Queensland (PQ) uses the Roche Cobas Taqman HBV assay to monitor DNA levels in known HBV positive patients over time, however the assay is not intended as a diagnostic test for HBV infection. Requests for HBV PCR, without the need for quantification, have been increasing over the last three years.

The success of molecular tests can be undermined by the high degree of sequence variability exhibited by HBV, and may lead to false-negative results. We aimed to design a reliable real-time qualitative assay for screening specimens in the diagnostic laboratory.

A multi-target HBV real-time PCR (mtHBV) assay was developed targeting highly conserved sequences on the HBV large S protein and X protein genes. The mtHBV assay was validated with 201 clinical specimens submitted to PQ for HBV testing, and the results were compared to those obtained using the quantitative COBAS Taqman HBV Test (Roche Diagnostics). The results show the mtHBV real-time PCR assay is suitable for routine detection of HBV DNA in clinical samples, and represents a significant cost saving compared to the Roche method. In addition, the two-target system of the mtHBV assay decreases the potential for sequence-related HBV false-negative results. This approach may enