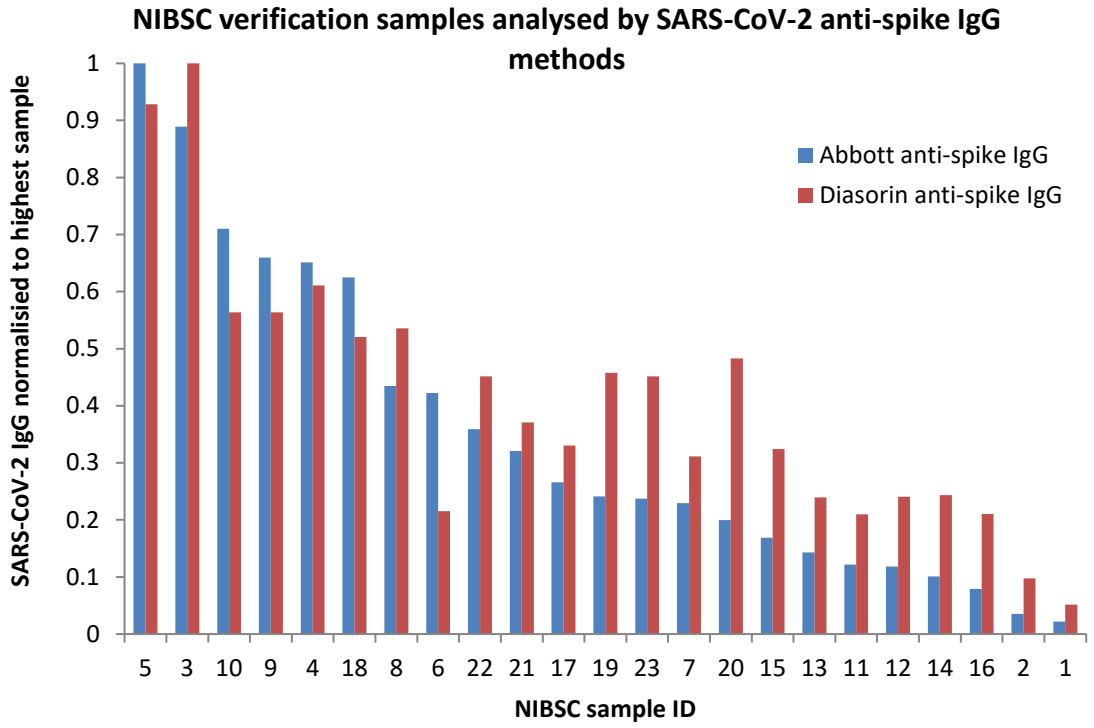
Figure 2.5 shows the NIBSC verification panel results obtained from the four SARS-CoV-2 IgG methods. The spike-based Abbott (quantitative) and DiaSorin methods performed similarly (A), and the nucleocapsid-based Abbott (qualitative) and EDI methods performed similarly (B). To compare the methods, results were normalised as a ratio to the highest value for each method to compare the magnitude of positive responses.

Figure 2.6 shows the Technopath linearity series measured by each of the four SARS-CoV-2 IgG methods. Only the Abbott SARS-CoV-2 anti-spike IgG (quantitative) method shows a linear response. The Abbott (qualitative), DiaSorin, and EDI methods were not linear using this material.

Cohen's Kappa (SE) **% Agreement** **Number of Samples**	DiaSorin	Abbott anti-nucleocapsid IgG (qualitative)	Abbott anti-spike IgG (quantitative)
EDI	0.899 (0.0014) 95.2% n=311	0.945 (0.0011) 97.4% n=314	0.956 (0.0009) 97.4% n=314
Abbott anti-spike IgG (quantitative)	0.930 (0.0012) 96.7% n=330	0.966 (0.0008) 98.4% n=314	
Abbott anti-nucleocapsid IgG (qualitative)	0.944 (0.0011) 97.4% n=311		

Figure 2.4: Cohen's Kappa concordance analysis of four SARS-CoV-2 IgG methods. The value of Kappa is shown with the standard error, as well as the percentage agreement of results, and the number of samples included in the comparison. The colours show those with the closest agreement (in green) to those with the poorest agreement (in red).

A



B

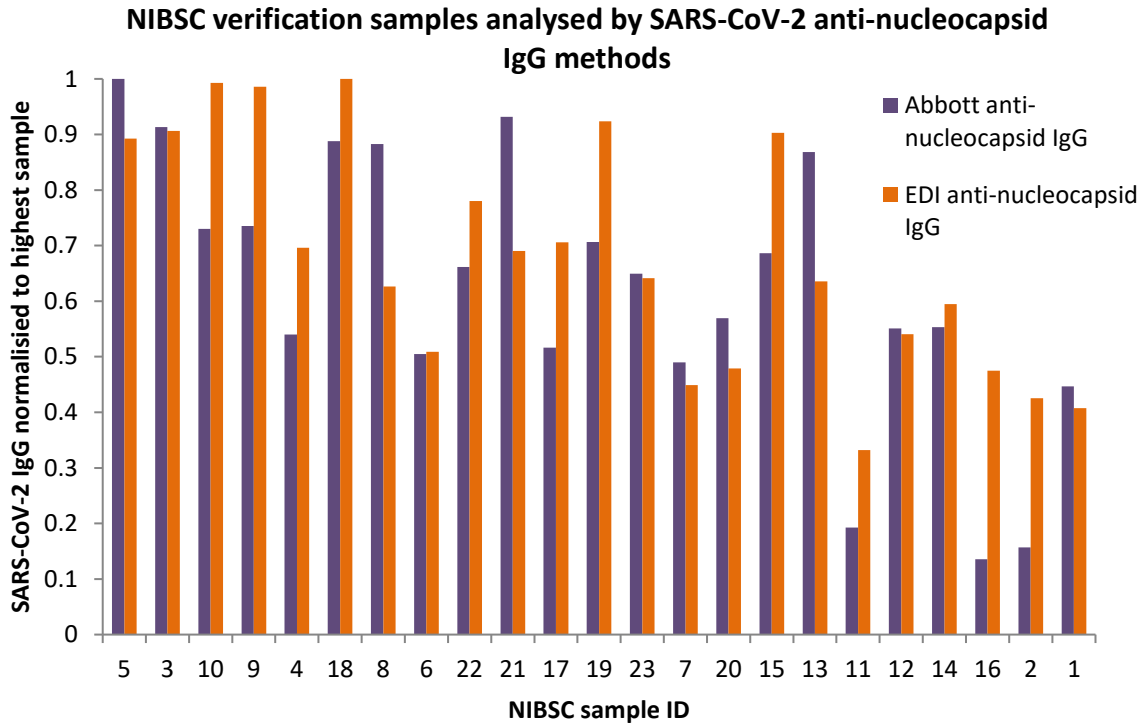


Figure 2.5: 23 anti-SARS-CoV-2 positive convalescent plasma samples from a CE-marked NIBSC verification panel (20/B770) were analysed on A) two spike-based SARS-CoV-2 IgG immunomethods and B) two nucleocapsid-based immunomethods.

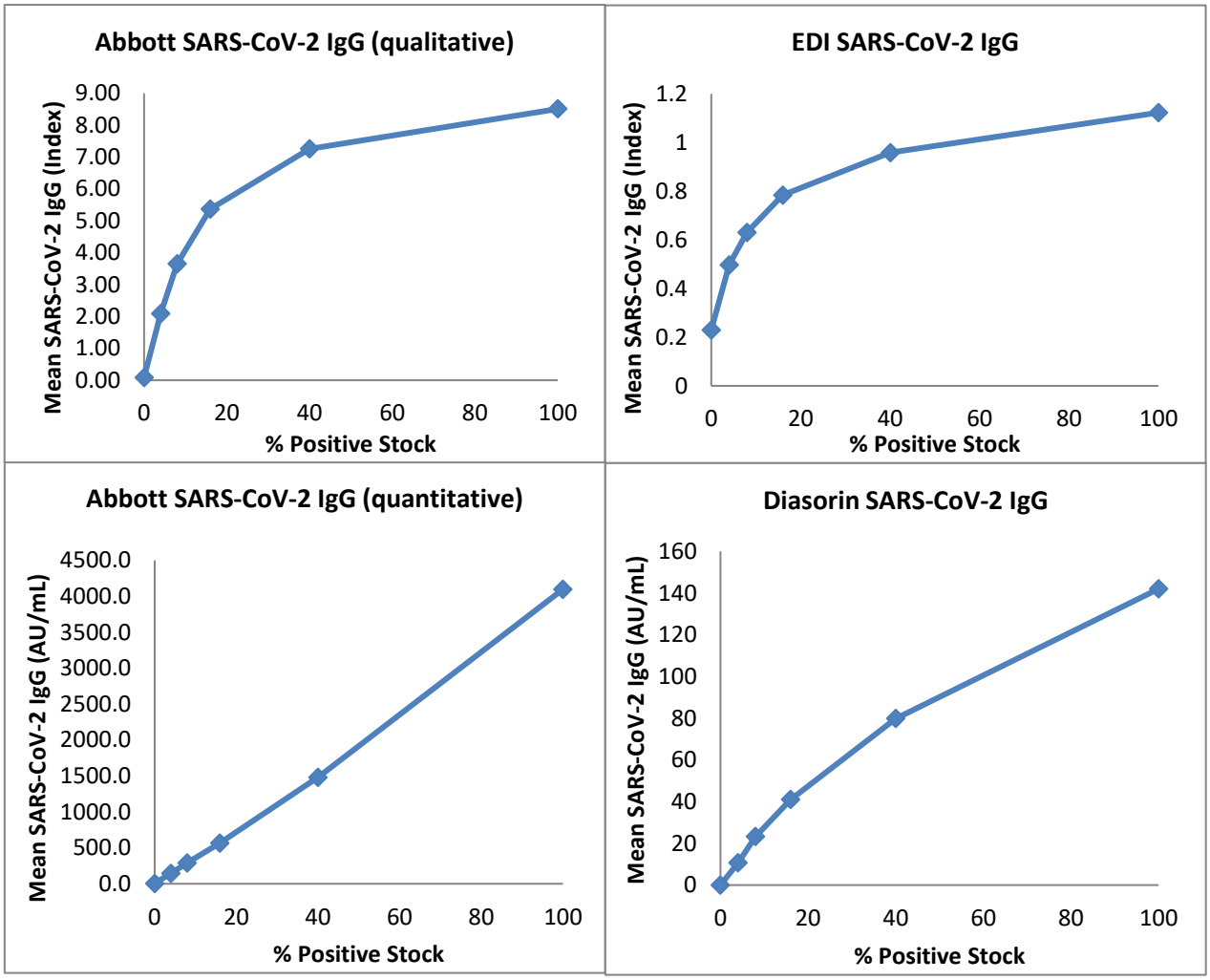


Figure 2.6: Analysis of a panel of six heat-inactivated SARS-CoV-2 reference materials (Technopath) evaluated according to CLSI EP-06 (CLSI, 2020). The samples were derived from human plasma and included a series of five positive vials and one negative vial, pre-diluted from positive stock. The linearity panel was analysed in triplicate on four SARS-CoV-2 IgG methods; 2 spike-based immunomethod methods (Abbott (quantitative) and DiaSorin), and 2 nucleocapsid-based immunomethod methods (Abbott (qualitative) and EDI).

2.3.8 Variants of concern *Figure 2.7* shows the rise and fall in SARS-CoV-2 IgG (quantitative) antibody concentrations for a patient with the Spanish B.1.177 strain of SARS-CoV-2. It also shows the antibody response in patients with two variants of concern (VOC 202012/V1 [UK] and VOC 202012/V2 [South African]). The antibody status has been compared with the number of days post positive RT-PCR result. Data for the number of days post infection or post symptom onset were not available.

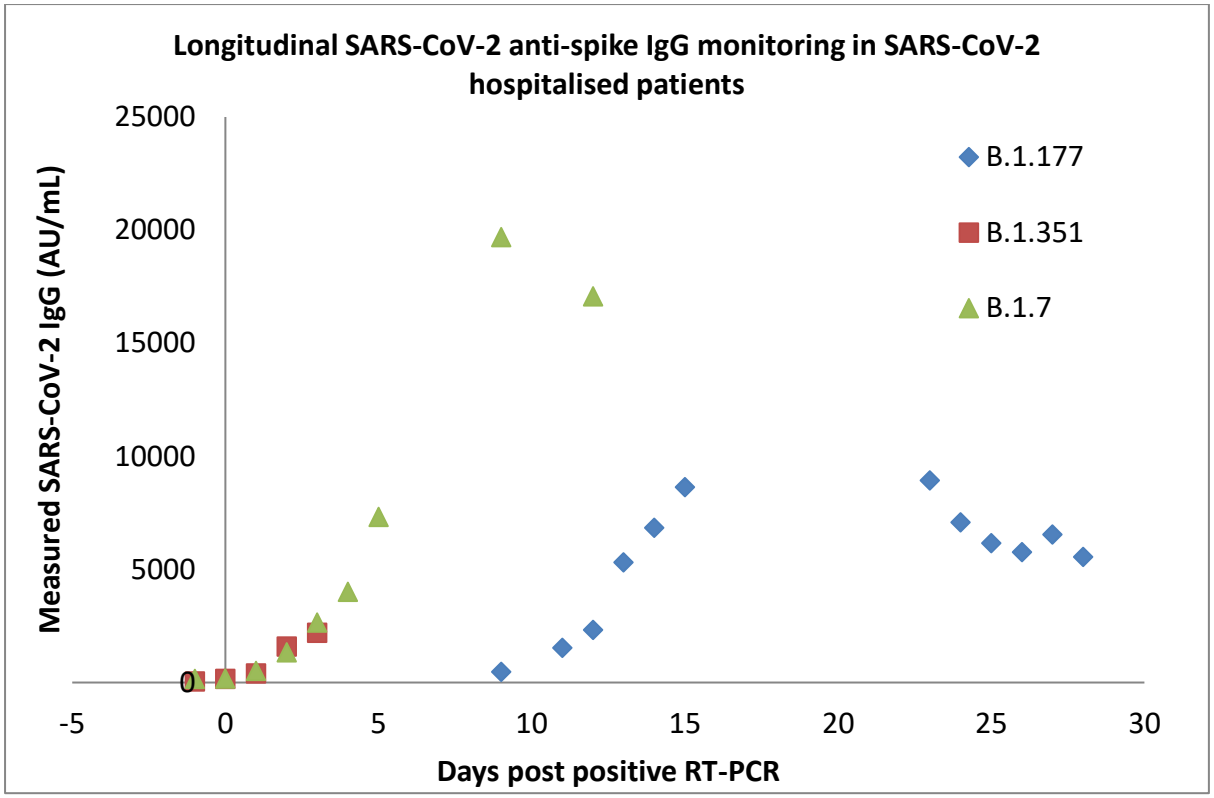


Figure 2.7: Longitudinal monitoring of the SARS-CoV-2 anti-spike IgG antibodies using the Abbott SARS-CoV-2 IgG (quantitative) method on the Abbott Alinity i system. The rise and fall in antibodies is shown for patients with three different strains of SARS-CoV-2 (Spanish B.1.177, UK B1.1.7, South African B.1.351).

2.4 Discussion

The Abbott SARS-CoV-2 anti-spike IgG (quantitative) method performed well across all parameters assessed in this evaluation. The imprecision was found to be excellent in the positive range (%CV ≤3.4% for mean concentrations ranging from 71.1 AU/mL to 2429.3 AU/mL). The method was also shown to perform well at the positivity cut-off (50.0 AU/mL) with a %CV of 3.9% achieved for a mean concentration of 52.2 AU/mL. The lowest concentration with good imprecision was 30.4 AU/mL (%CV = 5.4%). The higher imprecision around the bottom of the analytical range (%CV 13.6% for a mean concentration of 18.1 AU/mL) was also deemed acceptable. The poor imprecision (%CV 59.3% for a mean concentration of 3.8 AU/mL, and 64% for a mean concentration of 2.7 AU/mL) for concentrations below the analytical range (21.0 – 40,000 AU/mL) was not considered detrimental to the method performance, with concentrations <4.3 AU/mL being below the LOD quoted by Abbott (6.8 AU/mL). The imprecision was assessed in this evaluation using IQC material and patient serum pools. The IQC material was obtained from Abbott, and third party IQC material was not tested.

Recent studies have also reported excellent imprecision with the Abbott SARS-CoV-2 anti-spike IgG (quantitative) method. Narasimhan et al (2021) reported an imprecision less than 5% using two Abbott IQC levels. They do not state the mean concentration of the IQC samples used, but it is likely they reported the imprecision of the two Abbott positive IQC samples used in this evaluation (achieving an imprecision of ≤3.3%), and did not include data for the negative IQC sample due to poor imprecision. Jung et al (2021) found an imprecision of ≤3.5% using one IQC and two patient serum pools, although the lowest mean concentration sample tested was 413.4 AU/mL, with no imprecision studies performed around the positivity cut-off, or at the bottom of the analytical range.

The LOQ of the Abbott SARS-CoV-2 anti-spike (quantitative) method was found to be 15.4 AU/mL, suggesting the assay is capable of reporting results slightly lower than the manufacturer quoted LOQ of 21.0 AU/mL. The LOD was also found to be slightly lower (4.3 AU/mL) than the manufacturer reported LOD (6.8 AU/mL). Narasimhan et al (2021) stated an analytical measuring range of 4.2 to 50,000 AU/mL for the method, and a clinical

reporting range up to 200,000 AU/mL, but samples at this concentration were not tested in this evaluation.

The Abbott SARS-CoV-2 anti-spike IgG (quantitative) method was shown to be linear up to 38,365 AU/mL (the highest sample tested). It was the only method showing linearity when the Technopath linearity panel was analysed. The Abbott anti-nucleocapsid (qualitative) IgG, DiaSorin, and EDI methods are calibrated toward the positive/negative threshold and were not linear using this material. This was not unexpected, as the results for these three methods are reported as either positive or negative, and they are not marketed as quantitative methods. Despite the SARS-CoV-2 IgG anti-spike (quantitative) method being linear, care should be taken when diluting samples close to the positivity cut-off. One diluted sample was reported as positive (50 AU/mL) when it was expected to be negative (41 AU/mL). However, samples would not routinely be diluted unless they were >40,000.0 AU/mL, and this finding was not detrimental to the method performance. Other reports in the literature have also described the method as linear (Jung et al., 2021, Narasimhan et al., 2021).

The Abbott SARS-CoV-2 anti-spike IgG (quantitative) method showed a very high sensitivity of 98.3% [90.6 – 100.0] and specificity of 99.4% [97.1 – 100.0], suggesting few false positive or negative results will be reported. This performance is similar to a report by Jung et al (2021) who found a sensitivity of 97.6% and specificity of 99.3% with the same method. This is an improvement on other serology methods such as the Abbott SARS-CoV-2 anti-nucleocapsid IgG method and the DiaSorin method that were shown to have specificities of 95.1% and 94.9% respectively (Jääskeläinen et al., 2020).

The sensitivity of 98.3% [90.6 – 100.0] was based on samples collected more than 14 days post positive SARS-CoV-2 RT-PCR result. When all samples were included, the sensitivity dropped to 91.6% [85.8 – 95.6]. All SARS-CoV-2 RT-PCR positive samples were collected from hospital in-patients, and the number of days post-symptom onset was not available. Some patients may have been symptomatic for many days prior to hospital admission and RT-PCR testing, whereas others may have developed symptoms whilst in hospital.

One sample collected more than 14 days after a positive SARS-CoV-2 RT-PCR result gave a negative anti-spike IgG result. This patient may have been tested for SARS-CoV-2 very early in their illness, before IgG antibodies had been produced. However, Ma et al (2020) showed the medium seroconversion time for IgG was five to ten days post symptom onset and it would have been expected that this patient had developed antibodies. It is not known whether this patient was immunosuppressed, or whether they later went on to develop antibodies, as the samples were anonymised. Marklund et al (2020) reported patients with severe SARS-CoV-2 seroconvert earlier than those with mild symptoms, so perhaps this patient had a very mild case of SARS-CoV-2. Marklund et al (2020) also reported a number of SARS-CoV-2 patients that did not develop detectable SARS-CoV-2 IgG using two commercial methods for up to 90 days after symptom onset, but they were shown to have detectable neutralising antibodies, suggesting false negative results with the SARS-CoV-2 IgG methods.

One sample tested positive on the Abbott SARS-CoV-2 anti-spike IgG (quantitative) method in the negative control group. The sample was collected in 2019 pre-pandemic and had tested positive for a seasonal coronavirus method at that time. The result was reproducible and suggests potential interference in the method from other coronaviruses. It is not known which coronavirus the patient had, as samples were anonymised. It is also possible that this patient was an early case of SARS-CoV-2, although this is considered unlikely, as when tested on other methods (Abbott SARS-CoV-2 IgG (qualitative), DiaSorin, and EDI) the sample was reported as negative for SARS-CoV-2 IgG antibodies. This is the first example of cross-reactivity with the Abbott SARS-CoV-2 IgG (quantitative) method, and the sample was sent to Abbott for further investigation and confirmation. All pre-pandemic healthy samples from 2018 were reported as negative, but one sample was close to the cut-off, again suggesting possible cross reactivity with the method. All of the TSI samples were negative, suggesting the Abbott SARS-CoV-2 anti-spike IgG (quantitative) method does not suffer from nonspecific antibody binding. Since the method validation was performed, Jung et al (2021) have also reported a case of a pre-pandemic sample being reported as positive with the Abbott SARS-CoV-2 anti-spike IgG method. This suggests a risk of false positive results with the method, although Narasimhan et al (2021) analysed samples from 1,236 participants and found no cross-reactivity (100% specificity) and a sensitivity of 96% using samples

collected 15 days after a positive SARS-CoV-2 RT-PCR result. Narasimhan et al (2021) were able to increase the sensitivity of the method to 100% by combining results from the Abbott SARS-CoV-2 anti-spike IgG and IgM methods together.

Concordance between the four SARS-CoV-2 IgG methods showed currently available immunomethods give a range of results with no standardisation or harmonisation between methods. All the methods had different units and different positivity cut-offs, which made a true method comparison difficult. The Abbott SARS-CoV-2 IgG (quantitative) and the DiaSorin methods are immunomethods that use the spike protein as their binding antigen. The Abbott SARS-CoV-2 IgG (qualitative) and the EDI immunomethods are both based on the nucleocapsid protein. The poorest agreement in SARS-CoV-2 IgG results was between the DiaSorin and the EDI methods (Cohen's Kappa 0.899), and the closest agreement was between the two Abbott methods (qualitative and quantitative) (Cohen's Kappa 0.966), with even the two methods from the same manufacturer showing some discrepancy in how the results were reported. However, McHugh (2012) states a kappa value of 0.75 or greater represents excellent agreement, and the four SARS-CoV-2 IgG methods showed kappa values of 0.899 or greater.

With the aim of standardising SARS-CoV-2 IgG results, several materials were purchased from the NIBSC. The NIBSC verification panel consisted of 37 convalescent plasma samples (23 anti-SARS-CoV-2 positive and 14 anti-SARS-CoV-2 negative). The samples were analysed by the four SARS-CoV-2 IgG methods and the results normalised as a ratio to the highest value for each method. This was to compare the magnitude of positive responses, as the results were difficult to compare directly due to differences in values reported and units. Each method gave the expected positive or negative result, with a much clearer distinction in positive results obtained with the spike-based Abbott (quantitative) and DiaSorin methods, than the nucleocapsid-based Abbott (qualitative) and EDI methods.

There were no assigned values for the NIBSC verification panel samples and they could not be used for standardisation. The NIBSC calibrant and QC materials were also unsuitable for standardisation as the calibrant had an assigned arbitrary unit of 1000 U, but no volume unit, and the QC had no assigned value. Since the evaluation was completed, NIBSC have

released an international standard to allow assays to report in the same binding antibody units per ml (BAU/mL) and enable standardisation between methods (Kristiansen et al., 2021). However, Saker et al (2021) used this international standard to compare the results from four SARS-CoV-2 IgG methods, and showed antibody concentrations correlated well, but that harmonisation is not yet achieved due to differences in what the methods are measuring. The NIBSC international standard was not tested in this evaluation.

The Abbott SARS-CoV-2 (quantitative) method was capable of detecting a rise and fall in SARS-CoV-2 anti-spike IgG antibody concentrations raised against three different SARS-CoV-2 strains. Samples from patients with the Spanish SARS-CoV-2 strain (B.1.177), and two variants of concern (VOC 202012/V1 [UK] and VOC 202012/V2 [South African]) were analysed. The Spanish lineage B.1.117 was the major circulating strain of SARS-CoV-2 in the UK at the start of the evaluation (Autumn 2020), but by January 2021, the UK variant of concern (VOC) B1.1.7 (VOC 202012/V1 [UK]) had taken over as the predominant strain in the UK. The third strain tested was an imported case of the South African VOC 202012/V2. As the pandemic progresses, it will be advantageous if immunomethods are able to detect SARS-CoV-2 IgG antibodies to any new variants that may emerge. The most predominant virus in the UK during December 2021 was the Delta variant, but this strain was not tested in this evaluation. Further work is required to check the Abbott SARS-CoV-2 anti-spike IgG (quantitative) method is capable of measuring antibodies raised against the Delta variant, as well as the new Omicron variant, and any future variants that emerge.

Further work is also required to compare SARS-CoV-2 anti-spike IgG concentrations to neutralising antibody concentrations to provide confidence the method can be used as a marker of immunity. A study by Jääskeläinen et al (2020) showed the Abbott SARS-CoV-2 anti-nucleocapsid IgG method compared well with a neutralising method. Comparison with the anti-spike IgG method would allow the positivity cut-off to be adjusted to ensure positive antibody results correlate with SARS-CoV-2 immunity.

The method evaluation did not test for interference with rheumatoid factor (RF), an autoantibody against the Fc portion of IgG, but RF is a common cause of cross-reactivity in immunoassays (Jääskeläinen et al., 2020). Jääskeläinen et al (2020) showed RF did interfere

in some of the serology methods they tested (Acro IgG and IgM lateral flow methods), and it is unknown if RF interferes with the Abbott SARS-CoV-2 anti-spike IgG method.

2.5 Conclusion

The novel quantitative method for SARS-CoV-2 anti-spike IgG from Abbott was fully evaluated, and in addition to good analytical performance, the method demonstrated potential utility in monitoring the immune response to SARS-CoV-2 natural infections. The second part of this project will use this method to evaluate the immune response following vaccination in a group of health care workers.

Chapter 3

Evaluation of the antibody response following dose one of the BNT162b (Pfizer/BioNTech) SARS-CoV-2 vaccine

3.1 Introduction

In line with the Joint Committee on Vaccination and Immunisation (JCVI) prioritisation (Department of Health and Social Care, 2021b) the Norfolk and Norwich University Hospital (NNUH) began vaccinating its healthcare workers (HCW) against SARS-CoV-2 on Monday 11th January 2021. The NNUH aimed to vaccinate all staff by the end of the month, and by January 25th 2021, 98% of staff had been vaccinated. The majority were vaccinated using the BNT162b2 (Pfizer/BioNTech) SARS-CoV-2 vaccine.

The aim of this chapter is to use the fully validated Abbott SARS-CoV-2 anti-spike IgG (quantitative) method from chapter 2 to evaluate the immune response following SARS-CoV-2 vaccination in a cohort of HCW. Data from chapter 2 showed the method was capable of measuring the rise and fall in SARS-CoV-2 IgG antibody production following natural infection with three different SARS-CoV-2 strains. This part of the project will show whether the method is also capable of identifying SARS-CoV-2 IgG antibodies produced following vaccination. The Abbott SARS-CoV-2 IgG anti-nucleocapsid (qualitative) and IgM methods will also be used to fully evaluate the immune response to vaccination.

Previous studies have shown many factors, such as age, gender, Body Mass Index (BMI), ethnicity and vitamin D status can influence the immune response. Aging results in a diminished function of mature antibodies and a decline in immune function, a phenomenon known as immunosenescence (Montecino-Rodriguez et al., 2013, Kurupati et al., 2016). Vaccines have also been shown to be less effective in the 65 years and older population, due to a reduced ability of the immune system to respond (McElhaney and Dutz, 2008). Adult females have been shown to mount stronger immune responses than males, with a greater vaccine efficacy (Klein and Flanagan, 2016). Studies have also shown having an overweight BMI leads to reduced antibody responses to the hepatitis B vaccine in adults (Weber at al.,

1986), and the tetanus vaccine in children (Eliakim et al., 2006). Sheridan et al (2012) found those with an overweight BMI showed a significant decrease over time in antibody concentrations compared with healthy weight individuals. Ethnicity is another factor that has been reported as influencing the immune response, with African Americans shown to mount a greater antibody response to the influenza vaccine than Caucasians (Kurupati et al., 2016).

Age, gender, BMI, ethnicity, and vitamin D status prior to SARS-CoV-2 vaccination will be evaluated to identify factors leading to a greater antibody response. It is hypothesised that younger, female, non-Caucasian participants, with a healthy BMI and adequate vitamin D concentration prior to vaccination, will show the greatest antibody response.

3.2 Methodology

The CALM (COVID-19 antibody longitudinal monitoring) study is an observational cohort study performed at the NNUH in the United Kingdom (UK). HCW were recruited in January 2021, prior to the UK vaccination programme roll out, with the aim of evaluating the immune response to SARS-CoV-2 vaccination in a real world setting.

3.2.1 Study design and participants 111 HCW were recruited to the CALM study. Two participants were unable to provide the required blood samples and dropped out of the study; all of their results were removed. Two participants were vaccinated with vaccines other than Pfizer/BioNTech; their results were not included in the analysis. The study was approved by the Health Research Authority and Health and Care Research Wales (HCRW) ethical committee (IRAS project ID #292799) (Appendix E). Written informed consent was obtained from all study participants. A copy of the consent form, and the patient information sheet provided prior to consenting are shown in Appendix F and Appendix G respectively.

3.2.2 Procedures All participants were interviewed face-to-face by Good Clinical Practice (GCP) trained researchers. Participants were asked to complete a questionnaire (Appendix H) to provide demographic characteristics (age, gender, ethnicity), clinical characteristics

(weight, height, health issues, current medication), and the presence of SARS-CoV-2 symptoms within the last six months. The immune response generated after vaccination with the BNT162b (Pfizer Inc. [New York] and BioNTech SE [Mainz, Germany]) SARS-CoV-2 vaccine in 107 HCW was assessed. Baseline (week 0) venous blood samples were collected from all participants in the week prior to their first vaccination. The serum was analysed for SARS CoV-2 anti-spike IgG (quantitative), SARS CoV-2 anti-nucleocapsid IgG (qualitative), and IgM antibody concentrations. Subsequent venous samples were then collected at one, two, three, four, and eight weeks post vaccination, and in the week prior to their second vaccination (week ten for most participants). Blood samples were collected by trained NNUH phlebotomists within one day either side of the expected date. Total 25-hydroxy vitamin D and vitamin D metabolites (see below) were measured on the baseline and week ten samples. Some participants also provided blood samples at weeks five, six, and seven post dose one for analysis.

3.2.3 Abbott SARS-CoV-2 IgG (quantitative) method The Abbott SARS-CoV-2 anti-spike IgG (quantitative) method is an automated two-step chemiluminescent microparticle immunomethod (CMIA). It is used for the quantitative determination of IgG antibodies, including neutralising antibodies, to the receptor binding domain (RBD) of the S1 subunit of the spike protein of SARS-CoV-2 in human serum and plasma on the Abbott Alinity i system (Abbott Park, Chicago, IL, USA). The analytical measuring interval is 21.0 – 40,000.0 Arbitrary Units per mL (AU/mL). Samples above 40,000.0 AU/mL are automatically diluted by the Abbott Alinity i system, with Abbott Alinity i multi method manual diluent, and samples above 80,000.0 AU/mL are diluted manually. The positivity cut-off stated by Abbott is 50.0 AU/mL.

3.2.4 Abbott SARS-CoV-2 IgG (qualitative) method The SARS-CoV-2 anti-nucleocapsid IgG qualitative method from Abbott is an automated two step CMIA. It is used for the qualitative determination of IgG antibodies to the nucleocapsid protein of SARS-CoV-2 in human serum and plasma on the Abbott Alinity i system (Abbott Park, Chicago, IL, USA). The positivity cut-off is 1.40 Index, calculated by dividing the sample Relative Light Units (RLUs) by the calibrator RLUs.

3.2.5 Abbott SARS-CoV-2 IgM (qualitative) method The SARS-CoV-2 IgM qualitative method from Abbott is an automated two step CMIA. It is used for the qualitative determination of IgM antibodies to the spike protein of SARS-CoV-2 in human serum and plasma on the Abbott Alinity i system (Abbott Park, Chicago, IL, USA). The positivity cut-off is 1.00 Index, calculated by dividing the sample RLUs by the calibrator RLUs.

3.2.6 Vitamin D The total vitamin D method from Abbott is a delayed one step immunomethod. It is used for the quantitative determination of total 25-hydroxy vitamin D (25(OH)D) in human serum and plasma using CMIA technology. Total vitamin D ($25(OH)D_2$ and $25(OH)D_3$), $24,25(OH)_2D_2$ and $24,25(OH)_2D_3$ were measured simultaneously by a published mass spectrometry method (Tang et al., 2017). 1,25-dihydroxy vitamin D ($1,25(OH)_2D$) was measured by immunoassay (DiaSorin Liaison XL).

3.2.7 Statistics Microsoft Excel (2010) was used to perform all calculations and statistical analysis. Mean and SD were used to describe normally distributed data. Median and interquartile ranges were used to describe non-normally distributed data. Box and whisker plots were used to present antibody data at different time points following vaccination. Wilcoxon Rank Sum Tests (or Mann Whitney U tests) were used to evaluate participant vitamin D concentrations, as well as to compare differences between week three and ten SARS-CoV-2 anti-spike IgG values for two different age, vitamin D, and BMI categories. For statistical analyses two tailed p values <0.05 were considered to be statistically significant.

3.3 Results

The immune response of 107 HCW was assessed following vaccination with dose one of the SARS-CoV-2 (Pfizer/BioNTech) vaccine. The effect of participant age, gender, ethnicity, BMI, and vitamin D status prior to vaccination was evaluated to identify factors leading to an increased immune response.

3.3.1 CALM participant demographics The demographics of the 107 CALM participants are shown in *Table 3.1*. The mean age of participants was 41 ±11 years (mean ±SD), with the majority being white (91%) and female (78%). The median (IQR) BMI was 24 (21 – 29) kg/m^2,

with 44% of participants classified as having a healthy BMI (18.5 – 24.9 kg/m^2), 54% classified as overweight (BMI >24.9 kg/m^2), and 2% classified as underweight (BMI <18.5 kg/m^2)(ranges from NHS, 2019). No participants reported current SARS-CoV-2 symptoms (cough/fever/loss of taste or smell) at the time of recruitment, and 2% reported these symptoms during the previous six months. 21% of participants described themselves as suffering health problems, and 46% were taking medication at the time of the study.

3.3.2 CALM participant vitamin D status Participant total 25(OH)D concentrations at baseline prior to dose one of the SARS-CoV-2 vaccine, and then ten weeks later prior to dose two are shown in *Table 3.2*. The median (IQR) baseline 25(OH)D was 37 (21 – 62) nmol/L, with 31% of participants classified as being vitamin D insufficient (<25 nmol/L), 35% deficient (25 – 50 nmol/L) and 35% replete (>50 nmol/L) (ranges from Royal Osteoporosis Society, 2020). As 65% of participants were vitamin D deficient, voluntary supplementation guidance (Appendix I) was issued to all participants.

Following ten weeks of supplementation by most participants, the median (IQR) 25(OH)D increased significantly ($p<0.001$) by 49% to 73 (547 – 96) nmol/L, with 4% classified as insufficient, 16% deficient, and 80% replete *(Figure 3.1)*. Analysis of the 25(OH)D_3 and 25(OH)D_2 fractions showed the increase in total 25(OH)D was due to the 25(OH)D_3 fraction with a significant ($p<0.001$) increase of 50.0% from baseline. There was no increase in 25(OH)D_2 concentration following supplementation. Median (IQR) 1,25(OH)$_2$D increased from 104.0 (83.2 – 128.2) pmol/L to 115.0 (96.3 – 141.5) (+9.6%; $p=0.02$) following supplementation (reference range 55 – 139 pmol/L), while the median 25(OH)D:24,25(OH)$_2$D ratio (reference range 7 – 23) decreased slightly but remained within the reference range (16.8 and 15.2 at baseline and following supplementation respectively).

Figure 3.2 shows the total 25(OH)D results from the Abbott Alinity immunomethod compared well with results obtained by a published mass spectrometry method (Tang et al., 2017) ($R^2 = 0.9667$). The Abbott Alinity 25(OH)D results have been used for all subsequent vitamin D analysis.

Table 3.1: Participant demographics at baseline prior to dose one of the BNT162b (Pfizer/BioNTech) SARS-CoV-2 vaccine for the healthcare workers recruited to the CALM study.

	Dose 1 Baseline
Number of participants	107
Age – mean years (SD)	41 (11)
Weight – median kg (IQR)	72 (60 - 89)
Height – median cm (IQR)	167 (162 - 170)
BMI – median kg/m^2 (IQR)	24 (21 - 29)
Underweight (<18.5) – n (%)	2 (2%)
Healthy (18.5 – 24.9) – n (%)	47 (44%)
Overweight (>24.9) – n (%)	57 (54%)
Race – n (%)	
White	97 (91)
BAME	9 (8)
Did not disclose	1 (1)
Sex – n (%)	
Female	83 (78)
Male	24 (22)
Health Problems – n (%)	
Yes	23 (21)
No	84 (79)
Current COVID-19 symptoms – n (%)	
No	107 (100)
COVID-19 symptoms in past 6 months – n (%)	
Yes	2 (2)
No	105
Taking medication – n (%)	
Yes	49 (46)
No	57 (53)
Did not answer	1 (1)

Table 3.2: CALM participant vitamin D metabolite concentrations at baseline prior to dose one of the BNT162b (Pfizer/BioNTech) SARS-CoV-2 vaccine, and ten weeks later following vitamin D supplementation. Percentage change and two tailed p-values are shown. P-values were calculated using Wilcoxon Rank Sum Tests.

	Baseline	10 Weeks post supplementation	Percentage Change (%)
25(OH)D nmol/L – median (IQR)	37 (21 – 62)	73 (54 – 96)	+49 (p<0.001)
Insufficient (<25 nmol/L) – n (%)	33 (31)	4 (4)	–725
Deficient (25 – 50 nmol/L) – n (%)	37 (35)	17 (16)	–118
Replete (>50 nmol/L) – n (%)	37 (35)	84 (80)	+56
$25(OH)D_2$ nmol/L – median (IQR)	2.6 (1.8 – 3.8)	2.0 (1.4 – 3.2)	–30.0 (p=0.008)
$25(OH)D_3$ nmol/L – median (IQR)	36.8 (21.6 – 63.5)	73.6 (56.5 – 90.1)	+50.0 (p<0.001)
$1,25(OH)_2D$ pmol/L – median (IQR) (reference range 55 – 139 pmol/L)	104.0 (83.2 – 128.2)	115.0 (96.3 – 141.5)	+9.6 (p=0.02)
$24,25(OH)_2D_2$ nmol/L – median (IQR)	0.0 (0.0 – 0.0)	0.0 (0.0 – 0.0)	0.0
$24,25(OH)_2D_3$ nmol/L – median (IQR)	2.4 (1.2 – 5.0)	5.3 (3.5 – 7.0)	+54.7 (p<0.001)
$25(OH)D:24,25(OH)_2D$ – median (IQR) (reference range 7 – 23)	16.8 (13.2 – 21.1)	15.2 (13.1 – 18.2)	–10.5 (P=0.05)

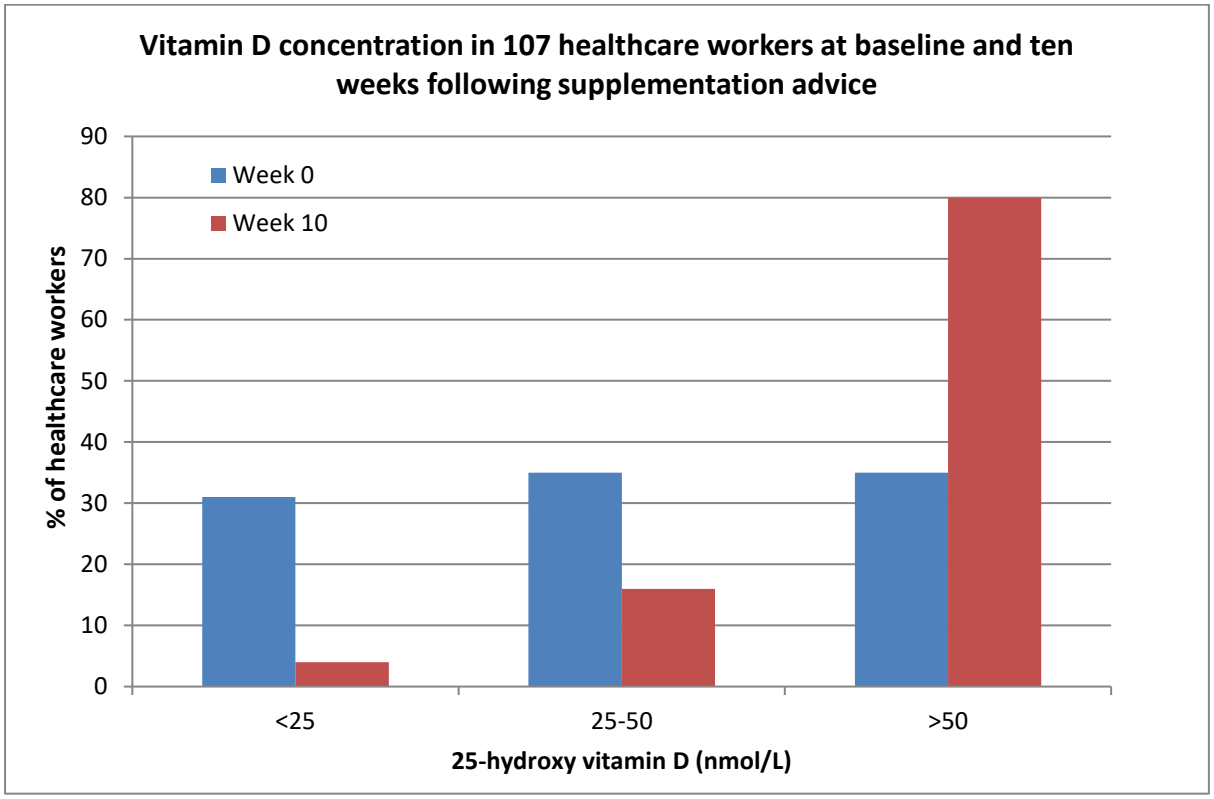


Figure 3.1: Total 25-hydroxy vitamin D (nmol/L) concentration of 107 healthcare workers, measured by Abbott Alinity i immunomethods, at baseline prior to dose one of the BNT162b2 (Pfizer/BioNTech) SARS-CoV-2 vaccine, and ten weeks later following vitamin D supplementation advice.

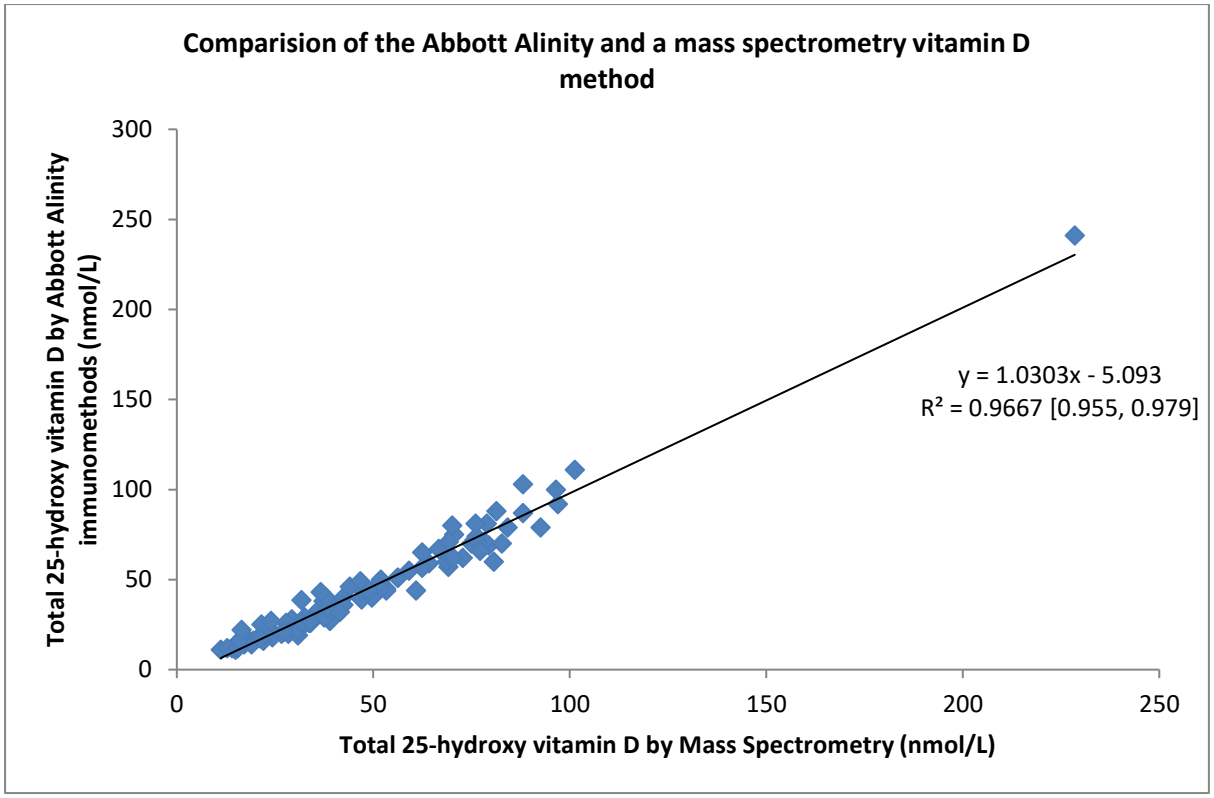


Figure 3.2: Comparison of total 25-hydroxy vitamin D (nmol/L) concentration of 107 healthcare workers measured by Abbott Alinity i immunomethods and a published mass spectrometry method (Tang et al., 2017).

3.3.3 CALM participant antibody response to dose one of the BNT162b (Pfizer/BioNTech) SARS-CoV-2 vaccine 105 CALM participants (98%) had a measured SARS-CoV-2 anti-spike IgG (quantitative) concentration above the positivity cut-off (50.0 AU/mL) following dose one of the SARS-CoV-2 (Pfizer/BioNTech) vaccine. Two participants did not develop antibody concentrations above the positivity cut-off, with peaks of 31.5 AU/mL and 11.8 AU/mL respectively. Six of the 107 participants had a positive SARS-CoV-2 anti-spike IgG (quantitative) result at baseline, with concentrations ranging from 58.1 to 5,496.9 AU/mL.

Of the 107 CALM participants, 75 (70%) had a measured SARS-CoV-2 IgM concentration above the positivity cut-off (1.00 Index) following dose one of the SARS-CoV-2 (Pfizer/BioNTech) vaccine, with 30% of participants not developing an IgM response. Four participants had a positive IgM result at baseline, with concentrations ranging from 1.09 to 9.18 Index.

Three CALM participants (2.8%) had a measured SARS-CoV-2 anti-nucleocapsid IgG (qualitative) concentration above the positivity cut-off (1.40 Index) following dose one of the SARS-CoV-2 (Pfizer/BioNTech) vaccine, with concentrations ranging from 1.41 to 7.78 Index.

The median SARS-CoV-2 anti-spike IgG (quantitative), anti-nucleocapsid IgG (qualitative), and IgM concentrations measured at baseline, and ten weeks later prior to dose two, are shown in *Table 3.3*. The median concentrations for all three antibody methods were negative at baseline. Ten weeks later, the median anti-spike IgG (quantitative) concentration had increased by 99.9% to 526.6 AU/mL (positivity cut-off = 50.0 AU/mL). The median anti-nucleocapsid IgG (qualitative) concentration remained below the positivity cut-off (0.03 to 0.03; positivity cut-off = 1.4 Index). There was a 66.7% increase in the median IgM concentration (0.08 to 0.24 Index), but the median concentration remained below the positivity cut-off (1.00 Index).

Table 3.3: Median and interquartile ranges for SARS-CoV-2 antibody concentrations measured by the Abbott Alinity immunomethod in 107 CALM participants, prior to dose one of the Pfizer/BioNTech SARS-CoV-2 vaccine, and ten weeks later prior to dose two, together with the percentage change.

	Antibody concentration – median (IQR)		
	Baseline	Week 10	Percentage Change (%)
SARS-CoV-2 anti-spike IgG quantitative (AU/mL) [cut-off = 50.0]	0.75 (0.03 – 2.73)	526.6 (298.7 – 949.5)	99.9
SARS-CoV-2 anti-nucleocapsid IgG qualitative (Index) [cut-off = 1.40]	0.03 (0.02 – 0.09)	0.03 (0.02 – 0.08)	0
SARS-CoV-2 IgM qualitative (Index) [cut-off = 1.00]	0.08 (0.05 – 0.12)	0.24 (0.09 – 0.39)	66.7

Table 3.4 shows the median SARS-CoV-2 anti-spike IgG (quantitative), anti-nucleocapsid IgG (qualitative), and IgM concentrations at each week following vaccination. The median SARS-CoV-2 anti-spike IgG (quantitative) concentration became positive at week two (693.0 AU/mL; positivity cut-off = 50.0), peaked at week three (1410.8 AU/mL), and was still positive at week ten (526.6 AU/mL). The median SARS-CoV-2 anti-nucleocapsid IgG (qualitative) concentration remained below the positivity cut-off (1.40 Index) with a peak of 0.07 Index at week seven. The median IgM peaked at week two (1.56 Index; positivity cut-off = 1.00), and then fell below the positivity cut-off at week five post vaccination.

Box plots showing the immune response following vaccination for SARS-CoV-2 anti-spike IgG (quantitative), SARS-CoV-2 anti-nucleocapsid IgG (qualitative), and IgM are shown in *Figures 3.3, 3.4, and 3.5* respectively. Data is shown for the 99 participants that developed an antibody response; those who had positive baseline concentrations (n=6), and those who did not develop antibodies above the positivity cut-off (n=2) were excluded. *Figure 3.3* shows the Abbott SARS-CoV-2 anti-spike IgG (quantitative) method is capable of measuring the rise and fall in antibody concentrations following vaccination with the SARS-CoV-2 (Pfizer/BioNTech) vaccine. The same rise and fall was not observed with the qualitative anti-nucleocapsid IgG method *(Figure 3.4)*. An earlier rise and fall in IgM was observed with the Abbott SARS-CoV-2 IgM method *(Figure 3.5)*.

Figure 3.6 shows the anti-spike IgG (quantitative) and IgM immune response in example participants who had, and had not, been previously exposed to SARS-CoV-2 natural infection respectively.

Table 3.4: Median SARS-CoV-2 anti-spike IgG quantitative (AU/mL), anti-nucleocapsid qualitative (Index), and IgM (Index) concentrations at baseline and number of weeks following dose one of BNT162b (Pfizer/BioNTech) SARS-CoV-2 vaccine. Median concentrations above the positivity cut-offs are shown in yellow. 107 CALM participant samples were measured using the Abbott Alinity immunomethod. Weeks five, six, and seven only had samples for 27, 32, and 14 CALM participants respectively.

	Weeks following dose one of SARS-CoV-2 (Pfizer/BioNTech) vaccine									
	0	1	2	3	4	5	6	7	8	10
Anti-s IgG (AU/mL) Cut-off = 50.0	0.8	1.4	693.0	1410.8	1277.2	1077.9	916.1	629.7	695.6	526.6
Anti-n IgG (Index) Cut-off = 1.40	0.03	0.03	0.03	0.03	0.03	0.06	0.06	0.07	0.03	0.03
IgM (Index) Cut-off = 1.00	0.08	0.08	1.56	1.49	1.04	0.76	0.40	0.32	0.24	0.24

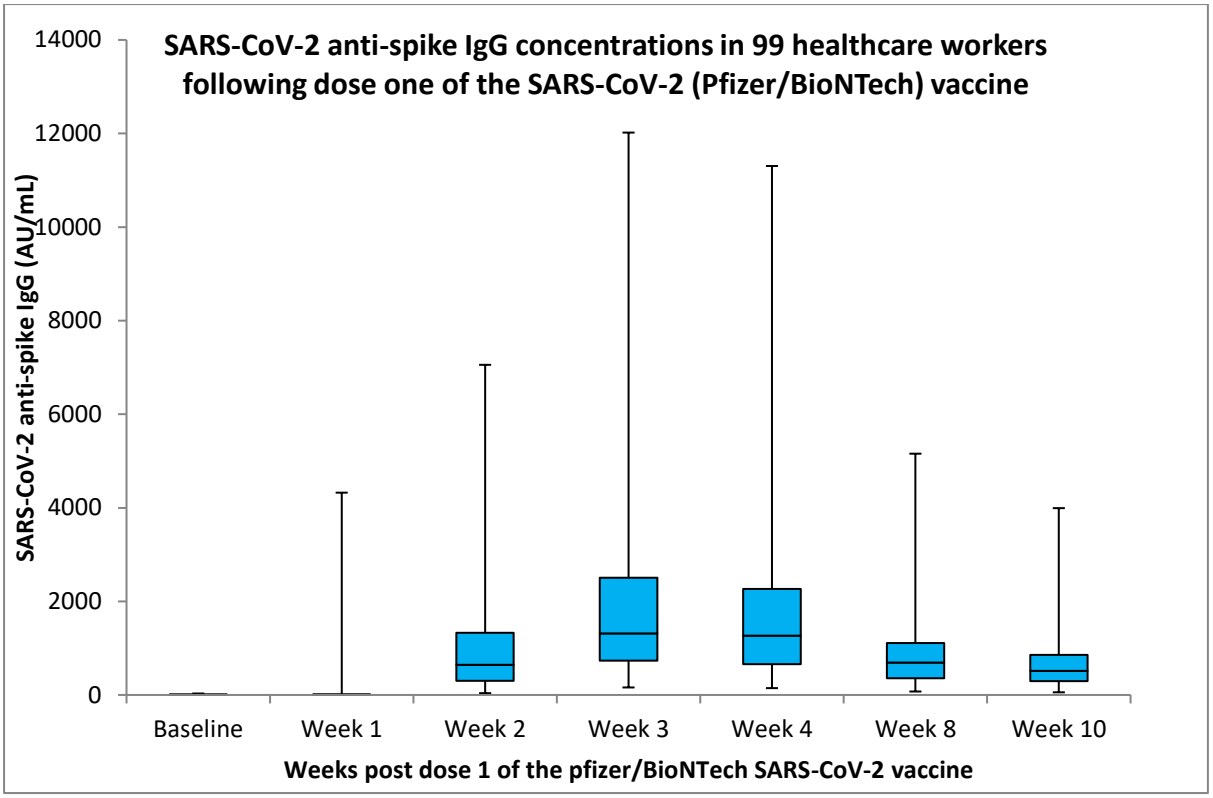


Figure 3.3: Box plot of SARS-CoV-2 anti-spike IgG (quantitative) (AU/mL) following dose one of SARS-CoV-2 (Pfizer/BioNTech) vaccine, measured by Abbott Alinity i system for 99 CALM participants. Positivity cut-off = 50.0 AU/mL.

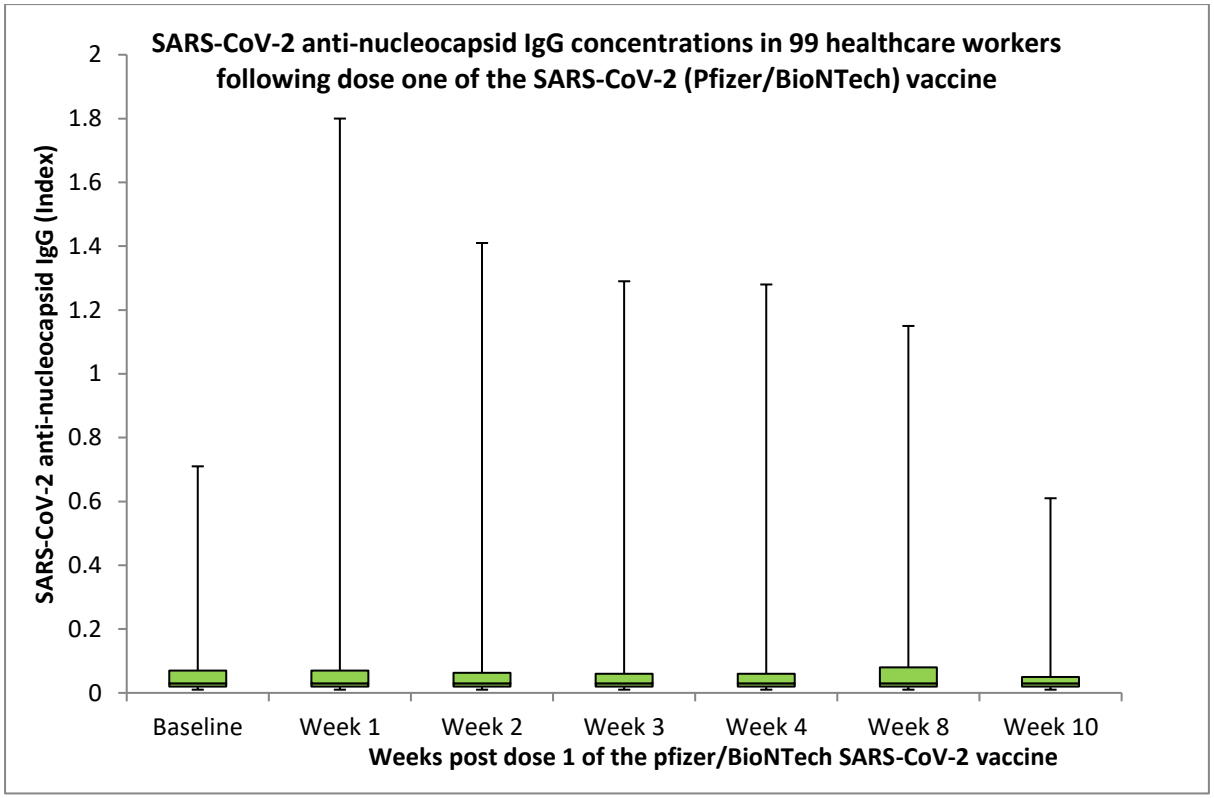


Figure 3.4: Box plot of SARS-CoV-2 anti-nucleocapsid IgG (qualitative) (Index) following dose one of SARS-CoV-2 (Pfizer/BioNTech) vaccine, measured by Abbott Alinity i system for 99 CALM participants. Positivity cut-off = 1.40.

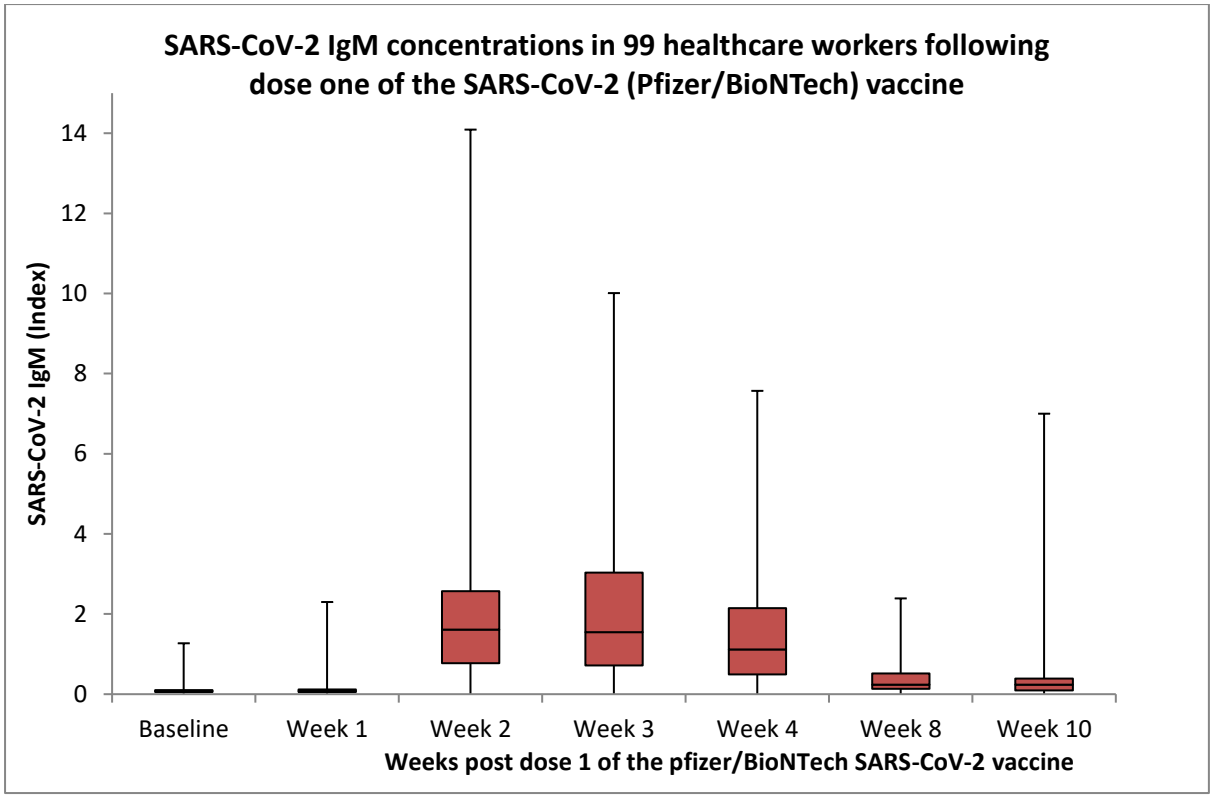
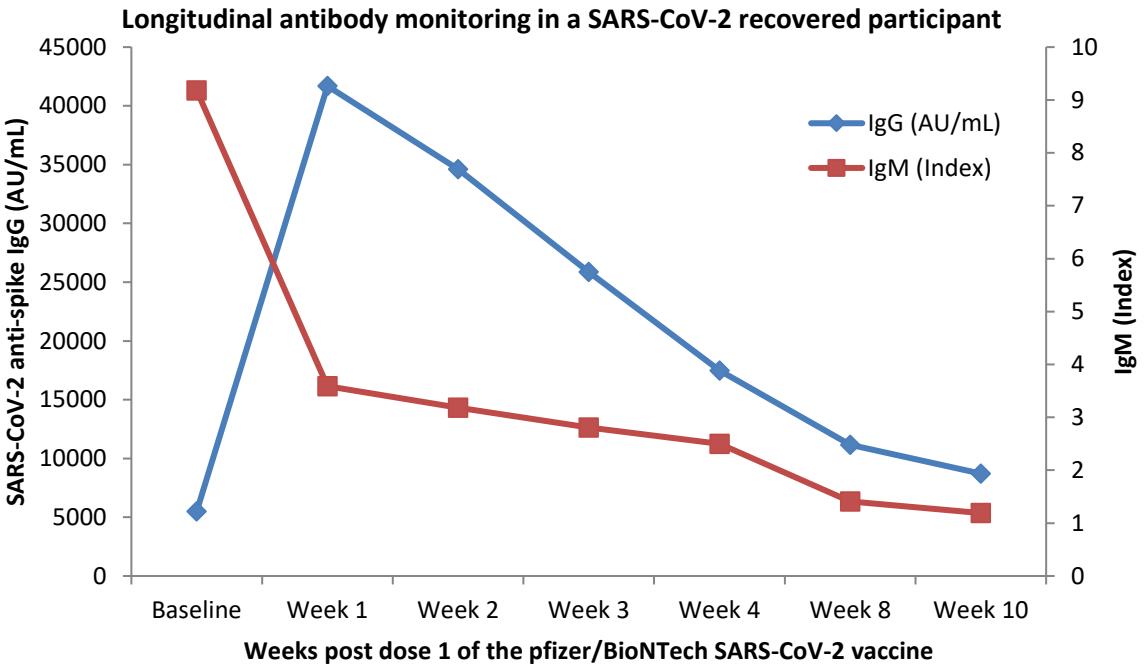


Figure 3.5: Box plot of SARS-CoV-2 anti-spike IgM (qualitative)(Index) following dose one of SARS-CoV-2 (Pfizer/BioNTech) vaccine, measured by Abbott Alinity i system for 99 CALM participants. Positivity cut-off = 1.00.

A



B

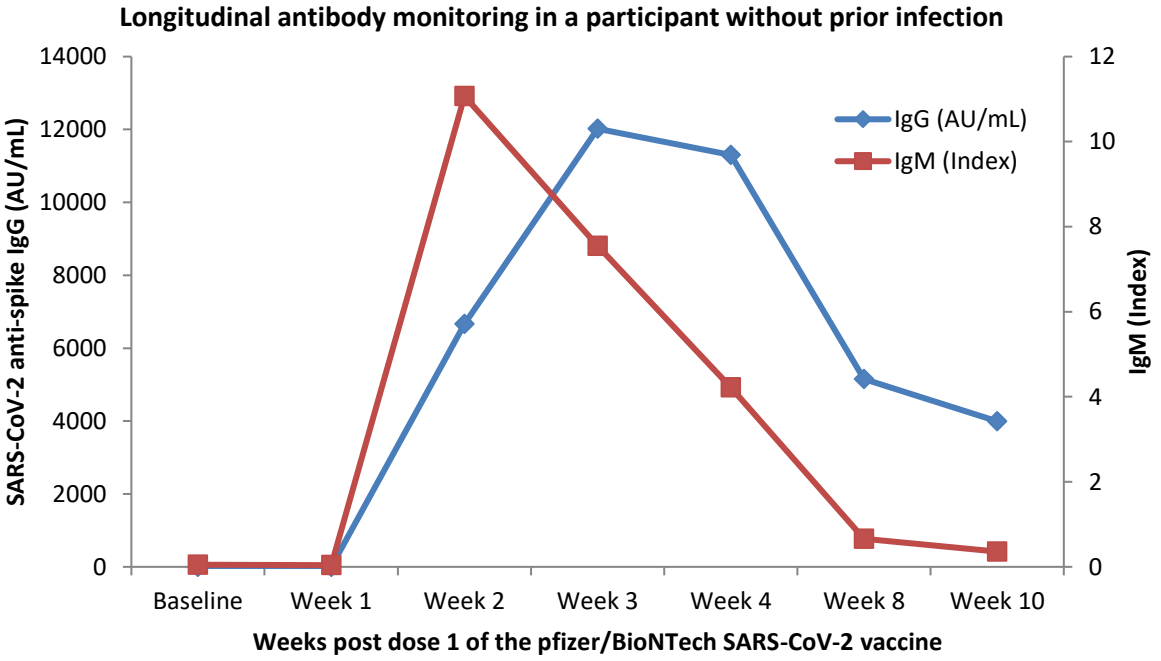


Figure 3.6: Abbott SARS-CoV-2 anti-spike IgG (quantitative) and IgM concentrations in the weeks following dose one of the Pfizer/BioNTech SARS-CoV-2 vaccine in A) a CALM participant who had previously been infected with SARS-CoV-2, and B) a CALM participant who had not previously been infected with SARS-CoV-2.

3.3.4 Effect of CALM participant demographics on SARS-CoV-2 anti-spike IgG (quantitative) antibody concentrations The effect of total 25(OH)D, BMI, and age on SARS-CoV-2 anti-spike IgG antibody development following dose one of the Pfizer/BioNTech SARS-CoV-2 vaccine are shown in *Figures 3.7, 3.8, and 3.9* respectively. Data shown is for the 99 CALM participants who developed an antibody response; those who had positive baseline concentrations (n=6), and those who did not develop antibodies above the positivity cut-off (n=2) were excluded from analysis.

Figure 3.7 splits the 99 CALM participants into those that were vitamin D insufficient (<25 nmol/L; n=33), deficient (25 – 50 nmol/L; n=35), and replete (>50 nmol/L; n=31)(ranges from Royal Osteoporosis Society, 2020). Wilcoxon Rank Sum tests showed the results were not significantly different between vitamin D replete (>50 nmol/L; n=37) or deficient (<50 nmol/L; n=70) participants at week three peak concentrations (p=0.32) or week ten concentrations (p=0.25).

Figure 3.8 splits the same 99 participants into those that were underweight (BMI <18.5 kg/m^2; n=2), healthy weight (18.5 – 24.9 kg/m^2; n=43), and overweight (>24.9 kg/m^2; n=54)(ranges from NHS, 2019). Wilcoxon Rank Sum Tests showed the results were not significantly different between participants with an overweight BMI >24.9 kg/m^2 (n=54) or a BMI <24.9 kg/m^2 (n=45) at week three peak concentrations (p=0.93) or week ten concentrations (p=0.91). There were only two individuals in the underweight category and significance was not estimated.

Figure 3.9 splits the participants by age (<30 years; n=22, 30 – 40 years; n=23, 40 – 50 years; n=35, and >50 years; n=19). Wilcoxon Rank Sum Tests showed the results were significantly different between participants over 40 years of age (n=57) and those under 40 years of age (n=42) at week three peak concentrations (p<0.0001) and week ten concentrations (p<0.0001).

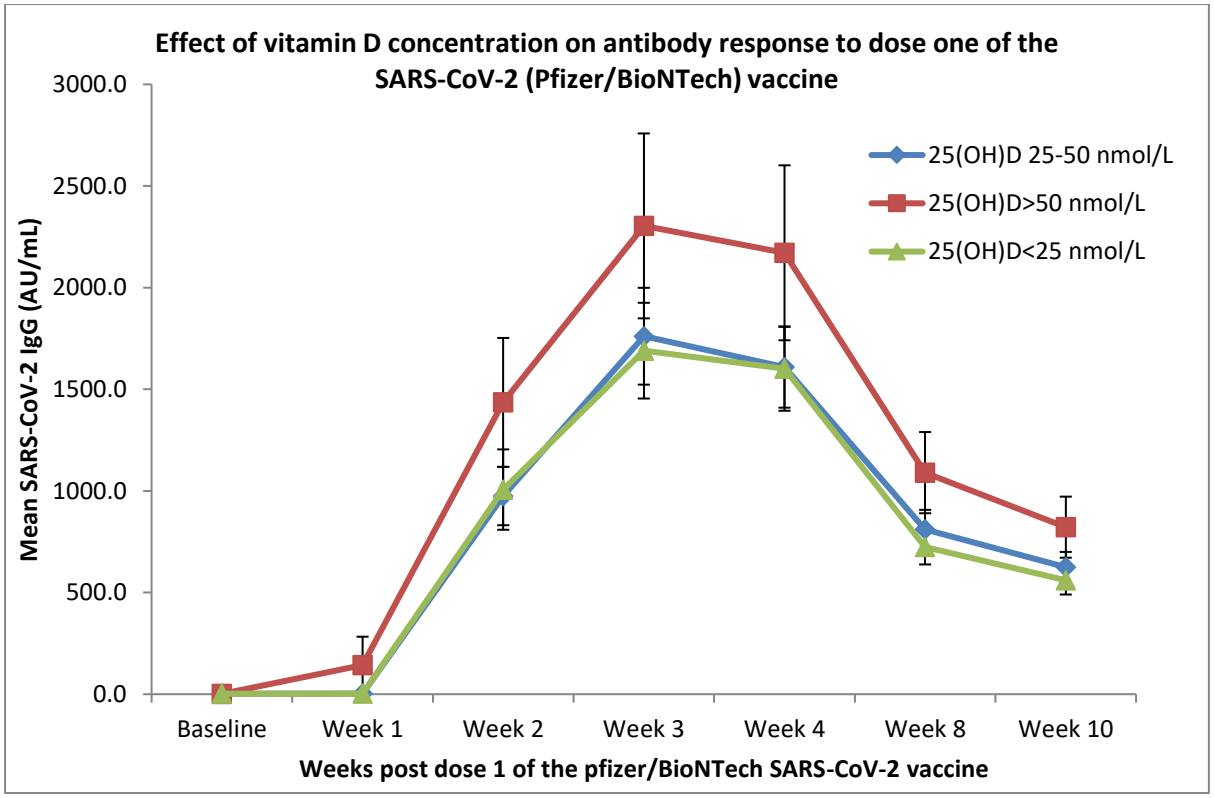


Figure 3.7: Effect of total 25-hydroxy vitamin D (nmol/L) on SARS-CoV-2 IgG development in 99 CALM participants following dose one of the SARS-CoV-2 (Pfizer/BioNTech) vaccine. Error bars represent standard error of the mean. IgG results measured by the Abbott SARS-CoV-2 anti-spike IgG method on the Alinity i system. Wilcoxon Rank Sum Tests showed the results were not significantly different between vitamin D replete (>50 nmol/L; n=37) or deficient (<50 nmol/L; n=70) participants at week three peak concentrations (p=0.32) or at week ten concentrations (p=0.25).

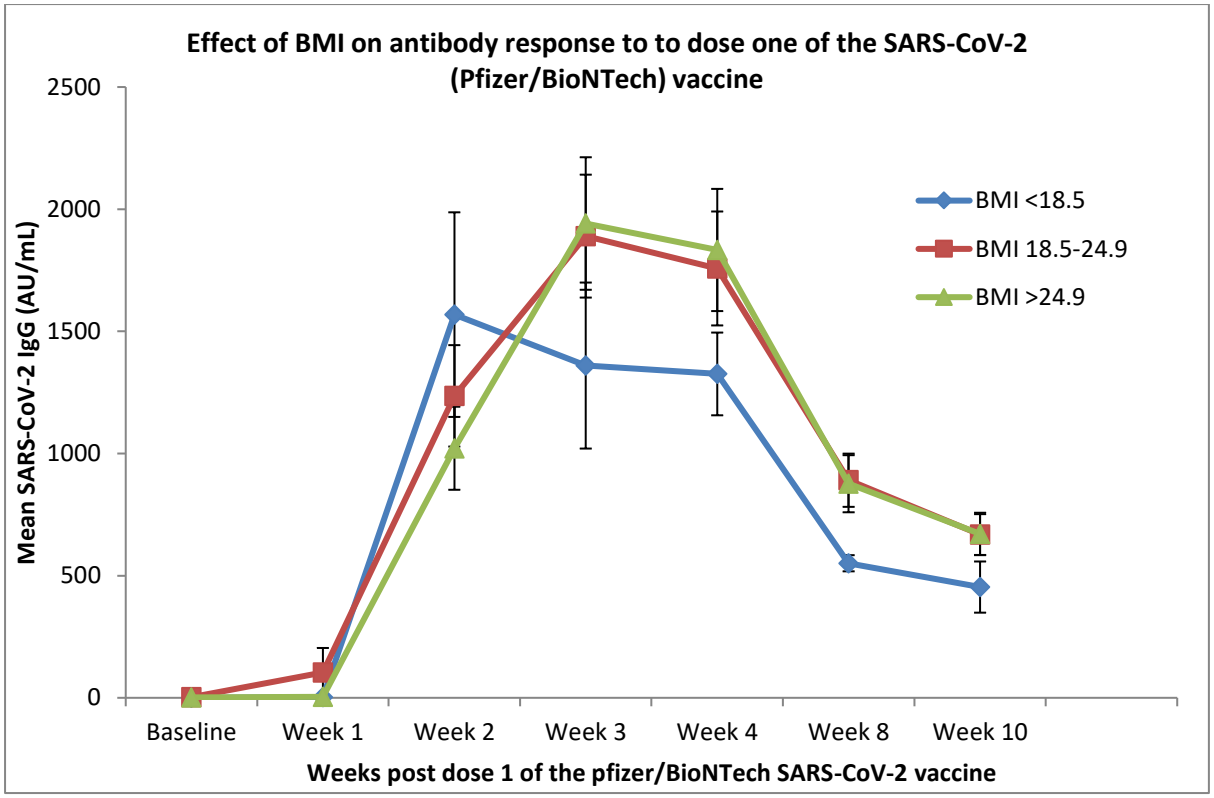


Figure 3.8: Effect of BMI (kg/m^2) on SARS-CoV-2 IgG development in 99 CALM participants following dose one of the SARS-CoV-2 (Pfizer/BioNTech) vaccine. Error bars represent standard error of the mean. IgG results measured by the Abbott SARS-CoV-2 anti-spike IgG method on the Alinity i system. Wilcoxon Rank Sum Tests showed the results were not significantly different between participants with an overweight BMI >24.9 kg/m^2 (n=54) or a BMI <24.9 kg/m^2 (n=45) at week three peak concentrations (p=0.93) or week ten concentrations (p=0.91). There were only two individuals in the underweight category and significance was not estimated.

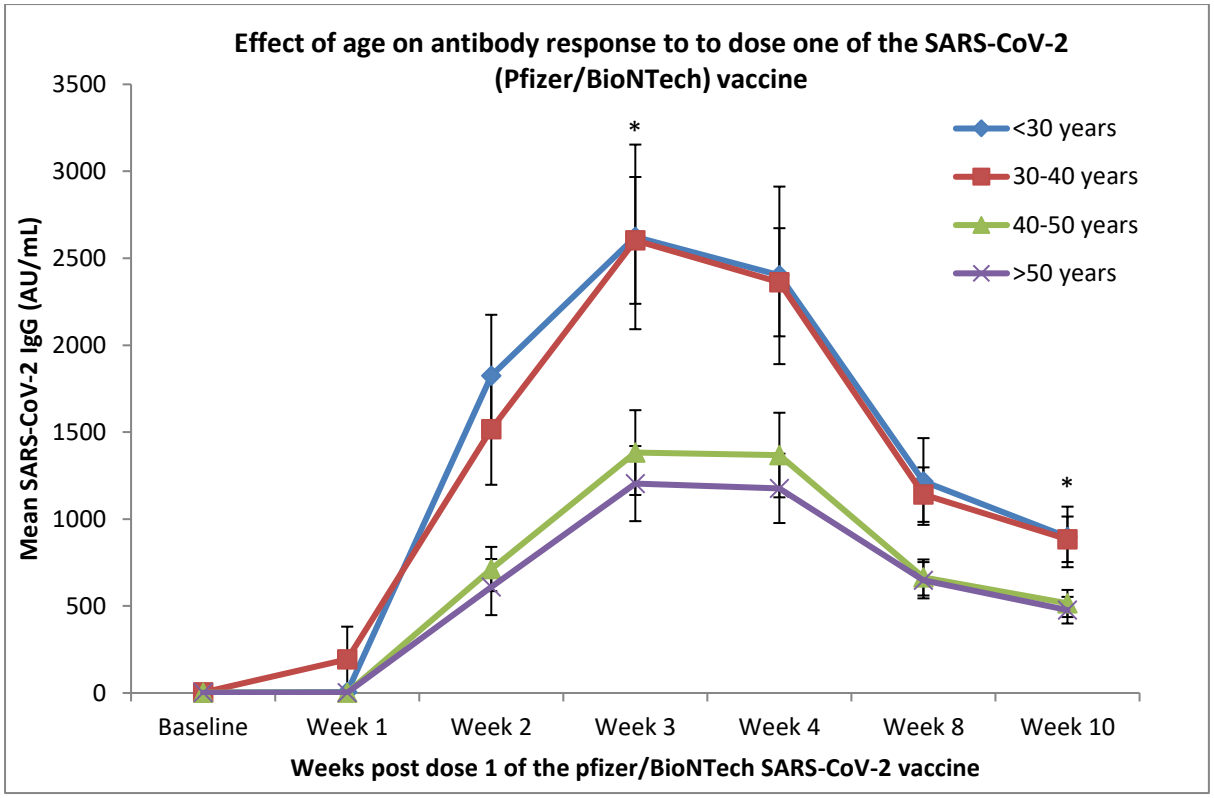


Figure 3.9: Effect of age (years) on SARS-CoV-2 IgG development in 99 CALM participants following dose one of the SARS-CoV-2 (Pfizer/BioNTech) vaccine. Error bars represent standard error of the mean. IgG results measured by the Abbott SARS-CoV-2 anti-spike IgG method on the Alinity i system. Wilcoxon Rank Sum Tests showed the results were significantly different between participants over 40 years of age (n=57) and those under 40 years of age (n=42) at week three peak concentrations ($p<0.0001$) and week ten concentrations ($p<0.0001$).

3.4 Discussion

The Abbott SARS-CoV-2 anti-spike IgG (quantitative) method was capable of measuring the rise and fall in antibody response in HCW following vaccination with the BNT162b (Pfizer/BioNTech) SARS-CoV-2 vaccine. 98% of CALM participants responded to the first dose of the vaccine, with a measured SARS-CoV-2 anti-spike IgG antibody concentration above the positivity cut-off.

Two CALM participants did not respond to the first dose of the SARS-CoV-2 (Pfizer/BioNTech) vaccine. One of these participants was a 48 year old Caucasian female taking Ustekinumab, an immunosuppressant for Crohn's disease. Their peak SARS-CoV-2 anti-spike IgG was 31.5 AU/mL (positivity cut-off = 50.0). Immunosuppressants have previously been shown to impair the immune response to vaccination in patients with inflammatory bowel disease (Cao et al., 2015), and the results for this participant suggest patients taking immunosuppressant drugs should be offered a second dose of the SARS-CoV-2 vaccine at the manufacturer recommended three week interval.

The other participant not responding to dose one of the SARS-CoV-2 (Pfizer/BioNTech) vaccine was a 57 year old Caucasian female taking a number of drugs (metformin and dapagliflozin for type 2 diabetes, amitriptyline, sertraline and pregablin for depression and anxiety, amlodipine, and Ramipril for hypertension, atorvastatin for hypercholesterolaemia, lansoprazole for acid reflux, asprin, and co-codamol); none of which are known to affect antibody production. Their peak SARS-CoV-2 anti-spike IgG was 11.8 AU/mL (positivity cut-off = 50.0). This participant subsequently reported not responding to their hepatitis B vaccination either. They may also have benefited from an earlier second dose of the vaccine, rather than waiting ten weeks without any protection from SARS-CoV-2 infection.

The Abbott SARS-CoV-2 anti-nucleocapsid IgG (qualitative) method was not able to detect the rise and fall in antibody response in HCW following vaccination. The BNT162b (Pfizer/BioNTech) SARS-CoV-2 vaccine is based on a SARS-CoV-2 spike glycoprotein antigen (Pfizer-BioNTech, 2020), and antibodies raised against the vaccine will only be detected by anti-spike based IgG methods. Anti-nucleocapsid based IgG methods will not detect

antibodies raised against the SARS-CoV-2 vaccine. A recent study reported the Abbott SARS-CoV-2 anti-nucleocapsid and anti-spike IgG methods were able to clearly differentiate naïve, SARS-CoV-2 infected, and vaccine-related immune responses (Narasimhan et al., 2021). Laboratories must be aware of the limitations of their methods to avoid misinterpretation of results. Once the population has been vaccinated, anti-nucleocapsid based IgG methods will be useful in identifying patients exposed to SARS-CoV-2, as these methods will only detect antibodies raised against natural infection, and not from vaccination.

70% of CALM participants developed an IgM response to dose one of the SARS-CoV-2 (Pfizer/BioNTech) vaccine. Ma et al (2020) found the medium seroconversion time for SARS-CoV-2 IgM antibodies was four to six days post symptom onset, and it is unlikely samples collected at week one missed the IgM peak concentration. It is possible that the 30% of participants not developing an IgM response had been previously exposed to SARS-CoV-2 natural infection, as the IgM concentration did not increase following vaccination in the one SARS-CoV-2 RT-PCR positive participant recruited to the study. Further work is required to confirm this.

For those participants that did develop an IgM response, the median IgM concentration peaked at week two, and remained positive until week five post vaccination. The findings from this study differ to the findings by Jalkanen et al (2021), who reported relatively few individuals with an increased IgM concentration following dose one of the SARS-CoV-2 (Pfizer/BioNTech) vaccine. They did not publish the percentage of participants developing, and not developing, an IgM response, but the difference may be explained by the different analytical methods used in the studies. Jalkanen et al (2021) used a manual enzyme immunoassay with an unstated sensitivity and specificity, which may not have been capable of measuring IgM antibodies raised against the SARS-CoV-2 vaccine.

The median SARS-CoV-2 anti-spike IgG concentrations peaked later than IgM concentrations, with the majority of participants peaking at week three post vaccination (67%), and smaller numbers peaking at week two (10%), and week four (23%). After peaking, concentrations of SARS-CoV-2 anti-spike IgG fell each week for all participants to a trough concentration at week ten. All 105 participants who developed an antibody response

to the vaccine tested positive for SARS-CoV-2 anti-spike IgG on the baseline sample prior to dose two, ten weeks following dose one. The anti-spike IgG concentration for one participant peaked at week one post vaccination. As this participant had previously been exposed to natural infection (RT-PCR positive), it suggests there is a faster immune response if previously exposed to the virus. Further work is required to determine whether there is any significance in achieving a peak IgG antibody concentration at week one, two, three, or four post vaccination.

SARS-CoV-2 IgG and IgM antibodies have been shown to increase over time in SARS-CoV-2 patients, with the IgM suggesting acute infection (Zhou et al., 2020). In this study, SARS-CoV-2 IgM concentrations peaked at week two post dose one, and SARS-CoV-2 IgG concentrations peaked at week three post dose one. Ma et al (2020) found the medium seroconversion time for SARS-CoV-2 IgA, IgM and IgG antibodies were four to six, four to six, and five to ten days post symptom onset respectively. The simultaneous early detection of IgG and IgM antibodies following vaccination was also reported by Narasimhan et al (2021), who commented that this, together with the presence of IgG in the early period suggests seroconversion might occur simultaneously, or shortly after IgM production in addition to the archetypal sequential class switching response (Narasimhan et al., 2021).

Only three CALM participants developed a peak SARS-CoV-2 anti-nucleocapsid IgG concentration above the positivity cut-off (1.40 Index). This included the one SARS-CoV-2 RT-PCR positive participant (peak 7.71 Index at week three), and two non-symptomatic participants (peaks 1.41 and 1.80 Index at week two and week one respectively). For the majority of participants the SARS-CoV-2 anti-nucleocapsid IgG concentration remained negative, confirming the qualitative method from Abbott was unable to detect IgG antibodies raised against the vaccine. It was however, able to measure IgG antibodies in participants who had been exposed to SARS-CoV-2 infection. The two SARS-CoV-2 anti-nucleocapsid IgG positive results in participants not known to have had SARS-CoV-2 may suggest previous exposure to natural infection, or may show non-specific binding in the method, but this was not investigated further in this study.

One CALM participant reported SARS-CoV-2 symptoms on their initial questionnaire, but was RT-PCR negative at that time. They had negative IgG antibodies on both the SARS-CoV-2 anti-nucleocapsid and anti-spike IgG methods at baseline, but they did have a positive SARS-CoV-2 IgM concentration (1.27 Index), which peaked at week two following vaccination (7.78 Index). The initial positive SARS-CoV-2 IgM concentration may have been due to cross-reactivity with the method, but this was not evaluated in this study. Recent exposure to the virus was thought unlikely as a SARS-CoV-2 RT-PCR sample processed at the same time was negative, and their anti-nucleocapsid IgG remained negative throughout the study.

Six of the 107 CALM participants had a positive anti-spike SARS-CoV-2 IgG (quantitative) concentration measured at baseline. One of these participants reported symptoms on their initial questionnaire, and was SARS-CoV-2 RT-PCR positive at this time; they were positive for all three antibody methods (anti-spike IgG (quantitative) = 5,496.9 AU/mL, anti-nucleocapsid IgG (qualitative) = 6.45 Index, and IgM = 9.18 Index). Their SARS-CoV-2 IgM did not increase following vaccination but continued to fall over the weeks following dose one of the SARS-CoV-2 vaccine. Their SARS-CoV-2 anti-spike IgG increased following vaccination, with a peak concentration at week one of 41,688 AU/mL. The five other participants with a positive SARS-CoV-2 anti-spike IgG (quantitative) baseline did not describe SARS-CoV-2 symptoms in the prior six months. One weakness of the recruitment questionnaire was the six month cut-off for describing symptoms. On reflection the questionnaire should have asked for the presence of symptoms since the start of the pandemic, as antibodies may have remained elevated for more than six months. Van Elslande et al (2021) reported 92.4% of their HCW cohort still had positive anti-spike IgG antibodies at seven to ten months following infection, compared to 17.8% for anti-nucleocapsid IgG antibodies. On further questioning, several of the participants with a positive SARS-CoV-2 anti-spike IgG baseline had had RT-PCR confirmed SARS-CoV-2 in the past (n=1), or thought it highly likely they had previously contracted the virus (n=2). Information for the other three participants was not available, but it is likely they had been previously exposed to SARS-CoV-2 in the past. All six participants were excluded from the analysis looking at the effects of age, BMI, and vitamin D on antibody development. This was to ensure any effects observed were due to antibodies produced following vaccination. The two participants that did not respond to vaccination were also excluded as outliers.

It was hypothesised that younger, female, non-Caucasian participants, with a healthy BMI, and adequate vitamin D concentration would show the greatest antibody response to vaccination. The effects of gender and ethnicity could not be evaluated, however, due to the small numbers of males and non-Caucasian participants recruited to the study. The majority of CALM participants were white (91%) females (78%). This reflects the current workforce within the Clinical Biochemistry laboratory at NNUH, but may have biased the data. Age, BMI, and vitamin D status at baseline prior to vaccination were evaluated to identify any factors leading to a greater antibody response to vaccination.

Wilcoxon Rank Sum Tests showed age had a significant ($p<0.0001$) effect on antibody response, with greater SARS-CoV-2 anti-spike IgG peak concentrations achieved at week three in those under 40 years of age than those older than 40 years of age. This significant difference in antibody concentration was maintained ten weeks post vaccination ($p<0.0001$). This was not unexpected, as aging is known to result in a decline in immune function (Montecino-Rodriguez et al., 2013). McElhaney and Dutz (2008) stated vaccines were less effective in the 65 years or older population, but the CALM study only recruited one participant in this age bracket. Further work is required to determine the immune response in those aged 65 years or older. McElhaney and Dutz (2008) also stated using antibody concentrations as a predictor of vaccine efficacy may fail to detect important changes in cellular immunity that enhance vaccine-mediated protection in this age group. Other markers of immune function, such as T-cells were not tested in the CALM study.

The mean BMI of the CALM participants (26 kg/m^2) was above the target adult range of 18.5 – 24.9 kg/m^2 (NHS, 2019). However, in this study Wilcoxon Rank Sum Tests showed no significant difference in week three or ten SARS-CoV-2 anti-spike IgG concentrations between those classified as overweight (mean = 1941.4 AU/mL), and those not overweight (1889.8 AU/mL) ($p >0.5$). This finding contradicts Frasca et al (2021), who found SARS-CoV-2 IgG antibodies were negatively associated with BMI in SARS-CoV-2 obese patients. Frasca et al (2021) reported obese patients as having a BMI >30 kg/m^2, whereas the CALM study evaluated all participants with a BMI >24.9 kg/m^2 without creating a separate obese category. The CALM study results appear to show an earlier (week two rather than week

three) lower SARS-CoV-2 IgG peak concentration at week three in those that were underweight (418.7 AU/mL). The significance of this could not be determined as only two CALM participants had an underweight BMI. Further work is required to investigate the immune response in those with a BMI <18 kg/m^2 or >30 kg/m^2.

Vitamin D appeared to have an effect on the antibody response following vaccination, with greater SARS-CoV-2 anti-spike IgG peak concentrations achieved in vitamin D replete participants than those that were deficient. However, Wilcoxon Rank Sum Tests showed antibody concentrations at week three (p=0.32) and week ten (p=0.25) were not significantly different in vitamin D replete or deficient participants. Chillon et al (2021) also found no significant difference in the dynamic increase or decrease of SARS-CoV-2 IgG concentration following vaccination as a function of vitamin D status.

The high rate of vitamin D deficiency (65%) observed within this healthy population at baseline was unexpected. Previous observational data has suggested a prevalence of 40% in Europeans during the winter months (Cashman et al., 2016). The increased proportion seen in the CALM study could be a result of the SARS-CoV-2 pandemic, characterised by multiple national lockdowns, reduced sun exposure, and increased time spent inside the workplace for HCW. Due to the large proportion of vitamin D deficiency, voluntary supplementary guidance (Appendix I) was issued to all participants. Of the vitamin D replete participants, 20% were already known to be taking vitamin D supplements. One participant had a toxic 25(OH)D concentration of 228 nmol/L at baseline. The participant was found to be taking 10,000 IU vitamin D daily, and they were recommended to stop straight away as toxic vitamin D concentrations can lead to chronic hypercalcaemia, calcification, and renal and cardiovascular damage (Royal Osteoporosis Society, 2020). Ten weeks later the participant's 25(OH)D concentration had fallen by 37% to 144 nmol/L.

Vitamin D supplementation had a significant (p<0.001) effect on participant's vitamin D concentration. After just ten weeks of supplementation the median 25(OH)D concentration had increased from 37 nmol/L to 73 nmol/L (+49%), with only 20% of participants classified as vitamin D deficient following supplementation guidance. Analysis by a mass spectrometry method showed the increase in total 25(OH)D concentration was due to an increase in the

25(OH)D_3 fraction, which is the recommended form of vitamin D supplementation. The 25(OH)D_2 fraction did not increase following supplementation. The 1,25$(OH)_2$D increased by 7.5% following supplementation but remained within the reference range. This is not surprising as 1,25$(OH)_2$D is the most potent physiologically active circulating metabolite of vitamin D and its concentration is tightly regulated by calcium and parathyroid hormone concentrations (Royal Osteoporosis Society, 2020). This, plus the short half-life of four to fifteen hours, makes 1,25$(OH)_2$D a poor indicator of vitamin D status (Royal Osteoporosis Society, 2020). The 25(OH)D:24,25$(OH)_2$D ratio also remained within the reference range before and after supplementation. 24,25$(OH)_2$D is the inactive product of 25(OH)D metabolism and the ratio to 25(OH)D is an indicator of vitamin D catabolic status (Tang et al., 2017).

3.5 Conclusion

The Abbott SARS-CoV-2 anti-spike IgG (quantitative) method is capable of measuring an immune response to vaccination. There was a 98% antibody response following the first dose of the SARS-CoV-2 (Pfizer/BioNTech) vaccine. The Abbott SARS-CoV-2 anti-nucleocapsid IgG (qualitative) method was not able to monitor the immune response to vaccination.

Following the first dose of the SARS-CoV-2 (Pfizer/BioNTech) vaccine there was a significantly greater antibody response in participants under 40 years of age than over 40 years. The next chapter will evaluate the immune response in the same 107 HCW following their second dose of the SARS-CoV-2 (Pfizer/BioNTech) vaccination.

Chapter 4

Evaluation of the antibody response following dose two of the BNT162b (Pfizer/BioNTech) SARS-CoV-2 vaccine

4.1 Introduction

Following UK government advice, the Norfolk and Norwich University Hospital (NNUH) began vaccinating its healthcare workers (HCW) with a second dose of the SARS-CoV-2 vaccine ten to twelve weeks after a first dose (Department of Health and Social Care, 2021a). This was against the published protocol by Pfizer/BioNTech that recommended a three week interval between doses (Pfizer-BioNTech, 2020).

The aim of this chapter is to evaluate the antibody response in 107 HCW following their second dose of the SARS-CoV-2 (Pfizer/BioNTech) vaccine. Data from chapter 3 showed the SARS-CoV-2 anti-spike IgG antibodies remained positive in all participants who developed an antibody concentration above the positivity cut-off for ten weeks following dose one. The aim of this chapter is to investigate how long antibodies remain positive following a second dose of the vaccine. It will also show whether the two participants who did not respond to dose one of the vaccine responded to a second dose, and provide evidence for or against prolonging the interval between doses. The data will provide an insight into the most beneficial time booster vaccines should be given. It will also look at whether peak antibody concentrations are higher following a second dose, and whether the anti-spike IgG concentrations peak faster following dose two, due to prior exposure.

4.2 Methodology

The CALM (COVID-19 antibody longitudinal monitoring) study is an observational cohort study performed at the NNUH in the United Kingdom (UK). The aim was to evaluate the immune response to SARS-CoV-2 vaccination in a group of HCW.

4.2.1 Study design and participants 111 HCW were recruited to the CALM study. Two participants were unable to provide the required blood samples and dropped out of the study; all of their results were removed. Two participants were vaccinated with vaccines other than Pfizer/BioNTech; their results were not included in the analysis. The study was approved by the Health Research Authority and Health and Care Research Wales (HCRW) ethical committee (IRAS project ID #292799) (Appendix E). Written informed consent was obtained from all study participants. A copy of the consent form and the patient information sheet provided prior to consenting are shown in Appendix F and Appendix G respectively.

4.2.2 Procedures Venous blood samples were collected from participants in the week prior to vaccination (baseline) with dose two of the BNT162b (Pfizer Inc. [New York] and BioNTech SE [Mainz, Germany]) SARS-CoV-2 vaccine. Samples were then collected in the week following vaccination, and each month for six months. Participants were given their second dose ten weeks after their first dose. Monthly blood samples were standardised for all participants and collected on the last week of the month. All serum samples were collected by trained NNUH phlebotomists, and analysed for SARS CoV-2 anti-spike IgG (quantitative), SARS CoV-2 anti-nucleocapsid IgG (qualitative), and SARS CoV-2 IgM to assess the immune response following vaccination.

4.2.3 Abbott SARS-CoV-2 IgG (quantitative) method The Abbott SARS-CoV-2 anti-spike IgG (quantitative) method is an automated two-step chemiluminescent microparticle immunomethod (CMIA). It is used for the quantitative determination of IgG antibodies, including neutralising antibodies, to the receptor binding domain (RBD) of the S1 subunit of the spike protein of SARS-CoV-2 in human serum and plasma on the Abbott Alinity i system (Abbott Park, Chicago, IL, USA). The analytical measuring interval is 21.0 – 40,000.0 Arbitrary Units per mL (AU/mL). Samples above 40,000.0 AU/mL are automatically diluted by the Abbott Alinity i system, with Abbott Alinity i multi method manual diluent (09P15), and samples above 80,000.0 AU/mL are diluted manually. The positivity cut-off stated by Abbott is 50.0 AU/mL.

4.2.4 Abbott SARS-CoV-2 IgG (qualitative) method The SARS-CoV-2 anti-nucleocapsid IgG qualitative method from Abbott is an automated two step CMIA. It is used for the

qualitative determination of IgG antibodies to the nucleocapsid protein of SARS-CoV-2 in human serum and plasma on the Abbott Alinity i system (Abbott Park, Chicago, IL, USA). The positivity cut-off is 1.40 Index, calculated by dividing the sample Relative Light Units (RLUs) by the calibrator RLUs.

4.2.5 Abbott SARS-CoV-2 IgM (qualitative) method The SARS-CoV-2 IgM qualitative method from Abbott is an automated two step CMIA. It is used for the qualitative determination of IgM antibodies to the spike protein of SARS-CoV-2 in human serum and plasma on the Abbott Alinity i system (Abbott Park, Chicago, IL, USA). The positivity cut-off is 1.00 Index, calculated by dividing the sample RLUs by the calibrator RLUs.

4.2.6 Statistics Microsoft Excel (2010) was used to perform all calculations and statistical analysis. Median and interquartile ranges were used to describe antibody concentrations as the data were not normally distributed. Box and whisker plots were used to present antibody data at different time points following vaccination. Wilcoxon Rank Sum Tests (or Mann Whitney U tests) were used to compare differences between the median SARS-CoV-2 anti-spike IgG values for two different age, vitamin D, and BMI categories, as well as differences in baseline anti-spike IgG concentrations. Spearman's correlation looked at the effects of age, vitamin D, and BMI on peak SARS-CoV-2 anti-spike IgG concentration. For statistical analyses two tailed p values <0.05 were considered to be statistically significant.

4.3 Results

The immune response of 107 HCW was assessed following vaccination with dose two of the SARS-CoV-2 (Pfizer/BioNTech) vaccine. Participant age, vitamin D concentration, and BMI were evaluated to identify factors leading to an increased immune response.

4.3.1 Antibody response to dose two of the BNT162b Pfizer/BioNTech SARS-CoV-2 vaccine All 107 CALM participants (100%) had a measured SARS-CoV-2 anti-spike IgG (quantitative) concentration above the positivity cut-off (50.0 AU/mL) following dose two of the SARS-CoV-2 (Pfizer/BioNTech) vaccine. The two CALM participants who did not respond to dose one did develop SARS-CoV-2 anti-spike IgG antibodies following their second dose. *Table 4.1*

shows their peak SARS-CoV-2 anti-spike IgG concentrations increased from 11.8 and 31.5 AU/mL following the first dose of the vaccine, to 11,591.4 and 2,831.3 AU/mL following the second dose (positivity cut-off = 50.0 AU/mL). For all 107 CALM participants, SARS-CoV-2 anti-spike IgG concentrations remained positive for six months following the second dose.

Two of the 107 CALM participants had a measured SARS-CoV-2 anti-nucleocapsid IgG concentration above the positivity cut-off (1.4 Index) following dose two of the SARS-CoV-2 vaccine. One was RT-PCR positive for SARS-CoV-2 prior to recruitment (peak SARS-CoV-2 anti-nucleocapsid IgG = 7.71 Index). The other participant developed SARS-CoV-2 symptoms after their second dose of the vaccine and was shown to be SARS-CoV-2 RT-PCR positive (peak SARS-CoV-2 anti-nucleocapsid IgG = 6.72 Index). During the CALM study this was the only participant to develop SARS-CoV-2 following vaccination with the SARS-CoV-2 (Pfizer/BioNTech) vaccine, showing 99% vaccine effectiveness. SARS-CoV-2 anti-nucleocapsid IgG concentrations remained negative following dose two in all other CALM participants.

Table 4.2 shows the median SARS-CoV-2 anti-spike IgG, anti-nucleocapsid IgG, and IgM concentrations at baseline before doses one and two of the SARS-CoV-2 (Pfizer/BioNTech) vaccine, and the peak concentrations following both doses. The median peak SARS-CoV-2 anti-spike IgG following the second dose was 19,925.4 AU/mL, which was 14-fold (1,312.4%) higher than the median peak concentration measured after dose one (1,410.8 AU/m L). There was no significant increase in the median SARS-CoV-2 anti-nucleocapsid IgG following either dose of the vaccine. The median SARS-CoV-2 IgM peak was 1.77 Index following dose one but did not reach the positivity cut-off following dose two (0.45 Index; positivity cut-off = 1.00 Index).

Of 107 CALM participants only 22% had a positive SARS-CoV-2 IgM concentration following dose two of the SARS-CoV-2 (Pfizer/BioNTech) vaccine. Of these, 83% had also developed an IgM response to dose one of the vaccine. Some participants (27%) did not develop an IgM response to either dose one or dose two of the SARS-CoV-2 vaccine.

Table 4.1: SARS-CoV-2 anti-spike IgG concentrations for the two CALM participants who did not respond to dose one of the SARS-CoV-2 (Pfizer/BioNTech) vaccine. Concentrations were measured on the Abbott Alinity i system. Participant one was a 57 year old Caucasian female taking a number of drugs (metformin and dapagliflozin for type 2 diabetes, amitriptyline, sertraline, and pregablin for depression and anxiety, amlodipine and Ramipril for hypertension, atorvastatin for hypercholesterolaemia, lansoprazole for acid reflux, asprin, and co-codamol). Participant two was a 48 year old Caucasian female taking Ustekinumab, an immunosuppressant for Crohn's disease.

	SARS-CoV-2 anti-spike IgG concentration (AU/mL) Positivity cut-off = 50.0 AU/mL				
	Dose 1 baseline	Dose 1 peak	Dose 2 baseline	Dose 2 peak	Month 6 post dose 2
Participant 1	0.1	11.8	5.1	11,591.4	810.7
Participant 2	2.8	31.5	23.9	2,831.3	735.7

Table 4.2: Median and interquartile ranges for SARS-CoV-2 anti-spike IgG, anti-nucleocapsid IgG, and IgM concentrations at baseline before doses one and two of the SARS-CoV-2 (Pfizer/BioNTech) vaccine, and peak concentrations following both doses. Samples were from 107 CALM participants and were measured on the Abbott Alinity i system. Median concentrations in yellow are above the positivity cut-off.

	Antibody concentrations – median (IQR)			
	Dose 1		Dose 2	
	Baseline	Peak	Baseline	Peak
SARS-CoV-2 anti-spike IgG quantitative (AU/mL) [cut-off = 50.0]	0.8 (0.03 – 2.7)	1,410.8 (739.6 – 2715.1)	526.6 (298.7 – 949.5)	19,925.4 (9661.5 – 33794.9)
SARS-CoV-2 anti-nucleocapsid IgG qualitative (Index) [cut-off = 1.40]	0.03 (0.02-0.09)	0.05 (0.02 - 0.15)	0.03 (0.02 – 0.08)	0.06 (0.03 -0.16)
SARS-CoV-2 IgM qualitative (Index) [cut-off = 1.00]	0.08 (0.05 - 0.12)	1.77 (0.75-3.22)	0.24 (0.09-0.39)	0.45 (0.21-0.87)

Box plots for the peak SARS-CoV-2 anti-spike IgG, anti-nucleocapsid IgG, and IgM concentrations following dose one and two of the SARS-CoV-2 (Pfizer/BioNTech) vaccine are shown in *Figures 4.1, 4.2, and 4.3* respectively. Data is shown for all 107 participants.

Table 4.3 shows the median SARS-CoV-2 anti-spike IgG, anti-nucleocapsid IgG, and IgM concentrations at each time point following the second dose of the SARS-CoV-2 (Pfizer/BioNTech) vaccination. The median SARS-CoV-2 anti-spike IgG (quantitative) peaked at week one post vaccination (18,592.1 AU/mL) and was still positive six months later (1,602.6 AU/mL) despite a 91.4% decrease. The median SARS-CoV-2 anti-nucleocapsid IgG (qualitative), and IgM concentrations remained below the positivity cut-offs.

Table 4.4 shows the percentage change in SARS-CoV-2 anti-spike IgG concentrations for each month following dose two of the BNT162b (Pfizer/BioNTech) vaccine. The average percentage decrease for all 107 CALM participants over the six month period was 32.8%.

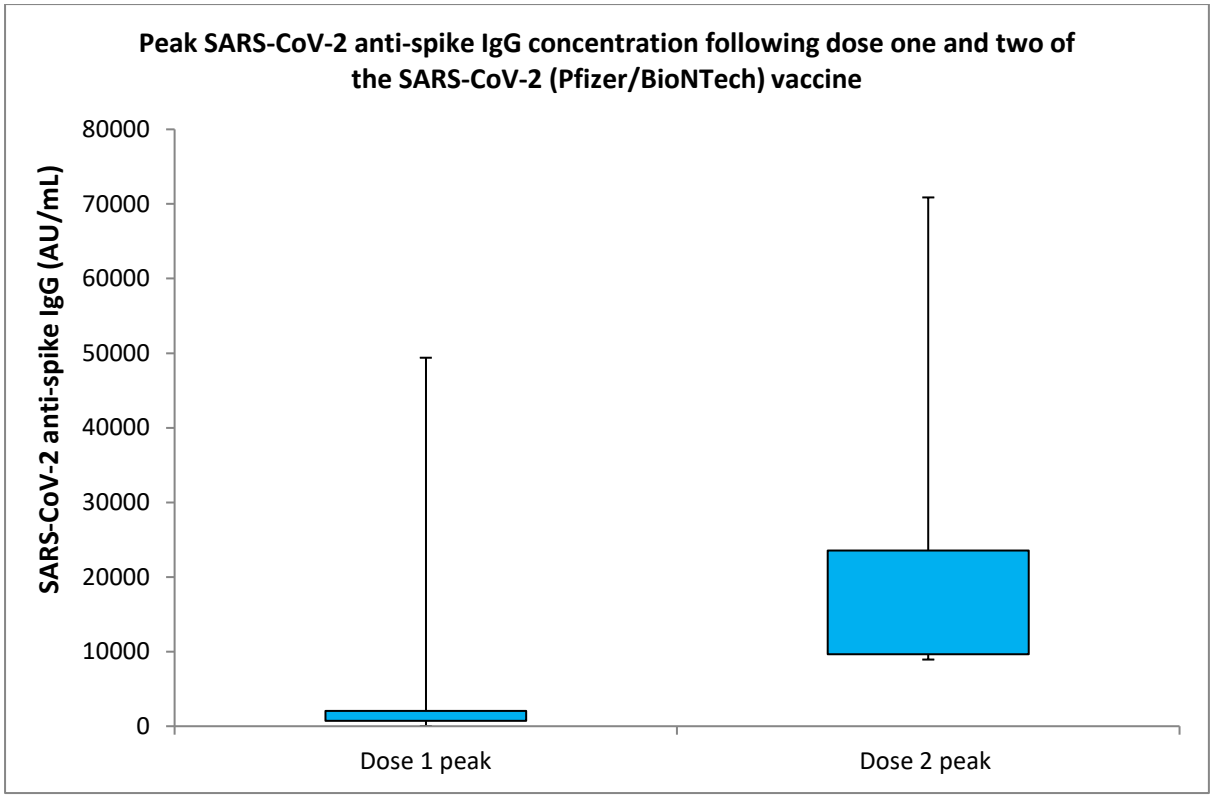


Figure 4.1: Box plot of the peak SARS-CoV-2 anti-spike IgG (AU/mL) concentrations following two doses of the SARS-CoV-2 (Pfizer/BioNTech) vaccine, measured by the Abbott Alinity i system for 107 CALM participants. Positivity cut-off = 50.0 AU/mL.

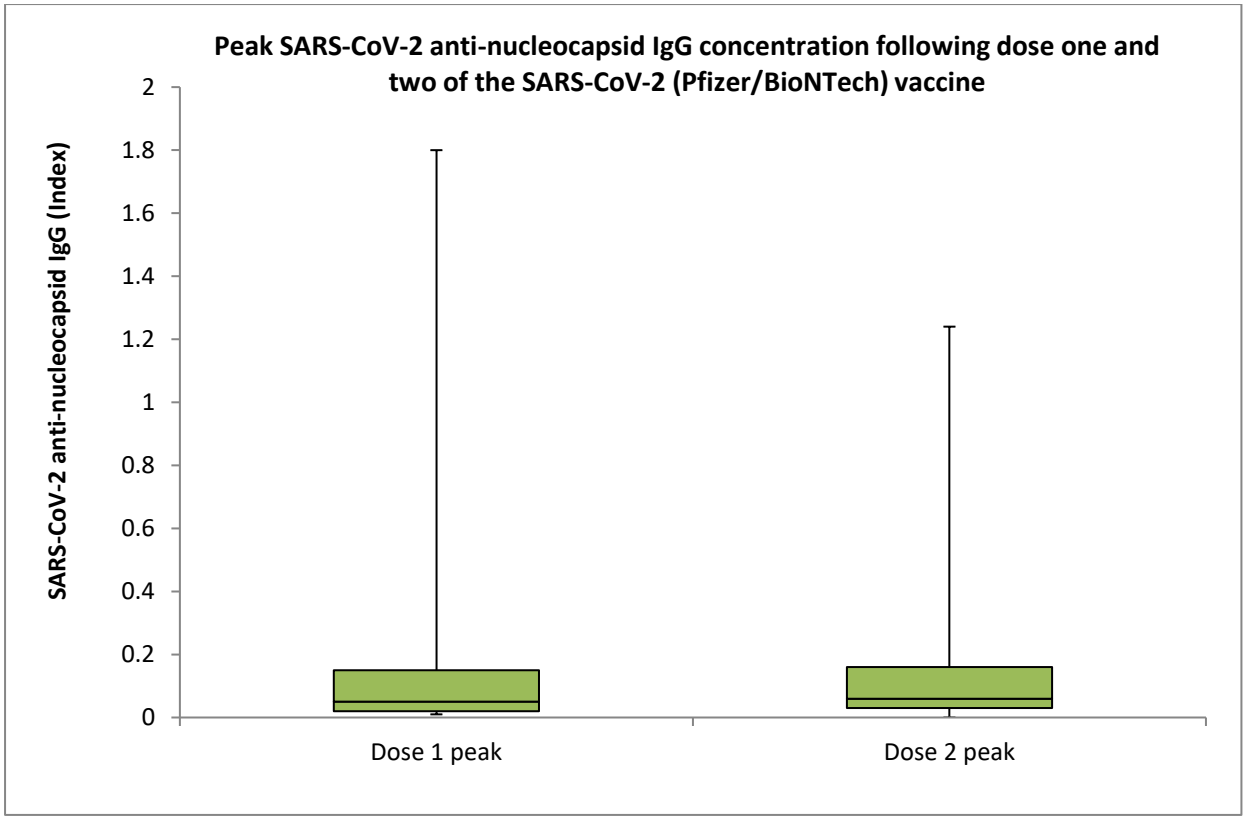


Figure 4.2: Box plot of the peak SARS-CoV-2 anti-nucleocapsid IgG (Index) concentrations following two doses of the SARS-CoV-2 (Pfizer/BioNTech) vaccine, measured by the Abbott Alinity i system for 107 CALM participants. Positivity cut-off = 1.40 Index.

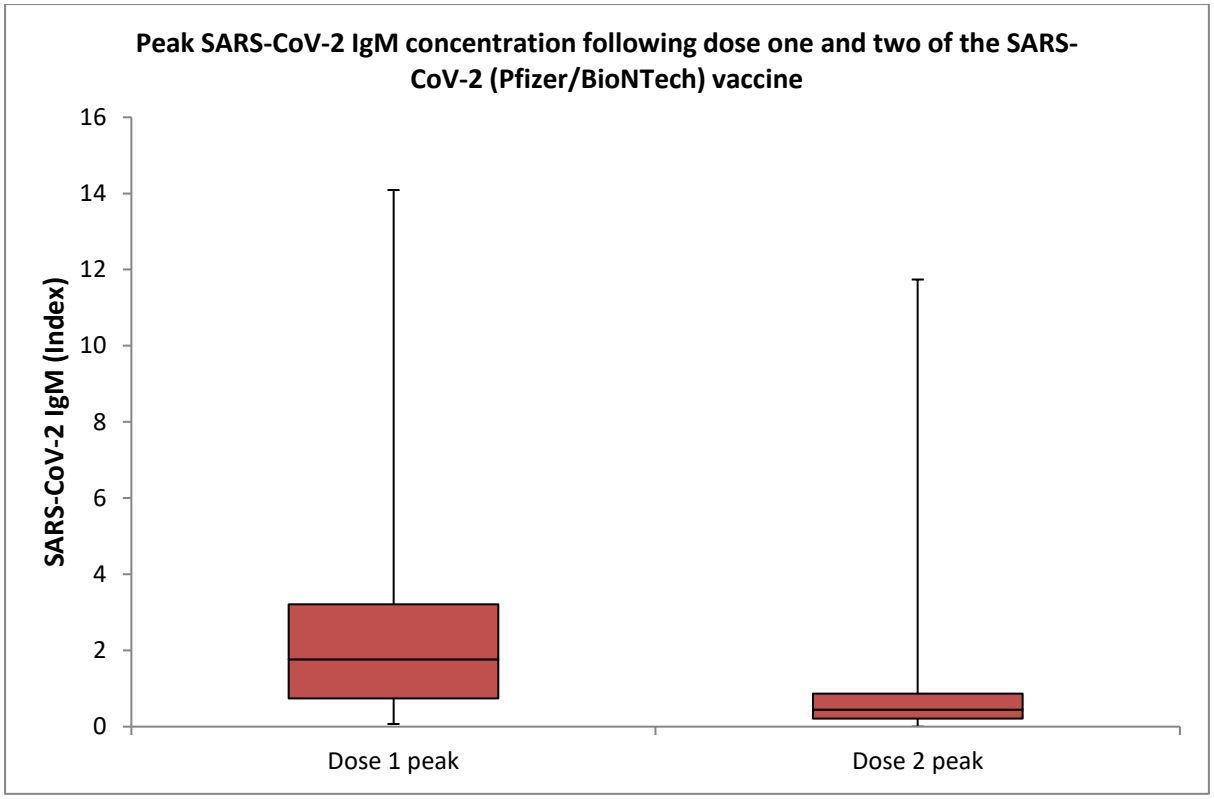


Figure 4.3: Box plot of the peak SARS-CoV-2 anti-spike IgM (Index) concentrations following two doses of the SARS-CoV-2 (Pfizer/BioNTech) vaccine, measured by the Abbott Alinity i system for 107 CALM participants. Positivity cut-off = 1.00 Index.

Table 4.3: Median SARS-CoV-2 anti-spike IgG quantitative (AU/mL), anti-nucleocapsid IgG qualitative (Index), and IgM (Index) at baseline, and number of weeks post dose two of the BNT162b (Pfizer/BioNTech) SARS-CoV-2 vaccine for all 107 CALM participants. Median concentrations in yellow are above the positivity cut-offs.

	Time post dose 2 of SARS-CoV-2 Pfizer/BioNTech vaccine							
	Base Line	Week 1	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Anti-s IgG (AU/mL) Cut-off = 50.0	526.6	18,592.1	15,274.4	9,016.0	4,687.5	3,143.1	2356.9	1,602.6
Anti-n IgG (Index) Cut-off = 1.40	0.03	0.04	0.05	0.04	0.05	0.04	0.04	0.05
IgM (Index) Cut-off = 1.00	0.24	0.25	0.26	0.17	0.07	0.06	0.05	0.06

Table 4.4: Median SARS-CoV-2 anti-spike IgG quantitative (AU/mL) at baseline, and number of weeks post dose two of the BNT162b (Pfizer/BioNTech) SARS-CoV-2 vaccine for all 107 CALM participants, together with the percentage change.

	Time post dose 2 of SARS-CoV-2 (Pfizer/BioNTech) vaccine							
	Baseline	Week 1	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
SARS-CoV-2 anti-spike IgG (AU/mL)	526.6	18,592.1	15,274.4	9,016.0	4,687.5	3,143.1	2,356.9	1,602.6
% Change		+3,435	–18	–41	–48	–33	–25	–32

4.3.2 Effect of CALM participant baseline SARS-CoV-2 anti-spike IgG (quantitative) antibody concentrations on response to vaccination *Figure 4.4* shows the log median SARS-CoV-2 anti-spike IgG concentration following two doses of the SARS-CoV-2 (Pfizer/BioNTech) vaccine, measured by the Abbott Alinity i system for 105 CALM participants. Participants are split by baseline SARS-CoV-2 anti-spike IgG concentration into those that were positive (≥50.0 AU/mL) (n=5), and those that were negative (<50.0 AU/mL) (n=100). The two SARS-CoV-2 RT-PCR positive participants were excluded.

A Wilcoxon Rank Sum Test showed there was a significant difference between the median SARS-CoV-2 anti-spike IgG concentrations in those with a negative baseline result, compared with those with a positive baseline result (p=0.015).

Table 4.5 shows the percentage change in SARS-CoV-2 anti-spike IgG concentrations in participants with a positive baseline concentration (≥50.0 AU/mL), and those with a negative baseline concentration (<50.0 AU/mL). The percentage decrease following vaccination was similar in both groups, with a 62 to 68% decrease following dose one, and a 92% decrease following dose two. However, the percentage increase was 10-fold higher in the negative baseline group (+218,900% and +3,683%), compared with the positive baseline group (+12,243% and +289%) for both doses of the vaccination. *Table 4.5* also shows the percentage change in the participant who had recovered from SARS-CoV-2 prior to recruitment.

Figure 4.5 shows the log median SARS-CoV-2 anti-spike IgG concentration following two doses of the SARS-CoV-2 (Pfizer/BioNTech) vaccine for all 107 participants measured by the Abbott Alinity immunomethod. Participants are split by baseline SARS-CoV-2 anti-spike IgG concentration into those that were positive (≥50.0 AU/mL) (n=5), and those that were negative (<50.0 AU/mL) (n=100). It also shows the two participants that were RT-PCR SARS-CoV-2 positive (one prior to recruitment, and one after dose two of the SARS-CoV-2 (Pfizer/BioNTech) vaccine).

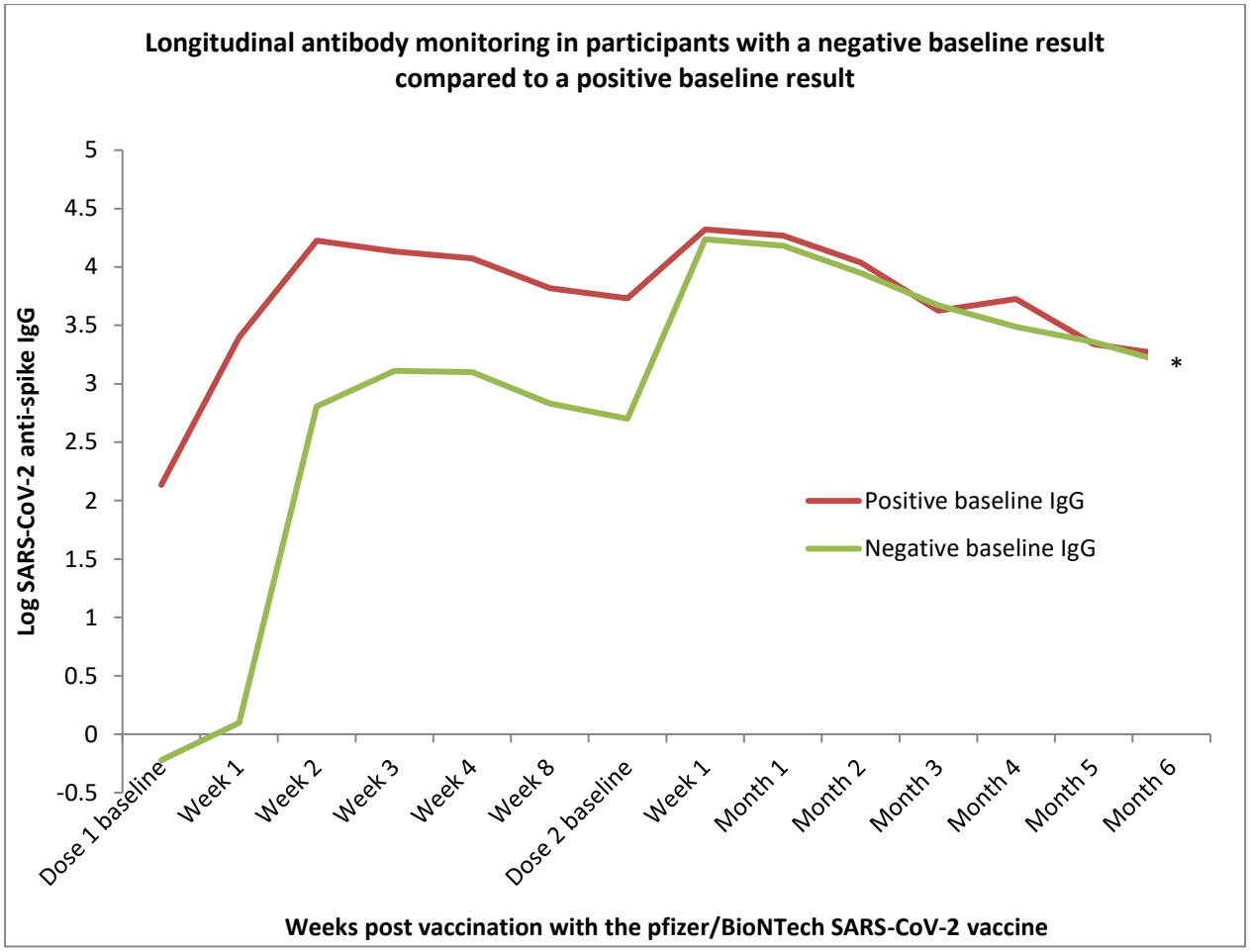


Figure 4.4: Log median SARS-CoV-2 anti-spike IgG following two doses of the SARS-CoV-2 (Pfizer/BioNTech) vaccine, measured by Abbott Alinity immunomethod for 105 CALM participants. Participants are split by baseline SARS-CoV-2 anti-spike IgG concentration, into those that were ≥50.0 AU/mL and positive (n=5), and those that were negative with a concentration <50.0 AU/mL (n=100). Two SARS-CoV-2 RT-PCR positive participants were excluded. A Wilcoxon Rank Sum Test showed a significant difference between the median SARS-CoV-2 anti-spike IgG concentrations in those with a negative baseline result, compared with those with a positive baseline result (p=0.015).

Table 4.5: Percentage change in SARS-CoV-2 anti-spike IgG concentrations in participants with a positive baseline concentration (≥50.0 AU/mL)(n=5), a negative baseline concentration (<50.0 AU/mL)(n=100), and a SARS-CoV-2 RT-PCR positive infection prior to vaccination (n=1). One participant who developed SARS-CoV-2 following dose two of the SARS-CoV-2 (Pfizer/BioNTech) vaccine has been excluded.

	% Change			
	Dose 1 Baseline to Dose 1 Peak	Dose 1 Peak to Dose 2 Baseline	Dose 2 Baseline to Dose 2 Peak	Dose 2 Peak to Month 6
Positive baseline SARS-CoV-2 IgG (n=100)	+12,243	–68	+289	–92
Negative baseline SARS-CoV-2 IgG (n-5)	+218,900	–62	+3,683	–92
RT-PCR positive SARS-CoV-2 (n=1)	+658	–79	+776	–94

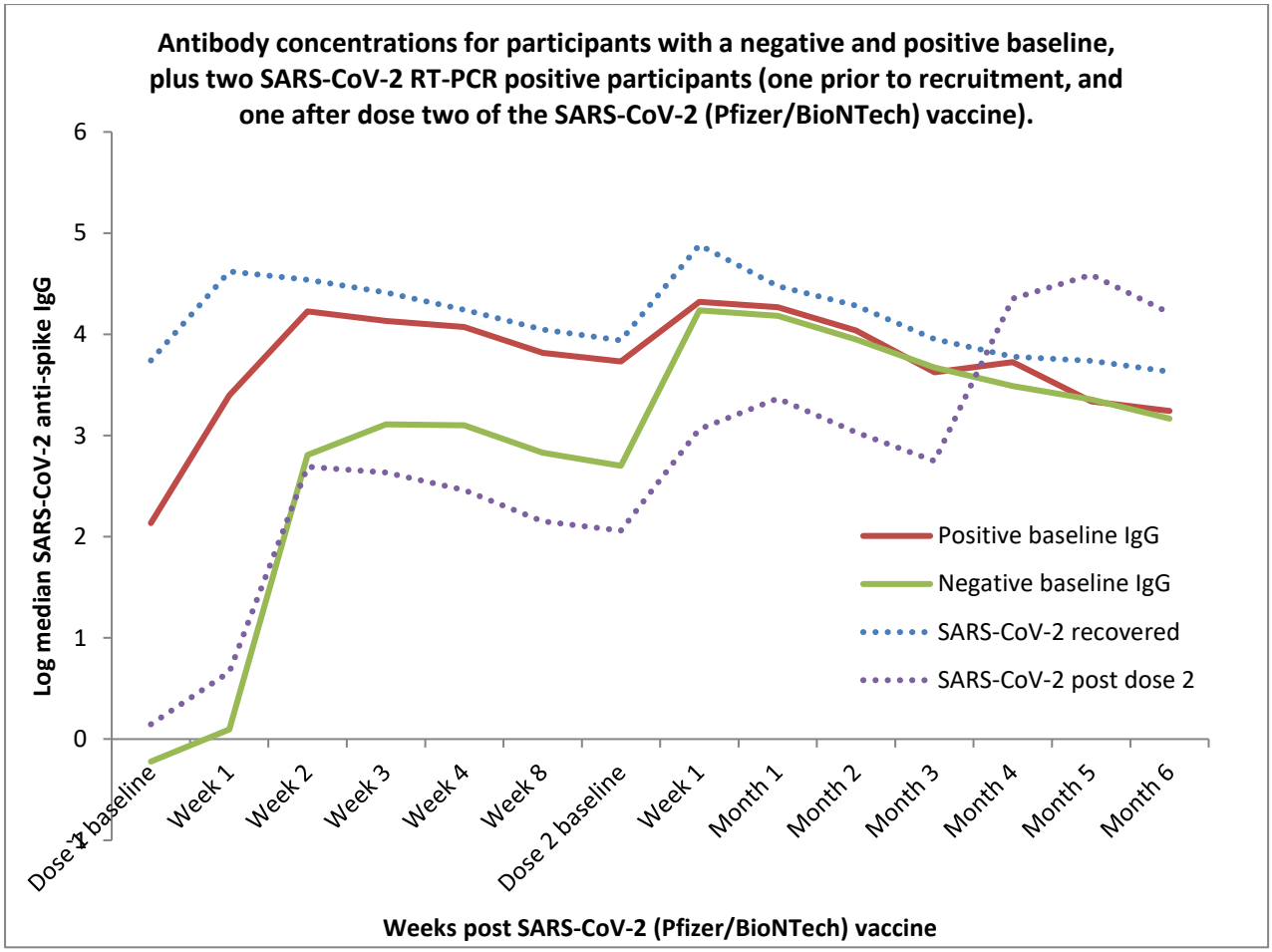


Figure 4.5: Log median SARS-CoV-2 anti-spike IgG following two doses of the SARS-CoV-2 (Pfizer/BioNTech) vaccine, measured by Abbott Alinity i system for 107 CALM participants. Participants are split by baseline SARS-CoV-2 anti-spike IgG concentration, into those that were ≥50.0 AU/mL and positive (n=5), and those that were negative with a concentration <50.0 AU/mL (n=100). It also shows the two participants that were SARS-CoV-2 RT-PCR positive (one prior to recruitment, and one after dose two of the SARS-CoV-2 (Pfizer/BioNTech) vaccine).

4.3.3 Effect of CALM participant demographics on SARS-CoV-2 anti-spike IgG (quantitative) antibody concentrations The effect of participant age, total 25(OH)D, and BMI on SARS-CoV-2 anti-spike IgG antibody development following vaccination with the SARS-CoV-2 (Pfizer/BioNTech) vaccine are shown in *Figures 4.6, 4.7, and 4.8* respectively. Data shown is for all 107 CALM participants.

Figure 4.6 splits the 107 CALM participants into those that were 40 years of age or older (n=44), and those that were under 40 years of age (n=63). *Figure 4.7*, splits the 107 CALM participants into those that were vitamin D deficient (<50 nmol/L; n=70), and those that were vitamin D replete (≥50 nmol/L; n=37). *Figure 4.8* splits the 107 participants into those that were underweight (BMI <18.5 kg/m^2; n=2), healthy weight (18.5 – 24.9 kg/m^2; n=47), overweight (24.9 – 30.0 kg/m^2; n=29), and obese (>30 kg/m^2).

Wilcoxon Rank Sum Tests looked for significance between median SARS-CoV-2 anti-spike IgG concentrations in participants younger or older than 40 years of age, vitamin D replete or deficient, and those with a BMI above or below the overweight category. Concentrations were found to not be significantly different for those under or over 40 years of age (p=0.43), for those classified as either vitamin D replete or deficient (p=0.62), or for those with a BMI above or below 24.9 kg/m^2 (p=0.92).

Spearman's correlation was used to assess significance between peak SARS-CoV-2 anti-spike IgG concentration following both doses of the vaccine, and participant age, vitamin D concentration, and BMI (*Figure 4.9*). There was a negative correlation between age and peak anti-spike IgG concentration (p=0.015), but the effects of vitamin D concentration (p=0.577), and BMI (p=0.806) were not significant.

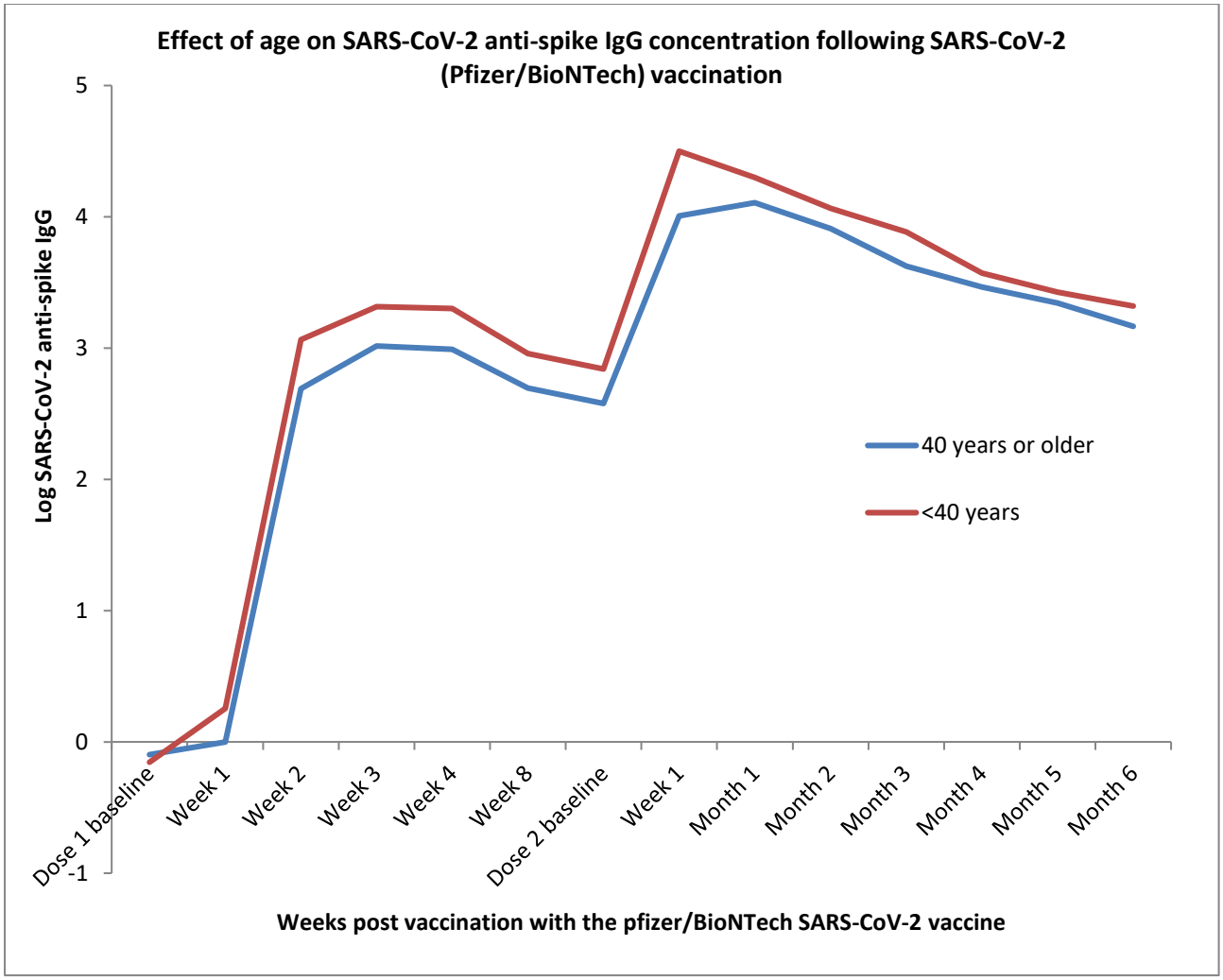


Figure 4.6: Log median SARS-CoV-2 anti-spike IgG following two doses of the SARS-CoV-2 (Pfizer/BioNTech) vaccine, measured by the Abbott Alinity i system for 107 CALM participants. Participants are split by age into those 40 years or over (n=44), and those under 40 years of age (n=63). A Wilcoxon Rank Sum Test showed concentrations were not significantly different for those under or over 40 years of age (p=0.43).

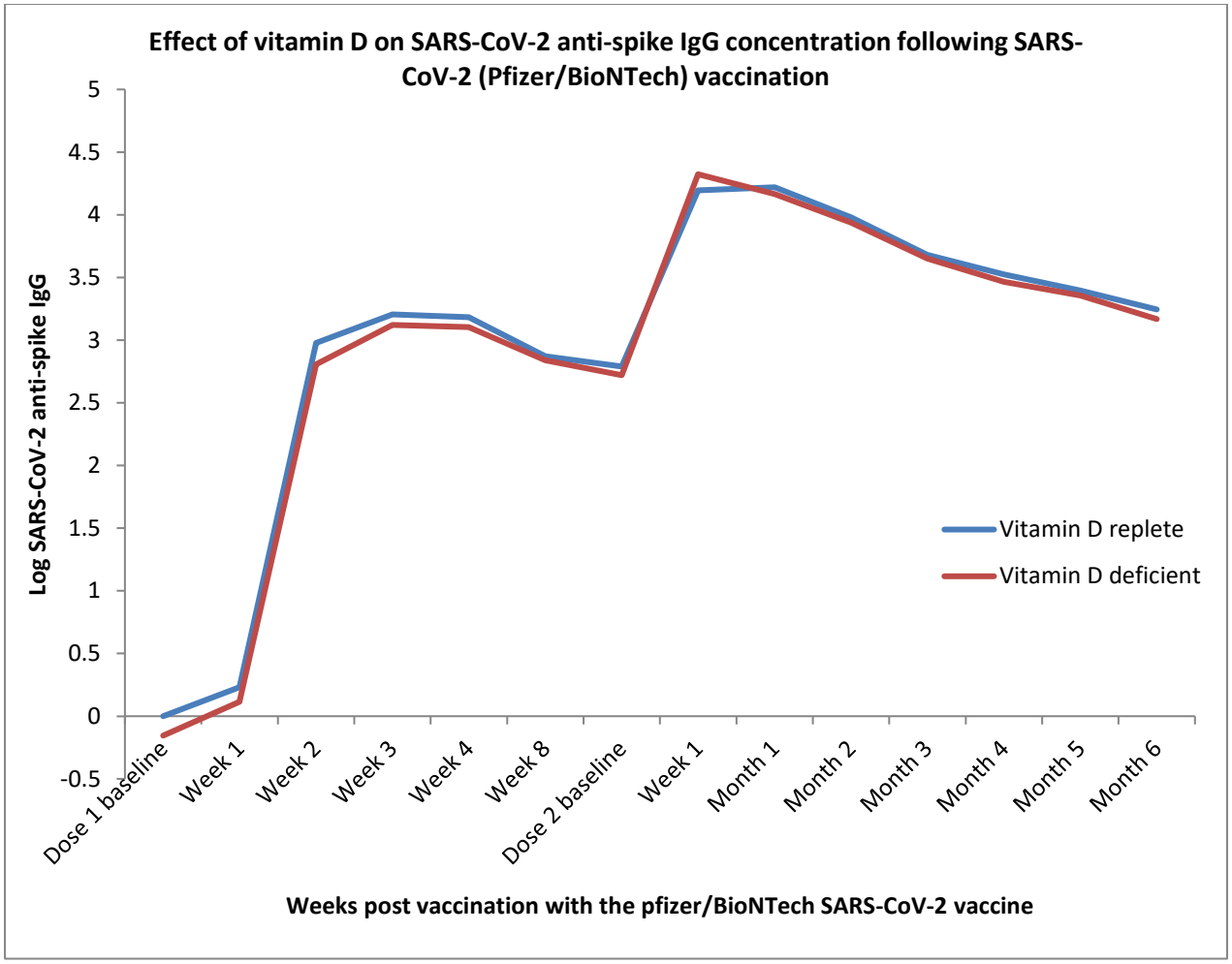


Figure 4.7: Log median SARS-CoV-2 anti-spike IgG following two doses of the SARS-CoV-2 (Pfizer/BioNTech) vaccine, measured by the Abbott Alinity i system for 107 CALM participants. Participants are split by baseline vitamin D concentration into those that are vitamin D replete with a 25(OH)D concentration ≥50 nmol/L (n=37), and those that are vitamin D deficient with a 25(OH)D concentration <50 nmol/L (n=70). A Wilcoxon Rank Sum Test showed concentrations were not significantly different for those classified as either vitamin D replete or deficient (p=0.62).

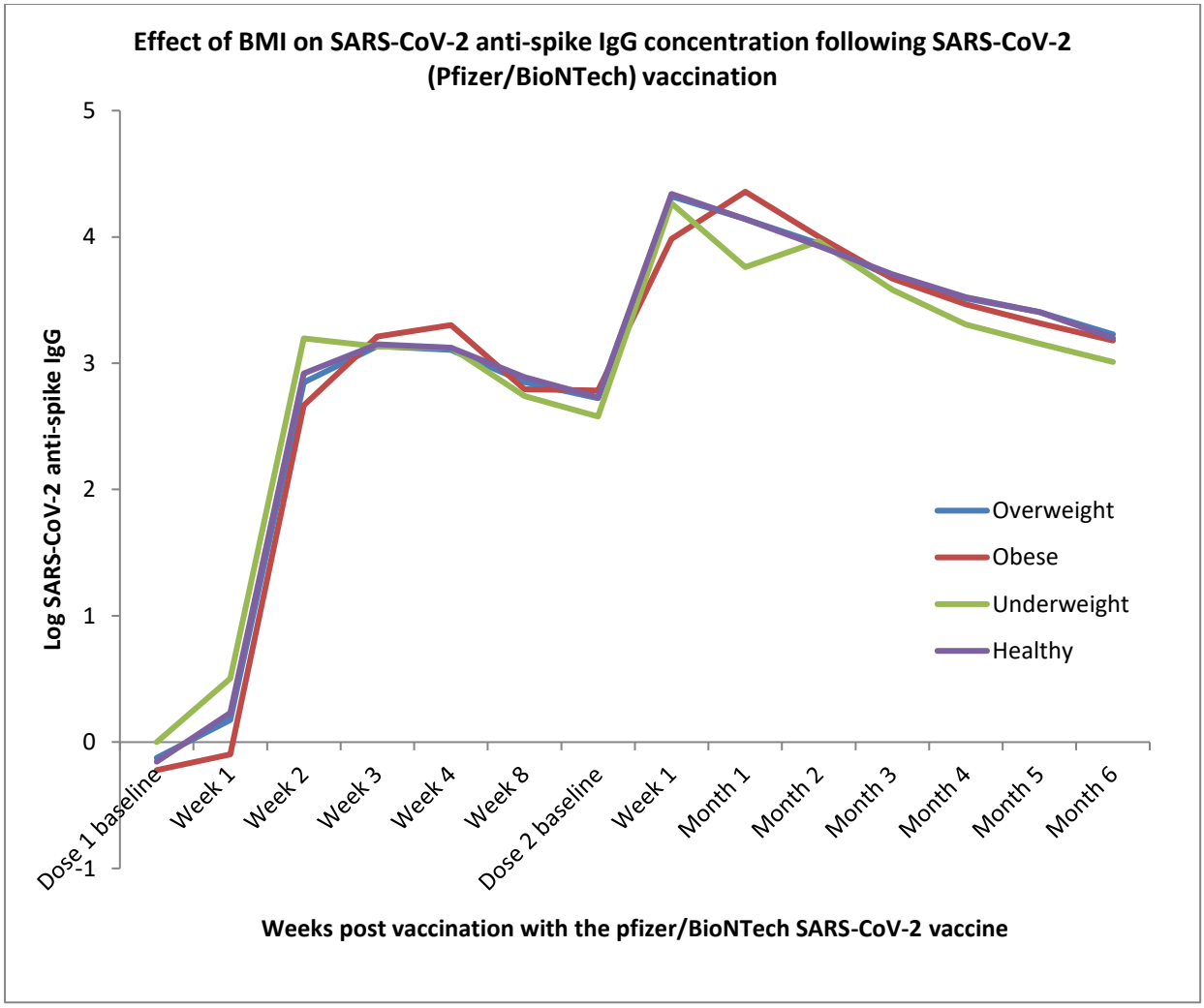


Figure 4.8: Log median SARS-CoV-2 anti-spike IgG following two doses of the SARS-CoV-2 (Pfizer/BioNTech) vaccine, measured by Abbott Alinity i system for 107 CALM participants. Participants are split by BMI into those with an obese BMI >30 kg/m^2 (n=29), an overweight BMI >24.9 kg/m^2 (n=29), a healthy BMI between 18.5 – 24.9 kg/m^2 (n=47), and an underweight BMI <18.5 kg/m^2 (n=2). A Wilcoxon Rank Sum Test showed concentrations were not significantly different for those with a BMI above or below 24.9 kg/m^2 (p=0.92).

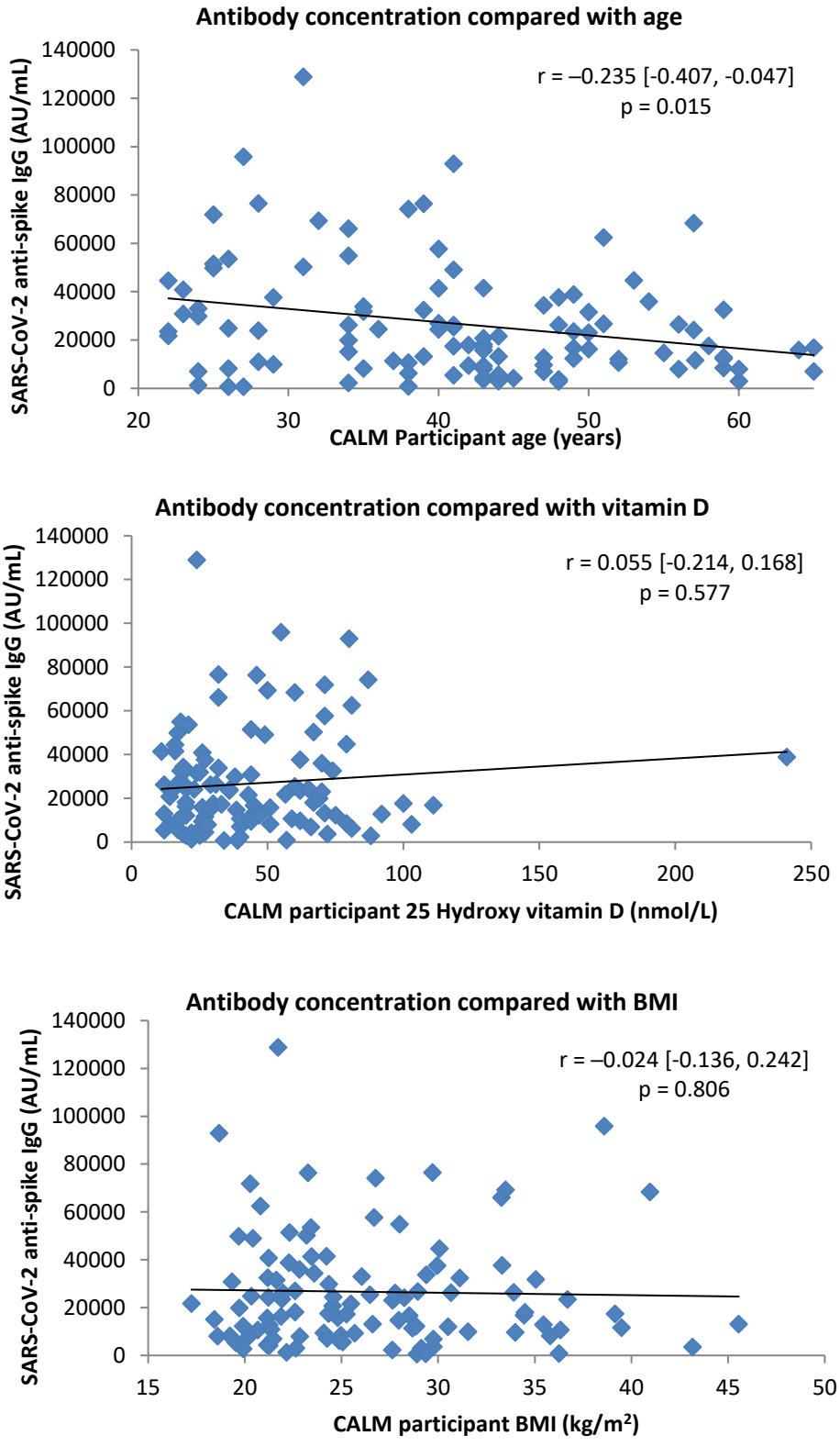


Figure 4.9: Peak SARS-CoV-2 anti-spike IgG (AU/mL) for 107 CALM participants following two doses of the SARS-CoV-2 (Pfizer/BioNTech) vaccine, compared with participant age (years), 25 hydroxy vitamin D (nmol/L), and BMI (kg/m^2). Spearman correlation coefficient (r) and exact two tailed p-values (p) are given with 95% confidence intervals.

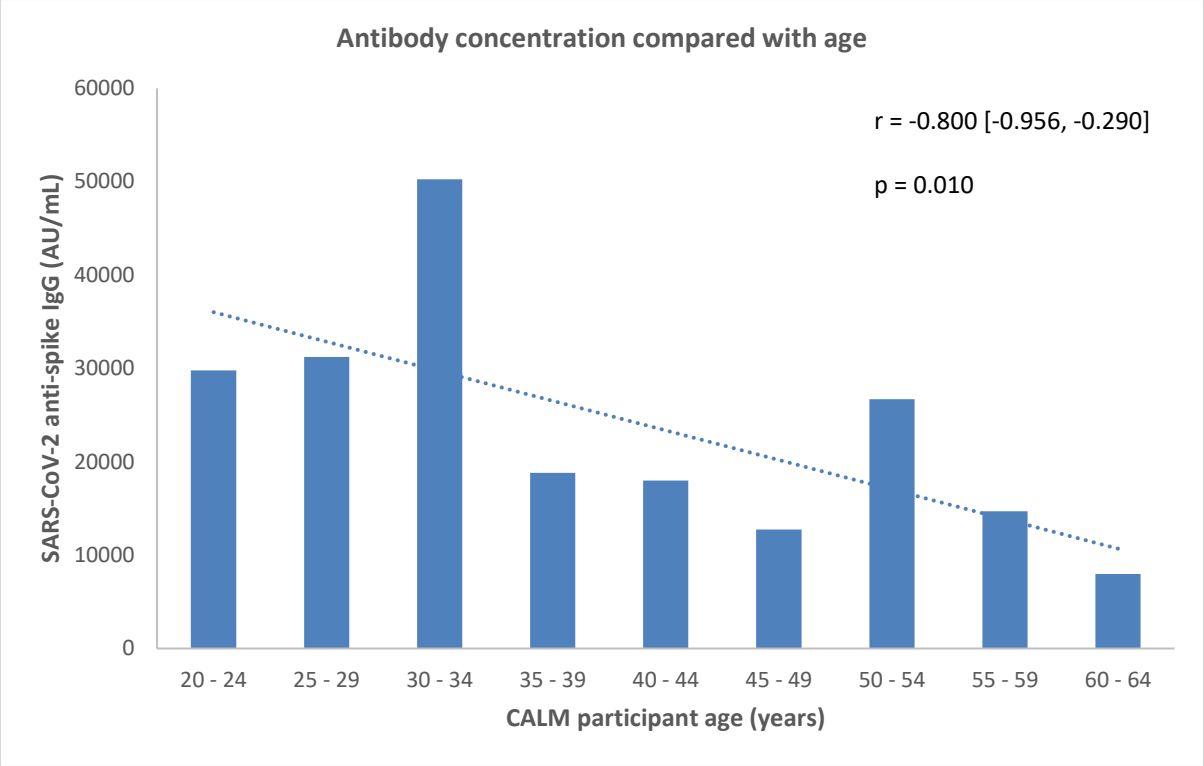


Figure 4.10: Peak SARS-CoV-2 anti-spike IgG (AU/mL) for 107 CALM participants following two doses of the SARS-CoV-2 (Pfizer/BioNTech) vaccine, compared with participant age (years). Spearman correlation coefficient (r) and exact two tailed p-values (p) are given with 95% confidence intervals.

4.4 Discussion

Following the second dose of the SARS-CoV-2 (Pfizer/BioNTech) vaccine there was 100% antibody response to vaccination in the CALM study, with all participants developing measurable SARS-CoV-2 anti-spike IgG concentrations above the positivity cut-off. The two participants who did not respond to dose one of the SARS-CoV-2 (Pfizer/BioNTech) vaccine did respond to a second dose. For the majority of the participants the UK government's decision to delay the dose interval to ten weeks rather than three was justified. Delaying the dose interval allowed a greater proportion of the population to be vaccinated with a first dose and receive some protection from the virus. However, for the two participants that did not respond to dose one, a second earlier dose at the recommended three week interval would have been more beneficial, rather than waiting ten weeks without any immune protection from the virus. This suggests a possible use for the quantitative SARS-CoV-2 anti-spike IgG method in measuring antibodies at three weeks following dose one. If antibodies are not detected at three weeks, a second dose could be given straight away, and if antibodies are detected, the ten week dose interval could remain. This is similar to other vaccinations where IgG antibodies are measured afterwards to evaluate the quality of the immune response (e.g. polio, pneumococcal polysaccharide, diphtheria toxoid and tetanus toxoid) (Justiz Vaillant et al., 2021).

A 14-fold increase in the median SARS-CoV-2 anti-spike IgG concentration was found following the second dose of the SARS-CoV-2 (Pfizer/BioNTech) vaccine compared to the first dose (1,410.8 to 19,925.4 AU/mL). Narasimhan et al (2021) also found a significant increase in the median SARS-CoV-2 anti-spike IgG concentration following the second dose of the vaccine in their cohort of naïve participants (2,217 AU/mL to 18,272 AU/mL), with similar concentrations achieved to those in the CALM study. Narasimhan et al (2021) reported a dampened response following the second dose in SARS-CoV-2 recovered individuals, but a several-fold higher response to the first dose. This finding could not be confirmed by the CALM study as only one participant was recruited who had recovered from SARS-CoV-2. This participant showed a reduced antibody response to both vaccine doses, compared to the negative baseline group of participants.

SARS-CoV-2 anti-spike IgG concentrations peaked at three weeks following the first dose for the majority of participants (67%). This is consistent with a recent report by Naaber et al (2021) who also found elevated antibodies in vaccinated serum three weeks after the first dose. Peak SARS-CoV-2 anti-spike IgG concentrations appeared to peak faster (week one) following dose two of the vaccine. However, samples were not collected at weeks two and three following the second dose, and so a direct comparison could not be made. It remains unknown if antibodies peaked faster following a second dose due to prior exposure, and further work is required.

Spearman's correlation showed age had a significant effect on peak SARS-CoV-2 anti-spike IgG concentrations ($p=0.015$), with participants under 40 years of age having a greater antibody response to both doses of the SARS-CoV-2 (Pfizer/BioNTech) vaccine. This confirms the findings from chapter three where Wilcoxon Rank Sum Tests showed the results were significantly different between participants over 40 years of age (n=57) and those under 40 years of age (n=42) at week three peak concentrations ($p<0.0001$) and week ten concentrations ($p<0.0001$). However, a Wilcoxon Rank Sum Test showed the median SARS-CoV-2 anti-spike IgG concentrations following both vaccine doses were not significantly different in the two age groups ($p=0.43$). Jalkanen et al (2021) reported a trend of declining immune response to vaccination by age, but predicted the trend was not strong in their study due to the ages of the HCW recruited (20 – 65 years). They predicted the age-related effect would be greater in those above 65 years of age (Jalkanen et al., 2021). The age of participants in the CALM study ranged from 22 to 65 years, and further work is required to confirm the effect of age on antibody development in those over 65 years of age.

Spearman's correlation and Wilcoxon Rank Sum Tests showed SARS-CoV-2 anti-spike IgG peak and median concentrations were not significantly different following vaccination in those classified as either vitamin D replete or deficient ($p=0.577$/$p=0.62$), or for those with a BMI above or below 24.9 kg/m^2 ($p=0.806$/$p=0.92$). This also confirms the findings from chapter three where Wilcoxon Rank Sum Tests showed the results were not significantly different between vitamin D or BMI categories at week three peak or week ten concentrations.

There was a significant difference between median SARS-CoV-2 anti-spike IgG concentrations in those with a negative baseline result, and those with a positive baseline result (p=0.015). Participants with a positive baseline were likely to have been exposed to SARS-CoV-2 natural infection prior to dose one of the Pfizer/BioNTech vaccination. The two known RT-PCR positive participants were not included in this analysis. Participants without prior exposure, with a negative SARS-CoV-2 anti-spike IgG baseline concentration, showed a 10-fold greater response to each dose of the vaccine than those with prior exposure and a positive baseline concentration. For the negative baseline group, the greatest response was following the initial dose of the vaccine with a 218,900% increase in median concentration from baseline to peak concentration, compared with a 3,683% increase following the second dose. In comparison, those with a positive baseline saw a 12,243% increase following dose one and a 289% increase following dose two. This difference allowed the median SARS-CoV-2 anti-spike IgG concentrations to be at similar concentrations following dose two, and both groups showed a similar rate of decline at six months (92%). The response in the one recovered SARS-CoV-2 RT-PCR positive participant prior to recruitment saw an increase of 658% following dose one and 776% following dose two. This data suggests the vaccination programme is most important in those individuals without any prior exposure to SARS-CoV-2 infection, and that these individuals may benefit from an earlier second dose to produce antibodies at a similar concentration to those with prior immunity. Further work is required to confirm these findings as there were relatively small numbers in each of the groups.

Recent studies showed antibody concentrations remained robust for six months following three week double mRNA vaccinations, and concentrations declined with a half-life of 52 days after day 43 (Doria-Rose et al., 2021). All participants in the CALM study had detectable SARS-CoV-2 anti-spike IgG concentrations six months following dose two of the SARS-CoV-2 (Pfizer/BioNTech) vaccine after a ten week interval, but there had been a 91.4% decrease from the median peak concentration, with an average percentage decrease of 32% each month. At this continued rate of decrease SARS-CoV-2 anti-spike IgG antibodies would be below the positivity cut-off at month 15 following dose two. A study by Naaber et al (2021) reported a similar rate of decrease with a 93% decrease in SARS-CoV-2 anti-spike IgG concentration at six months from peak median concentrations of 24,534 AU/mL. The

participants in the Naaber et al (2021) study were given the two vaccine doses three weeks apart, which suggests the time interval did not affect the peak SARS-CoV-2 anti-spike IgG peak concentrations, or six month percentage decrease. However, Parry et al (2021) showed that increasing the dose interval from three to ten weeks led to a 3.5-fold greater peak antibody response, and a longer period of clinical protection. They also reported a greater rate of decrease in the SARS-CoV-2 recovered participants, but this could not be confirmed in the CALM study, as there was only one participant recruited who had recovered from SARS-CoV-2 (Parry et al., 2021). These results suggest third booster vaccines are not required prior to six months following a second dose. Further work is required to determine how long antibodies remain detectable following vaccination, and when booster vaccinations will be required.

For the participants in the CALM study, 22% had a measured SARS-CoV-2 IgM concentration above the positivity cut-off (1.00 Index) following dose two of the Pfizer/BioNTech vaccine. Of these participants, 83% had also developed an IgM response to dose one of the vaccine. Further work is required to understand why some participants developed IgM antibodies following dose one (50%), some after dose two (3%), some after both doses (19%), and others not at all (28%). Narasimhan et al (2021) reported a significant increase in IgM response after the second dose of the vaccine, compared to the first dose, with median concentrations increasing from 1.1 Index to 1.95 Index. This differs to the results from the CALM study, where the median IgM concentration remained below the positivity cut-off (1.0 Index) following the second dose of the vaccine.

For the majority of participants, SARS-CoV-2 anti-nucleocapsid IgG concentrations remained negative following dose two of the SARS-CoV-2 (Pfizer/BioNTech) vaccine. Two participants had concentrations above the cut-off. One was the participant who had recovered from SARS-CoV-2 (RT-PCR positive) prior to recruitment, and the other participant developed SARS-CoV-2 symptoms during the study and was shown to be SARS-CoV-2 RT-PCR positive. This confirms findings from chapter three, that the Abbott SARS-CoV-2 anti-nucleocapsid IgG method is only capable of measuring antibodies to natural infection, and not vaccination.

The CALM participants were observed for nine months (January to September 2021), and during this time only one participant developed SARS-CoV-2 infection, and they described mild symptoms. The original Pfizer briefing document reported the true vaccine efficacy was at least 89.9%, with a 97.5% probability (Pfizer-BioNTech, 2020). The higher vaccine effectiveness seen in the CALM study may be explained by the majority of participants (78%) being females, as adult females have been shown to mount stronger immune responses than males, with greater vaccine efficacy (Klein and Flanagan, 2016). The immune response to vaccination is also known to decline with age (Montecino-Rodriguez et al., 2013), and the mean age of participants in the CALM study was 41 years compared to 52 years of age in the Pfizer/BioNTech study (Pfizer-BioNTech, 2020). The higher vaccine effectiveness may also have been due to a 91% Caucasian population, although it was hypothesised from work on other vaccines that Caucasians would have a worse response to vaccination (Kurupati et al., 2016). The Pfizer briefing document stated a lower observed vaccine primary efficacy of 89.3% for the 'all others' race subgroup, compared to >93% for all subgroups of age, sex, ethnicity, and country (Pfizer-BioNTech, 2020). As the CALM participants were all HCW, they were more likely to be aware of the importance of masks and personal protective equipment, good hand washing techniques, and social distancing than the general population were.

Further work is required to monitor the CALM participants over the coming months to identify any other cases of SARS-CoV-2, and the rate of antibody decrease. It is expected that the SARS-CoV-2 anti-spike IgG concentrations may reach a plateau before dropping below the positivity cut-off. Further work is required to evaluate the immune response following third booster doses of the vaccine, which are now being given to participants six months following their second doses.

Further work is also required to determine the SARS-CoV-2 anti-spike IgG concentration that corresponds to SARS-CoV-2 immunity. Neutralising antibody methods are expensive, manual, and labour intensive, requiring a large amount of time and expertise. The Abbott SARS-CoV-2 anti-spike IgG results could be compared with neutralising antibody concentrations to determine a new SARS-CoV-2 anti-spike IgG positivity cut-off for immunity. This cut-off could then be used to recommend earlier second or third doses in

individuals not reaching the immunity cut-off. This would tailor the vaccination programme to ensure only those that need extra immunity are vaccinated, and those that already have sufficient immunity are not given vaccines unnecessarily. For people with prior immunity from natural infection, one dose of the vaccine may be sufficient to reach the required level of immunity. This would help to distribute limited vaccine supplies globally to ensure equal access to immunity and reduce the threat from SARS-CoV-2.

4.5 Conclusion

The SARS-CoV-2 anti-spike IgG method from Abbott detected antibodies in all 107 CALM participants following the second dose of the SARS-CoV-2 (Pfizer/BioNTech) vaccine. Antibody concentrations remained elevated for up to six months after vaccination but continued to decline each month.

Chapter 5

Project summary and Final Conclusions

The novel quantitative SARS-CoV-2 anti-spike IgG method from Abbott was fully evaluated in this study. It was shown to perform well, with excellent imprecision, sensitivity and specificity. It was capable of measuring the immune response to natural infection from a range of SARS-CoV-2 strains, and to vaccination with the SARS-CoV-2 (Pfizer/BioNTech) vaccine. Use of a quantitative method, rather than a qualitative method, allows antibody development, peak concentration, and antibody decline over time to be evaluated.

This method was then used in the CALM (COVID-19 antibody longitudinal monitoring) study. The CALM study is an observational cohort study performed at the Norfolk and Norwich University Hospital. 111 healthcare workers (HCW) were recruited in January 2021, prior to the UK vaccination programme roll out, and their immune response to SARS-CoV-2 vaccination evaluated in a real-world setting.

The CALM study found one participant that contracted SARS-CoV-2 after two doses of the SARS-CoV-2 (Pfizer/BioNTech) vaccine, with a 99% vaccine effectiveness. 100% of participants developed a SARS-CoV-2 anti-spike IgG antibody response to two doses of the SARS-CoV-2 (Pfizer/BioNTech) vaccine. The study identified a potential benefit from measuring SARS-CoV-2 anti-spike IgG antibodies three weeks following dose one of the vaccine, and to offer individuals not responding an earlier second dose. For individuals responding to dose one, antibodies remained detectable for the ten week interval between doses. This supports the UK governments' decision to delay the dose interval and allow more individuals to be vaccinated with one dose. Antibodies then remained detectable six months following the second dose of the vaccine in all CALM participants.

Age was shown to have a significant effect on peak SARS-CoV-2 anti-spike IgG concentrations ($p=0.015$), with participants under 40 years of age having a greater antibody response to both doses of the SARS-CoV-2 (Pfizer/BioNTech) vaccine. Further work is required to confirm this.

The CALM study found a significant amount of vitamin D deficiency within the participants. Vitamin D has previously been shown to be important in generating an immune response (Kumar et al., 2021), and further studies are required to show what implications deficiency has on the immune response to SARS-CoV-2 natural infection and vaccination. Vitamin D deficient individuals did appear to have a lower antibody response to the vaccine in the CALM study, but the results were not significant. This may have been confounded by the majority of participants taking supplements following guidance advice at the beginning of the study. If vitamin D is found to improve the immune response to vaccination, it suggests the recommended national supplementation advice should be reinforced or better published to increase the average vitamin D concentration in the general population. Booster programmes could also be planned for summer months when the average vitamin D concentration is higher.

The CALM study has ethical approval to continue monitoring participants for 18 months or until antibody concentrations become undetectable. The next stage of the CALM study will be to evaluate the immune response following third booster vaccinations to show whether peak SARS-CoV-2 anti-spike IgG concentrations continue to increase, or if they reach a plateau.

The main limitations of the CALM study were the population demographics. All participants were HCW who were vaccinated as one of the first priority groups, and the majority were Caucasian females. Further work is required to evaluate the immune response following other vaccines, and in males, non-Caucasians, and people over 65 or under 20 years of age. There was also a potential difference in antibody development seen in participants with an underweight BMI, although the study only had two participants with a low BMI and further work is required.

To fully evaluate the immune response to vaccination, the SARS-CoV-2 anti-nucleocapsid IgG and IgM antibodies were also measured. The qualitative SARS-CoV-2 anti-nucleocapsid IgG method from Abbott was not able to monitor the immune response to vaccination, and was only able to detect antibodies raised against natural SARS-CoV-2 infection. Laboratories must understand what the different SARS-CoV-2 serology methods are capable of

measuring, and with the recent release of an international standard, methods can begin to be standardised. This will allow results to easily be compared between studies.
The SARS-CoV-2 IgM method from Abbott was able to monitor the immune response to natural infection and vaccination, with concentrations peaking earlier than IgG concentrations. Further work is required to understand why some participants developed IgM antibodies following dose one, some after dose two, some after both doses, and others not at all.

The SARS-CoV-2 pandemic has provided the opportunity to undertake research in this continually changing field. It has been a fantastic experience and is the final component of the Higher Specialist Scientific Training (HSST) programme.

References

Ahmed, W., et al. (2020). 'First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: A proof of concept for the wastewater surveillance of COVID-19 in the community', *Science of The Total Environment,* 728, 138764.

Allam, Z. (2020). 'The First 50 days of COVID-19: A Detailed Chronological Timeline and Extensive Review of Literature Documenting the Pandemic', *Surveying the Covid-19 Pandemic and its Implications,* pp. 1 – 7.

Andersson, M., et al. (2020). 'Rapid roll out of SARS-CoV-2 antibody testing—a concern', *BMJ,* 369, m2420.

Armbruster, D.A. and Pry, T. (2008). 'Limit of blank, limit of detection and limit of quantitation', *The Clinical Biochemist Reviews,* 29(Suppl1), pp. S49 – S52.

Assiri, A., et al. (2013). 'Epidemiological, demographic, and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study', *The Lancet Infectious Diseases*, 13(9), pp. 752 – 761.

Astuti, I. and Ysrafil. (2020). 'Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): An overview of viral structure and host response'*, Diabetes & Metabolic Syndrome,* 14(4), pp. 407 – 412.

Banerjee, A., Mossman, K. and Baker, M.L. (2021). 'Zooanthroponotic potential of SARS-CoV-2 and implications of reintroduction into human populations', *Cell Host & Microbe,* 29(2), pp. 160 – 164.

Bermingham, A., et al. (2012). 'Severe respiratory illness caused by a novel coronavirus, in a patient transferred to the United Kingdom from the Middle East, September 2012', *Euro Surveillance,* 17(40), 20290.

Callaway, E. (2021). 'Heavily mutated Omicron variant puts scientists on alert', *Nature,* 600 pp. 21.

Cao, Y., et al. (2015). 'Effects of immunosuppressants on immune response to vaccine in inflammatory bowel disease', *Chinese medical journal*, *128*(6), pp. 835 – 838.

Cashman, K.D., et al. (2016). 'Vitamin D deficiency in Europe: pandemic?', *The American Journal of Clinical Nutrition,* 103(4), pp. 1033 – 44.

Chan, J. F.W., et al. (2020). 'A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster', *The Lancet,* 395(10223), pp. 514 – 523.

Cheng, M.P., et al. (2020). 'Diagnostic Testing for Severe Acute Respiratory Syndrome–Related Coronavirus 2: A Narrative Review', *Annals of Internal Medicine,* 172(11), pp. 726 – 734.

Cheng, V.C.C, Lau, S.K.P., Woo, P.C.Y. and Yuen, K.Y. (2007). 'Severe acute respiratory syndrome coronavirus as an agent of emerging and reemerging infection', *Clinical Microbiology Reviews,* 20(4), pp. 660 – 694.

Chillon, T.S., et al. (2021). 'Relationship between Vitamin D Status and Antibody Response to COVID-19 mRNA Vaccination in Healthy Adults', *Biomedicines,* 9(11), 1714.

Claeson, M. and Hanson, S. (2020). 'COVID-19 and the Swedish enigma', *The Lancet,* 397(10271), pp. 259 – 261.

Clinical and Laboratory Standards Institute (2012). *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition.* Pennsylvanian, USA: Clinical and Laboratory Standards Institute (CLSI Document EP17-A2).

Clinical and Laboratory Standards Institute (2013). *Measurement Procedure Comparison and Bias Estimation Using Patient Sample; Approved Guideline – Third Edition.* Pennsylvanian, USA: Clinical and Laboratory Standards Institute (CLSI Document EP09-A3).

Clinical and Laboratory Standards Institute (2014a). *User verification of Precision and Estimation of Bias; Approved Guideline – Third Edition.* Pennsylvanian, USA: Clinical and Laboratory Standards Institute (CLSI document EP15-A3).

Clinical and Laboratory Standards Institute (2014b). *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guidelines – Third Edition.* Pennsylvanian, USA: Clinical and Laboratory Standards Institute (CLSI document EP05-A3).

Clinical and Laboratory Standards Institute (2020). *Evaluation of Linearity of Quantitative Measurement Procedures; Second Edition.* Pennsylvanian, USA: Clinical and Laboratory Standards Institute (CLSI Document EP06-Ed2).

Coccia, M. (2021). 'Optimal levels of vaccination to reduce COVID-19 infected individuals and deaths: A global analysis', *Environmental Research* [Preprint]. Available at: https://doi.org/10.1016/j.envres.2021.112314 (Accessed: 3rd December 2021).

Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. (2020). 'The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2', *Nature Microbiology,* 5, pp. 536 – 544.

Cyranoskim, D. (2020). 'What China's coronavirus response can teach the rest of the world', *Nature,* 579(7800), pp. 479 – 480.

Department for Transport, et al. (2020). *Test to Release: England introduces testing strategy for international arrivals.* Available at: https://www.gov.uk/government/news/test-to-release-england-introduces-testing-strategy-for-international-arrivals (Accessed: 1st December 2021).

Department of Health and Social Care (2020a). *Face coverings to be mandatory in shops and supermarkets from 24 July.* Available at: https://www.gov.uk/government/speeches/face-coverings-to-be-mandatory-in-shops-and-supermarkets-from-24-july (Accessed: 1st December 2021).

Department of Health and Social Care (2020b). *Coronavirus (COVID-19) Scaling up our testing programmes*. Available at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/878121/coronavirus-covid-19-testing-strategy.pdf (Accessed: 3rd December 2021).

Department of Health and Social Care (2021a). *Optimising the COVID-19 vaccination programme for maximum short-term impact.* Available at: https://www.gov.uk/government/publications/prioritising-the-first-covid-19-vaccine-dose-jcvi-statement/optimising-the-covid-19-vaccination-programme-for-maximum-short-term-impact (Accessed: 1st December 2021).

Department of Health and Social Care (2021b). *Joint Committee on Vaccination and Immunisation: advice on priority groups for COVID-19 vaccination, 30 December 2020.* Available at: https://www.gov.uk/government/publications/priority-groups-for-coronavirus-covid-19-vaccination-advice-from-the-jcvi-30-december-2020/joint-committee-on-vaccination-and-immunisation-advice-on-priority-groups-for-covid-19-vaccination-30-december-2020#references (Accessed: 1st December 2021).

Department of Health and Social Care (2021c). *JCVI statement regarding a COVID-19 booster vaccine programme for winter 2021 to 2022.* Available at: https://www.gov.uk/government/publications/jcvi-statement-september-2021-covid-19-booster-vaccine-programme-for-winter-2021-to-2022/jcvi-statement-regarding-a-covid-19-booster-vaccine-programme-for-winter-2021-to-2022 (Accessed: 1st December 2021).

Department of Health and Social Care (2021d). *Government takes decisive action against new COVID-19 variant.* Available at: https://www.gov.uk/government/news/government-takes-decisive-action-against-new-covid-19-variant (Accessed: 1st December 2021).

Department of Health and Social Care (2021e). *Interim Clinical Commissioning Policy: Casirivimab and imdevimab for patients hospitalised due to COVID-19.* Available at: https://www.england.nhs.uk/coronavirus/wp-content/uploads/sites/52/2021/09/C1421-interim-cc-policy-casirivimab-imdevimab-hospitalised-patients.pdf (Accessed: 1st December 2021).

Doria-Rose, N., et al. (2021). 'Antibody Persistence through 6 Months after the Second Dose of mRNA-1273 Vaccine for Covid-19', *The New England Journal of Medicine,* 384, pp. 2259 – 2261.

Drosten, C., et al. (2003). 'Identification of a novel coronavirus in patients with severe acute respiratory syndrome', *The New England Journal of Medicine,* 348(20), pp. 1967 – 1976.

Eliakim, A., et al. (2006). 'Reduced tetanus antibody titers in overweight children', *Autoimmunity,* 39(2), pp. 137 – 141.

English, E., et al. (2021). 'Performance of the Abbott SARS-CoV-2 IgG II Quantitative antibody method, including the new Variants of Concern, VOC 202012/V1 (United Kingdom) and VOC 202012/V2 (South Africa), and first steps towards global harmonization of COVID-19 antibody methods', *Journal of Clinical Microbiology,* 59(9), e00288 – 21.

Foreign & Commonwealth Office (2020). *Travel Advice against all non-essential travel: Foreign Secretary's statement, 17 March 2020.* Available at: https://www.gov.uk/government/news/travel-advice-foreign-secreatary-statement-17-march-2020 (Accessed: 1st December 2021).

Frasca, D., et al. (2021). 'Influence of obesity on serum levels of SARS-CoV-2-specific antibodies in COVID-19 patients', *PLOS ONE,* 16(3), e0245424.

Ghaffari, A., Meurant, R. and Ardakani, A. (2020). 'COVID-19 Serological tests: How well do they actually perform?', *Diagnostics,* 10(7), 453.

Giri, B., et al. (2021). ‘Review of analytical performance of COVID-19 detection methods’, *Analytical and Bioanalytical Chemistry*, 413(1), pp. 35 – 48.

Hu, B., Guo, H., Zhou, P. and Shi, Z.-L. (2021). ‘Characteristics of SARS-CoV-2 and COVID-19’, *Nature Reviews Microbiology,* 19(3), pp. 141 – 154.

Huang, C., et al. (2020). ‘Clinical Features of Patients Infected With 2019 Novel Coronavirus in Wuhan, China’, *The Lancet,* 395(10223), pp. 497 – 506.

Jääskeläinen A.J., et al. (2020). ‘Performance of six SARS-CoV-2 immunoassays in comparison with microneutralisation’, *Journal of Clinical Virology,* 129, 104512.

Jalkanen, P., et al. (2021). ‘COVID-19 mRNA vaccine induced antibody responses against three SARS-CoV-2 variants’, *Nature Communications,* 12(1), 3991.

Janeway, C.A., et al. (2001). *Immunobiology: the immune system in health and disease.* 5th edn. New York: Garland Science.

Jung, K., et al. (2021). ‘Performance evaluation of three automated quantitative immunoassays and their correlation with a surrogate virus neutralization test in coronavirus disease 19 patients and pre-pandemic controls’, *Journal of Clinical Laboratory Analysis,* 35(9), e23921.

Justiz Vaillant, A.A., Jamal, Z., Ramphul, K. (2021). *Immunoglobulin*. StatPearls Publishing. Available at: https://www.ncbi.nlm.nih.gov/books/NBK513460/. (Accessed 13th December 2021).

Kang, J., et al. (2020). ‘South Korea's responses to stop the COVID-19 pandemic’, *American Journal of Infection Control,* 48(9), pp. 1080 – 1086.

Kaza, M., et al. (2019). 'Bioanalytical method validation: new FDA guidance vs. EMA guideline. Better or worse?', *Journal of Pharmaceutical Biomedical Analysis,* 165, pp. 381 – 385.

Keaney, D., Whelan, S., Finn, K. and Lucey, B. (2021). 'Misdiagnosis of SARS-CoV-2: A Critical Review of the Influence of Sampling and Clinical Detection Methods', *Medical Sciences,* 9(2), 36.

Keshta, A.S., et al. (2021). 'COVID-19 versus SARS: A comparative review', *Journal of Infection and Public Health,* 14(7), pp. 967 – 977.

Khateeb, J., Li Y., and Zhang, H. (2021). 'Emerging SARS-CoV-2 variants of concern and potential intervention approaches', *Critical Care,* 25(244), pp. 1 – 8.

Klein, S.L. and Flanagan, K.L. (2016). 'Sex differences in immune responses', *Nature Reviews Immunology,* 16, pp. 626 – 638.

Koh, L.P., Li, Y. and Lee, J.S.H. (2021). 'The value of China's ban on wildlife trade and consumption', *Nature Sustainability,* 4, pp. 2 – 4.

Kristiansen, P.A., et al. (2021). 'WHO International Standard for anti-SARS-CoV-2 immunoglobulin', *The Lancet,* 397(10282), pp. 1347 – 1347.

Kumar, R., et al. (2021). 'Putative roles of vitamin D in modulating immune response and immunopathology associated with COVID-19', *Virus Research,* 15(292), 198235.

Kurupati, R., et al. (2016). 'Race-related differences in antibody responses to the inactivated influenza vaccine are linked to distinct pre-vaccination gene expression profiles in blood', *Oncotarget*, 7(39), pp. 62898 – 62911.

Lam, W.K., Zhong, N.S., and Tan, W.C. (2003). 'Overview on SARS in Asia and the world', *Respirology,* 8 Suppl(Suppl 1), pp. S2 – S5.

Last, J.M. (2001). *A dictionary of epidemiology*. 4th edn. New York: Oxford University Press.

Lau, H., et al. (2021). 'Evaluating the massive underreporting and undertesting of COVID-19 cases in multiple global epicentres', *Pulmonology,* 27(2), pp. 110 – 115.

Li, Q., et al. (2020). 'Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia', *The New England Journal of Medicine,* 382(13), pp. 1199 – 1207.

Lillie, P.J., et al. (2020). 'Novel coronavirus disease (Covid-19): The first two patients in the UK with person to person transmission', *Journal of Infection,* 80(5), pp. 578 – 606.

Liu, Y., et al. (2021). 'BNT162b2-Elicited Neutralization against New SARS-CoV-2 Spike Variants', *The New England Journal of Medicine,* 385(5), pp. 472 – 474.

Lu, R., et al. (2020a). 'Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding', *The Lancet,* 395(10224), pp. 565 – 574.

Lu, L., et al. (2020b). 'A comparison of mortality-related risk factors of COVID-19, SARS, and MERS: A systematic review and meta-analysis', *Journal of Infection,* 81(4), e18 – e25.

Ma, H., et al. (2020). 'Serum IgA, IgM, and IgG responses in COVID-19', *Cellular & Molecular Immunology,* 17, pp. 773 – 775.

Mahase, E. (2020a). 'Coronavirus: covid-19 has killed more people than SARS and MERS combined, despite lower case fatality rate', *BMJ,* 368, m641.

Mahase, E. (2020b). 'Covid-19: UK records first death, as world's cases exceed 100 000', *BMJ,* 368, m943.

Marklund, E., et al. (2020). 'Serum-IgG responses to SARS-CoV-2 after mild and severe COVID-19 infection and analysis of IgG non-responders', *PLOS ONE*, 15(10), e0241104.

McElhaney, J.E. and Dutz, J.P. (2008). 'Better Influenza Vaccines for Older People: What Will It Take?', *The Journal of Infectious Diseases,* 198(5), pp. 632 – 634.

McHugh, M.L. (2012). 'Interrater reliability: the kappa statistic', *Biochemia medica*, 22(3), pp. 276- 282.

Medicines & Healthcare products Regulatory Agency (2020). *Vaccine BNT162b2 – conditions of authorisation nder regulation 174.* Available at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1022642/Conditions_of_authorisation_for_Pfizer_BioNTech_vaccine__27_Sept.pdf (Accessed: 14th December).

Mohamadian, M., et al. (2021). 'COVID-19: Virology, biology and novel laboratory diagnosis', *The Journal of Gene Medicine,* 23(2), e3303.

Montecino-Rodriguez, E., Berent-Maoz, B. and Dorshkind, K. (2013). 'Causes, consequences, and reversal of immune system aging', *The Journal of Clinical Investigation.* 123(3), pp. 958 – 965.

Moore, S., et al. (2021). 'Modelling optimal vaccination strategy for SARS-CoV-2 in the UK', *PLoS Computational Biology,* 17(5), e1008849.

Mullol, J., et al. (2020). 'The Loss of Smell and Taste in the COVID-19 Outbreak: a Tale of Many Countries', *Current Allergy and Asthma Reports,* 20(10), 61.

Naaber, P., et al. (2021). 'Dynamics of antibody response to BNT162b2 vaccine after six months: a longitudinal prospective study', *The Lancet Regional Health – Europe,* 10(100208).

Narasimhan, M., et al. (2021). 'Clinical evaluation of the Abbott Alinity SARS-CoV-2 spike-specific quantitative IgG and IgM assays in infected, recovered, and vaccinated groups', *Journal of Clinical Microbiology,* 59(7), e00388 – 21.

NHS (2019). *What is the body mass index (BMI)?* Available at: https://www.nhs.uk/common-health-questions/lifestyle/what-is-the-body-mass-index-bmi/ (Accessed: 1st December 2021).

NHS (2020). *NHS to ramp up coronavirus testing labs.* Available at: https://www.england.nhs.uk/2020/03/nhs-to-ramp-up-coronavirus-testing-labs/ (Accessed: 1st December 2021).

NHS England and NHS Improvement (2020). *Antibody testing programme roll out for NHS staff and patients*. Available at: https://www.england.nhs.uk/coronavirus/wp-content/uploads/sites/52/2020/05/antibody-testing-programme-letter-25-may-2020.pdf (Accessed: 3rd December 2021).

Omer, S.B., et al. (2021). 'Promoting COVID-19 vaccine acceptance: recommendations from the Lancet Commission on Vaccine Refusal, Acceptance, and Demand in the USA', *The Lancet* [Preprint]. Available at: https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(21)02507-1/fulltext (Accessed: 3rd December 2021).

Parry, H., et al. (2021). 'Extended interval BNT162b2 vaccination enhances peak antibody generation in older people', *MedRxiv* [Preprint]. Available at: https://doi.org/10.1101/2021.05.15.21257017 (Accessed 30th December 2021).

Pfizer-BioNTech (2020). *Pfizer-BioNTech Covid-19 vaccine (BNT162, PF-07302048) vaccines and related biological products advisory committee briefing document meeting date 10th December 2020.* Available at: https://www.fda.gov/media/144246/download (Accessed: 3rd December 2021).

Piec, I., et al. (2021). 'Performance of SARS-CoV-2 serology tests: are they good enough?', *PLoS One,* 16(2), e0245914.

Polack, F.P., et al. for the C4591001 Clinical Trial Group. (2020). 'Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine', *The New England Journal of Medicine,* 383(27), pp. 2603 – 2615.

Prime Minister's Office (2020a). *Prime Minister's statement on coronavirus (COVID-19): 23 March 2020.* Available at: https://www.gov.uk/government/speeches/pm-address-to-the-nation-on-coronavirus-23-march-2020 (Accessed: 1st December 2021).

Prime Minister's Office (2020b). *Prime Minister's statement on coronavirus (COVID-19): 5 November 2020.* Available at: https://www.gov.uk/government/speeches/prime-ministers-statement-on-coronavirus-covid-19-5-november-2020 (Accessed: 1st December 2021).

Prime Minister's Office (2020c). *Prime Minister announces Tier 4: 'Stay At Home' Alert Level in response to new COVID variant.* Available at: https://www.gov.uk/government/news/prime-minister-announces-tier-4-stay-at-home-alert-level-in-response-to-new-covid-variant (Accessed: 1st December 2021).

Prime Minister's Office (2020d). *Prime Minister's statement to the House of Commons on COVID-19 regulations: 6 January 2021.* Available at: https://www.gov.uk/government/speeches/prime-ministers-statement-to-the-house-of-commons-on-covid-19-regulations-6-january-2021 (Accessed: 1st December 2021).

Prime Minister's Office (2020e). *PM statement at coronavirus press conference: 19 July 2021.* Available at: https://www.gov.uk/government/speeches/pm-statement-at-coronavirus-press-conference-19-july-2021 (Accessed: 1st December 2021).

Public Health England (2021). *COVID-19 vaccination programme Information for healthcare practitioners. Version 3.10.* Available at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1009174/COVID-19_vaccination_programme_guidance_for_healthcare_workers_6_August_2021_v3.10.pdf (Accessed: 3rd December 2021).

Royal Osteoporosis Society (2020). *Vitamin D and bone health: a practical clinical guideline for patient management.* Available at: https://strwebprdmedia.blob.core.windows.net/media/ef2ideu2/ros-vitamin-d-and-bone-health-in-adults-february-2020.pdf (Accessed: 29th December 2021).

Saker, K., et al. (2021). 'Evaluation of commercial anti-SARS-CoV-2 antibody assays and comparison of standardized titers in vaccinated healthcare workers', *Journal of Clinical Microbiology* [Preprint]. Available at: https://journals.asm.org/doi/10.1128/JCM.01746-21 (Accessed: 3rd December 2021).

Schoeman, D. and Fielding, B.C. (2019). 'Coronavirus envelope protein: current knowledge', *Virology Journal,* 16(69), pp. 1 – 22.

Sheridan, P., et al. (2012). 'Obesity is associated with impaired immune response to influenza vaccination in humans', *International Journal of Obesity - Nature,* 36(8), pp. 1072 – 1077.

Shiehzadegan, S., Alaghemand, N., Fox, M. and Venketaraman V. (2021). 'Analysis of the Delta Variant B.1.617.2 COVID-19', *Clinics and Practice,* 11(4), pp. 778 – 784.

Tang, J.C.Y., et al. (2017). 'Reference intervals for serum 24,25-Dihydroxyvitamin D and the ratio with 25-Hydroxyvitamin established using a newly developed LC-MS/MS method', *Journal of Nutritional Biochemistry,* 46, pp. 21 – 9.

UK Health Security Agency (2021a). *Vaccinations in the United Kingdom.* Available at: https://coronavirus.data.gov.uk/details/vaccinations (Accessed: 1st December 2021).

UK Health Security Agency (2021b). *Antibody testing for coronavirus: privacy information.* Available at: https://www.gov.uk/government/publications/coronavirus-covid-19-testing-privacy-information/antibody-testing-for-coronavirus-privacy-information (Accessed: 1st December 2021).

Van Elslande, J., Gruwier, L., Godderis, L., Vermeersch, P. (2021). 'Estimated half-life of SARS-CoV-2 anti-spike antibodies more than double the half-life of anti-nucleocapsidantibodies in healthcare workers', *Clinical Infectious Diseases,* 73(12), pp. 2366 – 2368.

Volz, E., et al. (2021). 'Assessing transmissibility of SARS-CoV-2 lineage B.1.1.7 in England', *Nature,* 599, pp. 266 – 269.

Voysey, M., et al. (2021). 'Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK', *The Lancet,* 397(10269), pp. 99 – 111.

Watson, J., Richter, A. and Deeks, J. (2020). 'Testing for SARS-CoV-2 antibodies', *BMJ,* 370, m3325.

Weber, D.J., et al. (1986). 'Impaired immunogenicity of hepatitis B vaccine in obese persons', *The New England Journal of Medicine,* 314(21), 1393.

Worldometer (2021a). *COVID-19 Coronavirus pandemic.* Available at: https://www.worldometers.info/coronavirus/ (Accessed: 1st December 2021).

Worldometer (2021b). *COVID-19 Coronavirus/Graphs.* Available at: https://www.worldometers.info/coronavirus/worldwide-graphs/ (Accessed: 1st December 2021).

Worldometer (2021c). *United Kingdom Coronavirus Cases.* Available at: https://www.worldometers.info/coronavirus/country/uk/ (Accessed: 1st December 2021).

World Health Organisation (2020a)*. Covid-19 –China.* Available at: https://www.who.int/emergencies/disease-outbreak-news/item/2020-DON229 (Accessed: 1st December 2021).

World Health Organisation (2020b). *Statement on the first meeting of the International Health Regulations (2005) Emergency Committee regarding the outbreak of novel coronavirus (2019-nCoV).* Available at: https://www.who.int/news/item/30-01-2020-statement-on-the-second-meeting-of-the-international-health-regulations-(2005)-emergency-committee-regarding-the-outbreak-of-novel-coronavirus-(2019-ncov) (Accessed: 1st December 2021).

World Health Organisation (2020c). *Statement on the second meeting of the International Health Regulations (2005) Emergency Committee regarding the outbreak of novel coronavirus (2019-nCoV).* Available at: https://www.who.int/news/item/23-01-2020-statement-on-the-meeting-of-the-international-health-regulations-(2005)-emergency-committee-regarding-the-outbreak-of-novel-coronavirus-(2019-ncov) (Accessed: 1st December 2021).

World Health Organisation (2020d). *WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020.* Available at: https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020 (Accessed: 1st December 2021).

World Health Organisation (2020e). *Past pandemics.* Available at: https://www.euro.who.int/en/health-topics/communicable-diseases/influenza/pandemic-influenza/past-pandemics (Accessed: 1st December 2021).

World Health Organisation (2020f). *WHO issues its first emergency use validation for a COVID-19 vaccine and emphasizes need for equitable global access.* Available at: https://www.who.int/news/item/31-12-2020-who-issues-its-first-emergency-use-validation-for-a-covid-19-vaccine-and-emphasizes-need-for-equitable-global-access (Accessed: 1st December 2021).

World Health Organization (2020g). *Laboratory testing strategy recommendations for COVID-19: interim guidance, 21 March 2020.* Available at: https://apps.who.int/iris/handle/10665/331509 (Accessed: 1st December 2021).

World Health Organisation (2021). *Tracking SARS-CoV-2 variants.* Available at: https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/ (Accessed: 1st December 2021).

Wu, F., et al. (2020). 'A new coronavirus associated with human respiratory disease in China', *Nature,* 579, pp. 265 – 269.

Xiang, N., et al. (2013). 'Use of national pneumonia surveillance to describe influenza A(H7N9) virus epidemiology, China, 2004 – 2013', *Emerging Infectious Diseases,* 19(11), pp. 1784 – 1790.

Zhang, W., et al. (2020). 'Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes', *Emerging Microbes & Infections,* 9(1), pp. 386 – 389.

Zhou, P., et al. (2020). 'A pneumonia outbreak associated with a new coronavirus of probable bat origin', *Nature,* 579, pp. 270 – 273.

Zhu, N., et al. (2020). 'A novel coronavirus from patients with pneumonia in China, 2019', *The New England Journal of Medicine,* 382(8), pp. 727 – 733.

Appendix A

Higher Specialist Scientific Training (HSST) Module A

Module A comprised five taught modules on Leadership and Management in the Healthcare Sciences. Two essays were submitted for each unit (28,847 words examined in total):

Unit A1 (Professionalism and Professional Development)

- A personal model of professionalism for healthcare scientists today **(2572 words)**
- Using social mobilising theory and in accordance with NHS governance requirements, construct a compelling and evidence based narrative for a proposed change or improvement in a service that your department or organisation provides **(3290 words)**

Unit A2 (Theoretical Aspects of Leadership)

- A critical evaluation of leadership models in relation to clinical biochemistry **(2945 words)**
- South West London Pathology through the lens of collective leadership theories **(3093 words)**

Unit A3 (Personal and Professional Development to Enhance Performance)

- My Personal Profile – Who am I? **(1485 words)**
- Continuing Personal Development portfolio **(3844 words)**

Unit A4 (Leadership and Quality Improvement in the Clinical and scientific Environment)

- A critical review of Quality Improvement in Clinical Blood Science Laboratories **(2997 words)**
- Quality Improvement in Practice **(2851 words)**

Unit A5 (Research and Development in Health and Social Care)

- Undertake a research needs assessment on a chosen topic or area, searching the literature and other sources to establish current knowledge and to assess, specify and justify the knowledge gaps that need to be addressed in that area **(2801 words)**
- An innovative pathway for women at risk of pre-eclampsia **(2996 words)**

The final unit marks were ratified by the University Board of Examiners and are presented below with the number of credits received for each unit:

MANCHESTER 1824
The University of Manchester
Alliance Manchester Business School

PGDip Leadership & Management in the Healthcare Sciences
Unit marks ratified by Board of Examiners, November 2017

Trainee name: Laura Tooth
Student ID: 7177951
Award: PG Credit

Unit	Unit Title	Mark	Credits
BMAN73511	Unit A1 Professionalism and Professional Development in the Healthcare Environment	67% **Pass**	**30**
BMAN73522	Unit A2 Theoretical Foundations of Leadership	66% **Pass**	**20**
BMAN73531	Unit A3 Personal and Professional Development to Enhance Performance	62% **Pass**	**30**
BMAN73542	Unit A4 Leadership and Quality Improvement in the Clinical and Scientific Environment	64% **Pass**	**20**
BMAN73550	Unit A5 Research and Innovation in Health and Social Care	63% **Pass**	**20**
			120 / 120

Appendix B

Higher Specialist Scientific Training (HSST) Module B

Module B comprised of the three Fellowship of the Royal College of Pathologist (FRCPath) examinations. These were all successfully passed first time, and the examination result letters are shown in this appendix.

The Royal College of Pathologists
Pathology: the science behind the cure

College Reference No: **20003108**
Candidate No: **735**

Miss Laura Tooth

22 May 2015

Dear Miss Tooth

FRCPath Part 1 Written Examination in Clinical Biochemistry - Spring 2015

I am pleased to inform you that you have satisfied the Examiners in the Part 1 Examination. You have therefore completed this part of the examination and you are now eligible to become an Associate of the College.

Council will formally ratify the nominations to Associateship on **11 June**. Please complete and return the enclosed consent form and address slip to the Membership Department, in the envelope provided, as soon as possible and no later than **Monday 27 July**.

The subscription year runs from January and once you have returned your consent form an invoice will be sent out for your subscription due. This will take into account any fee you may have already paid and pro-rata rates for the year. For current rates please refer to our website (http://www.rcpath.org/index.asp?PageID=1196).

On receipt of your payment and consent form, your certificate will be sent out to you with your full name as displayed at the top of this letter. If there is an error with your name please notify the Examinations Department in writing, at exams@rcpath.org, as soon as possible.

Please note the Associate grade holds no 'post-nominals'. If you do not wish to become an Associate of the College please indicate on the enclosed form or e-mail the Membership department on membership@rcpath.org

Details of the Part 2 Examinations can be found in the Examinations page of our website.

Congratulations on your success in this examination.

Yours sincerely

Dr Kevin West
Director of Examinations and Assessment

Enc: Consent form and address slip
Envelope addressed to the Membership Department

INVESTORS IN PEOPLE

The Royal College of Pathologists
Pathology: the science behind the cure

College Reference No: **20003108**
Candidate No: **392**

Miss Laura Elizabeth Tooth

20 May 2016

Dear Miss Tooth

FRCPath Part 2 in Clinical Biochemistry (Module 1) – Spring 2016

I am pleased to inform you that you have reached the required standard at this examination.

When you wish to enter for your module 2 examination, you should submit a further Part 2 application form, after the fee has been paid online.

Congratulations on your success in this examination.

Yours sincerely

Dr Andrew Day
Director of Examinations

INVESTORS IN PEOPLE

The Royal College of Pathologists
Pathology: the science behind the cure

College Reference Number:20003108
Candidate Number: 392

19 May 2017

Dear Miss Tooth

Part 2 Practical and Oral Examination in Clinical Biochemistry (module 2)– Spring 2017

I am pleased to inform you that you have satisfied the Examiners in the Part 2 Examination.

However, as you are aware, you are not eligible to become a Fellow of The Royal College of Pathologists yet as your Part 2 Project has not been approved.

We look forward to receiving the project in due course. In case you have any queries about your project please contact Stevie Davidson at stevie.davidson@rcpath.org.

Congratulations on your success in this examination.

Yours sincerely

Dr Andrew Day
Director of Examinations

INVESTORS IN PEOPLE

Appendix C

Higher Specialist Scientific Training (HSST) Module C1

Innovation Project (70 Credits)
Assignment: Literature Review, Business Case, and Lay Presentation

Module C1 consisted of an innovation project, with a literature review (4,401 words), and business case (1,534 words) submitted for examination. My business case was for the implementation of a novel urine oxalate service that was also able to detect the Primary Hyperoxaluria (PH) metabolites, and act as a PH screen on all urine oxalate requests. The marking proforma is shown in this appendix.

An oral presentation about this innovation project was given to a lay audience, and the feedback received is also shown in this appendix.

Work on this innovation project began, and a working mass spectrometry method was successfully developed that was able to detect three PH metabolites (glycolate, glycerate, and DHG). Unfortunately, the method was not fully evaluated due to difficulties getting clinical samples, the SARS-CoV-2 pandemic, and moving hospitals to begin a new role as a Principal Clinical Scientist. The pandemic did however give me the opportunity to undertake the serology research project written up for my C2 thesis.

Marking Criteria and Proforma

Please mark each section as Pass/Fail

All sections MUST be passed for an overall pass to be achieved. If any section is failed this results in an overall "fail" outcome. The clinical scientist has one opportunity to resubmit any failed sections to obtain an overall pass.

Please provide written feedback under ***each*** criteria. If any section is failed it is particularly important to give detailed feedback that will allow the errors to be corrected.

Student Name: Laura Tooth

1. **Clinical Relevance – [Clinical Supervisor to lead assessment]**

   Is the proposed innovation convincing as *'An idea, service or product, new to the NHS or* **to be** *applied in a way that is new to the NHS, which* **has the potential to** *significantly improve the quality of health and care wherever it* **might be** *applied?'*

Feedback: Yes this proposal will potentially improve a diagnostic pathway for primary hyperoxaluria by introducing a service which will hopefully reduce unnecessary investigations which has obvious patient and financial benefits.

Please circle the outcome for this section

PASS

2. **Academic Standard – [Academic Supervisor to lead assessment]**

   Is the appropriate literature critically reviewed at a level consistent with doctoral standards? *Does the student demonstrate a deep level of understanding of the topic? Have a range of appropriate sources, including peer reviewed primary sources, been used? Is the literature analysed and interpreted, with limitations in current knowledge and practice highlighted?*

Feedback: The literature review shows the student understands the topic area and clearly explains and discusses the current testing strategies very well. The review is supported by appropriate and relevant literature and also refers to current testing algorithms and recommendation. Limitations to studies and testing are highlighted which sets the scene for the innovation and the aims and objectives very nicely.

Please circle the outcome for this section

PASS

3. **The Innovation**

   Has the business case for implementation and any barriers for implementation been clearly articulated? The trainees have been advised the business case can take whatever format they find most appropriate. In most cases this will include financial costings but in some it may not. The important criterion to mark against is whether the business case convinces (either for or against) the innovation. **[Both supervisors have equal weighting]**

Feedback: The innovation was clear and covered all the main areas required. It included a risk/benefit analysis and had a good implementation plan with stakeholder consideration. Overall the innovation and business case was convincing.

Please circle the outcome for this section

PASS FAIL

4. Is the proposal clearly written and understandable and is the executive summary accessible to non-experts **[Academic Supervisor to lead]**

Feedback: The proposal was well written and flowed very nicely. The executive summary was good but could have been more accessible to the non-expert in places, particularly with some of the technical terms.

Please circle the outcome for this section

☐ PASS

Overall Outcome: ☐ PASS FAIL

Date Completed: 13/07/2017

Academic Supervisor: Phil Macdonald

Clinical Supervisor:

MANCHESTER 1824

The University of Manchester

C1 Oral Presentation Feedback

Name of Trainee: Laura Tooth

Date of assessment: 30/09/17

Specialism: ____ Clinical Biochemistry___

Innovation Title: Novel urine oxalate service

Assessment criteria:

- Quality and clarity of explanation of the innovation for a lay audience (awareness of the use of jargon, scientific language and acronyms)
- Synthesis of relevant scientific evidence for a lay audience
- Ability to persuade a lay audience of the merits (or otherwise) of the innovation and its potential role in healthcare science services
- Style of presentation (slides, delivery; body language, eye contact, voice, confidence) and appropriateness for a lay audience
- Demonstration of values, attitudes and behaviours expected of a leader in clinical science

Summary comments for feedback:

What was good:
This was a very good presentation. You explained your innovation clearly and without slipping into jargon, your descriptions of mass spectrometry and standard curves was particularly good. The style and content of your delivery was good and your slides were mostly clear and made appropriate use of diagrams which were helpful in explaining the ideas. You answered the questions very well and clearly demonstrated your empathy to patients and your focus on NHS values. A good, clear talk easily understandable to non-specialists.

What could be improved:
A few slides were a bit busy and occasionally you turned away from the audience to read from the screen, but this was a minor part of an overall good presentation.

Overall:
A very good talk that met all of the above criteria

Recommendation: Pass

Appendix D

Publication list

Throughout the HSST programme I have attended conferences presenting oral and poster presentations, published papers, and completed two book chapters. A list is shown below:

Poster presented at EUROMEDLAB 2021 MUNICH:

Cook, L.E., Piec, I., English, E. (2021). 'Vitamin D deficiency in healthcare workers during the COVID-19 pandemic'.

Journal article in review 2021:

Piec, I., Cook, L.E., Dervisevic, S. et al. (2021). 'Age and vitamin D affect the magnitude of the antibody response to the first dose of the SARS-CoV-2 BNT162b2 vaccine'.

Journal article published 2021:

English, E., Cook, L.E., Piec, I. et al. (2021). 'Performance of the Abbott SARS-CoV-2 IgG II Quantitative antibody method, including the new Variants of Concern, VOC 202012/V1 (United Kingdom) and VOC 202012/V2 (South Africa), and first steps towards global harmonization of COVID-19 antibody methods', *Journal of Clinical Microbiology,* 59(9), e00288-21. http://doi.org/10.1128/JCM.00288-21.

Text book chapter 'Biochemistry' published 2018:

Crerar-Gilbert, A.A. and MacGregor, M. (2018). *Core Topics in preoperative anaesthetic assessment and management*. Cambridge press.

Poster presented at FOCUS 2018:

Tooth, L., Whitlock, M., Walker, E. (2018). 'An Unexpected Endocrine Abnormality'.

Journal article published 2017:

Tooth, L.E. and Collinson, P.O. (2017). 'The dilemma of finding a young patient with a raised cholesterol concentration'. *Journal of* Applied Laboratory Medicine, 1(5), pp. 572–575. https://doi.org/10.1373/jalm.2016.022426.

Text book chapter 'POCT – The UK Perspective' published 2017:

Luppa, P.B. and Junker R. (2017). *POCT – Patientennahe Labordiagnostik*. Springer.

Poster presented at FOCUS 2017:

Tooth, L. and Klammer, M. (2017). 'An underlying cause of iron overload in a bone marrow transplant patient'.

Poster presented at AACC 2016 ATLANTA:

Tooth, L. and Collinson, P. (2016). 'The LDL-R variant c.1426C>T; p.P476S as a novel cause of familial hypercholesterolaemia'.

Oral presentation at FOCUS 2016:

'Hospital induced metabolic acidosis'.

Poster presented at FOCUS 2015:

Tooth, L., Collinson, P. and Boa, F. (2015). 'Implementing the Acute Kidney Injury (AKI) NICE guidelines through the NOVA Statsensor Point Of Care Testing (POCT) creatinine device'.

Appendix E

CALM Ethics Approval

This Appendix contains the ethical approval letter for the CALM study.

Ymchwil Iechyd a Gofal Cymru
Health and Care Research Wales

NHS
Health Research Authority

Prof Garry John
Consultant Clinical Biochemist
Norfolk and Norwich University Hospital
Colney Lane
Norwich
Norfolk
NR4 7UY

Email: approvals@hra.nhs.uk
HCRW.approvals@wales.nhs.uk

07 January 2021

Dear Prof John

HRA and Health and Care Research Wales (HCRW) Approval Letter

Study title:	**Measurement of COVID-19 antibody concentrations following vaccination of NHS staff against COVID-19.**
IRAS project ID:	**292799**
REC reference:	**20/NE/0287**
Sponsor	**Norfolk and Norwich University Hospital**

I am pleased to confirm that HRA and Health and Care Research Wales (HCRW) Approval has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

Please now work with participating NHS organisations to confirm capacity and capability, in line with the instructions provided in the "Information to support study set up" section towards the end of this letter.

How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?
HRA and HCRW Approval does not apply to NHS/HSC organisations within Northern Ireland and Scotland.

If you indicated in your IRAS form that you do have participating organisations in either of these devolved administrations, the final document set and the study wide governance report (including this letter) have been sent to the coordinating centre of each participating nation. The relevant national coordinating function/s will contact you as appropriate.

Please see IRAS Help for information on working with NHS/HSC organisations in Northern Ireland and Scotland.

How should I work with participating non-NHS organisations?
HRA and HCRW Approval does not apply to non-NHS organisations. You should work with your non-NHS organisations to obtain local agreement in accordance with their procedures.

What are my notification responsibilities during the study?

The standard conditions document "*After Ethical Review – guidance for sponsors and investigators*", issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

Who should I contact for further information?
Please do not hesitate to contact me for assistance with this application. My contact details are below.

Your IRAS project ID is **292799**. Please quote this on all correspondence.

Yours sincerely,
Christie Ord

Approvals Specialist

Email: approvals@hra.nhs.uk HCRW.approvals@wales.nhs.uk

Copy to: *Ms Julie Dawson*

List of Documents

The final document set assessed and approved by HRA and HCRW Approval is listed below.

Document	*Version*	*Date*
IRAS Application Form [IRAS_Form_10122020]		10 December 2020
Letter from funder [Letter from funder]	1.0	05 November 2020
Letters of invitation to participant [Letter of invitation]	v1.1	06 January 2021
Non-validated questionnaire [First visit questionnaire]	v1.1	06 January 2021
Other [Response to Conditions of favourable opinion]	1	06 January 2021
Participant consent form [Consent form]	v1.1	06 January 2021
Participant information sheet (PIS) [PIS]	v1.1	06 January 2021
Research protocol or project proposal [Protocol]	v1.1	06 January 2021

IRAS project ID	292799

Information to support study set up

The below provides all parties with information to support the arranging and confirming of capacity and capability with participating NHS organisations in England and Wales. This is intended to be an accurate reflection of the study at the time of issue of this letter.

Types of participating NHS organisation	Expectations related to confirmation of capacity and capability	Agreement to be used	Funding arrangements	Oversight expectations	HR Good Practice Resource Pack expectations
This is a single site study sponsored by the participating NHS organisation therefore there is only one site type.	This is a single site study sponsored by the participating NHS organisation. You should work with your sponsor R&D office to make arrangements to set up the study. The sponsor R&D office will confirm to you when the study can start following issue of HRA and HCRW Approval.	This is a single site study sponsored by the participating NHS organisation therefore no agreements are expected.	External study funding has been secured	A Principal Investigator should be appointed at study sites	The sponsor has confirmed that local staff in participating organisations in England who have a contractual relationship with the organisation will undertake the expected activities. Therefore no honorary research contracts or letters of access are expected for this study.

Other information to aid study set-up and delivery

This details any other information that may be helpful to sponsors and participating NHS organisations in England and Wales in study set-up.
The applicant has indicated that they do not intend to apply for inclusion on the NIHR CRN Portfolio.

Appendix F

CALM Consent form

This Appendix contains the consent form for the CALM study.

Assessing immune response to COVID-19 vaccine

I confirm

Please initial each box
Do not tick.

- I have read the participant information Sheet [version 1.1] and had the opportunity to ask questions. ☐
- I am happy to supply some personal and medical data as shown on the short questionnaire [First visit version 1.1] ☐
- I am happy for the research team to use this information in the study, and in any publications that may result in an anonymised form so I cannot be identified ☐
- I am happy to have blood samples collected as described in the Patient information Sheet [version 1.2] for use in the research described. ☐
- I consent to any residual material remaining after the study to be stored in the Norwich Research Park Biorepository and be used in other ethically approved research into COVID-19. ☐
- I wish to receive results of the tests on my blood ☐
- I wish to receive a summary of the study findings when it is concluded ☐
- I confirm my participation in this study was totally voluntary ☐

Sign .....

Print name .....

Witnessed by .....

Print name and
Role in research group .....

It is recommended that you take 24 hours to consider if you wish to participate in this study.

A COPY OF SIGNED CONSENT FORM TO BE GIVEN TO PARTICIPANT.

Appendix G

CALM Patient information sheet

This appendix contains a copy of the patient information sheet (PIS) that was distributed to all potential CALM participants prior to their consent. The PIS briefly explains the background to the project, what the project aims to do, and what it involves from the participants. It also details where to ask for more information, and what happens with the study samples after analysis.

Assessing immune response to COVID-19 vaccine

Invitation

You are being invited to take part in this research project, which aims to investigate people's immune response to the COVID-19 vaccine being rolled out nationally. The study will involve having several blood samples taken over a period of time to see if you make antibodies to the virus after having the vaccine and if so how high the antibody concentration is and how long it lasts (serology testing). Before you decide to participate in this study, it is important you understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish, feel free to ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

This project is being sponsored by Norfolk and Norwich University Hospital

What is the project's purpose?

SARS-CoV-2 is the virus that results in the disease COVID-19 and is an entirely new virus which has affected many millions of people worldwide. Because it is so new little is known about the human body's immune response to the virus or the vaccinations that have been developed to protect us against it. The development of an immune response is shown by the presence of antibodies in people's blood (serology testing).

Recent research indicates that a person's Vitamin D levels may affect their susceptibility to the disease and their immune response to the disease or the vaccination. Therefore, we will also measure Vitamin D on the first sample, taken just prior to vaccination.

In this project we are investigating 5 things:

1. Do people show an immune response in the form of antibodies to COVID-19 following vaccination?
2. How high is the concentration of the antibodies in the blood after vaccination?
3. How long do the antibodies last?
4. Is the response to the vaccine different in people who have had the disease before they were vaccinated?
5. Is the immune response to the vaccine different depending on your Vitamin D status?

Why have I been chosen?

You have been chosen because you will be receiving a vaccination against COVID-19 as part of the National vaccination programme.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you should keep a copy of this information sheet and indicate your agreement on the consent form; you will be given a copy of your signed consent form. You can withdraw from the study at any time via the details at the end of this information sheet. You do not have to give a reason if you choose not to participate, or withdraw early. If you withdraw from the study we will keep any data or samples we currently hold on you, but will not collect any further samples or data.

What will happen to me if I take part?

Using a short questionnaire we will collect and record some basic information about you including information about your general health and relevant medication. You will be asked to give a blood sample of 6 ml (teaspoon full) which will be tested for COVID-19 antibody concentrations. You will be asked to give further samples of 6 ml (teaspoon full) weekly (1, 2, 3, 4 and 8 weeks) until your second injection then monthly for 18 months or until you no longer have antibodies detected in your blood.

We will measure Vitamin D on the blood sample collected prior to vaccination and this result will be reported to you. This is why we need to collect your name and date of birth.

You will be offered the opportunity to have your antibody results. This is your choice and you can change your mind at any time during the study. These results will be reported using your study number. There will be no reference as to the interpretation of results as **we do not currently know the significance of antibody concentration in an individual, or even if the presence antibodies confirm immunity to COVID-19.**

What will happen to my blood after the tests?

When the study team receive your sample, it will be quickly processed by Clinical Biochemistry at the Norfolk and Norwich University Hospital and will be stored there until the end of the study. Samples will be processed to determine COVID-19 antibody concentration. At the end of the study, samples will be incinerated, unless you consent to store your samples at the Norwich Research Park Biorepository. This is entirely optional. If you would like more information about the Norwich Research Park Biorepository, please contact the team at BioRepository@nnuh.nhs.uk

All data and samples will be anonymised using a unique code which will enable the research team to identify you and pass relevant information to you. Your name or any other details will not be released to anyone outside the research team.

What do I have to do?

We will collect the blood samples and ask you to complete the questionnaire at a time and place suitable to you. This will be agreed with you at the time of first contact.

What are the possible disadvantages and risks of taking part?

There is always a small chance of some bruising when blood is taken, other than this participating in the research is not anticipated to cause you any disadvantages or discomfort.

What are the possible benefits of taking part?

We will give you the results of all tests if you wish to receive them so you will know if you have made antibodies to the COVID-19 vaccine. This study will help us to understand what a good response to the vaccine is but this is not known at the moment and you are still required to follow the Government's current COVID-19 guidelines even if you have antibodies.

What if something goes wrong?

If you have any comments or complaints about study then you can contact Professor John using the contact details at the end of this patient information sheet.

Will my taking part in this project be kept confidential?

In this research study we will use information from you. We will only use information that we need for the research study. We will let very few people know your name or contact details, and only if they really need it for this study.

Everyone involved in this study will keep your data safe and secure. We will also follow all privacy rules.

At the end of the study we will save some of the data in case we need to check it and for future research.

We will make sure no-one can work out who you are from the reports we write.

Information provided by you in the questionnaire will be held securely on a password protected computer on the Sponsors site. There will be two data bases:

Central Database (data held)

- Name and email address
- Date of birth
- Study number

This will be used to inform you of your Vitamin D result. This will be stored on a secure database separate from the working data. This database will be destroyed at the end of the study.

Working Database (data held)

- Study number
- Age
- Gender
- Ethnicity
- Basic clinical information
- All laboratory results

People will use this information to do the research or to check your records to make sure that the research is being done properly.

People who do not need to know who you are will not be able to see your name or contact details. Your data will have a study number instead.

We will keep all information about you safe and secure. Your data will be stored on a secure PC within the NNUH network and held to the same level of protection as your NHS records and will be stored for 15 years following the end of the study.

Once we have finished the study, we will keep some of the data held in the working database so we can check the results. We will write our reports in a way that no-one can work out that you took part in the study.

What are your choices about how your information and samples are used?

- You can stop being part of the study at any time, without giving a reason, but we will keep samples and information about you that we already have.
- If you agree to take part in this study, you will have the option to take part in future research using your data/samples saved from this study. Samples will be stored in the Norwich Biorepository.

Where can you find out more about how your information is used?

- at www.hra.nhs.uk/information-about-patients/
- by asking one of the research team
- by sending an email to g.john@nnuh.nhs.uk
- by ringing us on 01602 286933

What will happen to the results of the research project?

Results of the research will be published to inform ourselves, other researchers in the field, particularly those working on vaccine development, and the doctors treating patients with COVID-19. You will not be identified in any report or publication. If you wish to be given a copy of any reports resulting from the research, please ask us to put you on our circulation list when you see us.

Who is organising and funding the research?

The research will be organised through the Norfolk and Norwich University Hospital and Norwich Medical School, University of East Anglia. Financial support and materials for the project will be provided by Abbott Laboratories (Chicago, USA).

Who has ethically reviewed the project?

NHS Research Ethics Service (NRES)

Further questions / withdrawal from study

This Patient Information Sheet should be kept safely and can be referred to during the study and will hopefully answer any questions you may have; but if you have any further questions or wish to withdraw from the study please feel free to contact me at (g.john@nnuh.nhs.uk) or phone (01603 286933).

If you have any complaints relating to this study please contact the Patient Advice and Liaison Service (PALS): email address pals@nnuh.nhs.uk; telephone 01603 289036 or 01603 289045.

How do I sign up?

Send an email to (g.john@nnuh.nhs.uk) or phone (01603 286933) and leave a message. We will contact you and arrange to meet, collect the signed consent form and fill in a short information questionnaire before taking the first blood sample

Thank you for considering taking part in this research.

Professor Garry John
Department of Clinical Biochemistry & Immunology
Norfolk and Norwich University Hospital
Colney Lane
Norwich
NR4 7UY
Direct dial: 01603 286933
e-mail: g.john@nnuh.nhs.uk

Appendix H

CALM Participant questionnaire

This Appendix contains the questionnaire for the CALM study that was completed at recruitment.

University of East Anglia

NHS
Norfolk and Norwich University Hospitals
NHS Foundation Trust

Study code

Assessing immune response to COVID-19 vaccine

First visit

Participant details:

Participant's full name: _____

Participant's email address: _____

Date of birth (age) _____

Ethnicity: _____

Male ☐ Female ☐

Weight: _____kg or stone/lbs Height _____cm or feet/inches

DO YOU WANT TO BE INFORMED OF YOUR RESULTS? YES / NO

Overall health at time of blood collection	YES	NO
Any health Problems		
Current COVID-19 Symptoms		
COVID-19 Symptoms in past 6 months		
Current medication (excluding contraception medication)		

If answering YES to any of the above please detail here.	Specify medication if known

Specimen collection

Date __/__/____ time: ___:___

Phlebotomist Initials: _____

Appendix I

Vitamin D supplementation guidance

This Appendix contains the voluntary vitamin D supplementation guidance issued to all CALM participants following dose one baseline vitamin D results that identified 65% of participants as being vitamin D deficient.

University of East Anglia

Norfolk and Norwich University Hospitals NHS Foundation Trust

Guidance for Supplementation of Vitamin D3 in Subjects with Varying Concentrations of Circulating 25 hydroxy vitamin D (25 OHD)

Prof William D Fraser (w.fraser@uea.ac.uk)

- When supplementing with vitamin D it is best to use vitamin D3, as vitamin D2 has a shorter half life in circulation, and is not as potent as vitamin D3. (25 mcg = 1000 IU Vitamin D3).
- The recommendation made is to ensure all body stores are fully replete. Boots is a good source of vitamin D3.

Concentration of 25 OHD	Recommendation
0 – <30 nmol/L	Commence 20,000 IU Vitamin D3 once weekly for 3 months, then decrease to 1,000 IU daily for 9 months.
30 – <50 nmol/L	Commence 1,000 IU Vitamin D3 daily for 9 months.
50 – 75 nmol/L	In winter, or if symptomatic with bone aches, mild pains, or with pigmented skin, consider 400-1000 IU daily for 9 months.
75 – 120 nmol/L	No supplement required, unless already on supplementation. (If already on supplementation, maintain same level of supplement).
>120 nmol/L	If on supplement, reduce dose. If not on supplement contact Doctor (use email above).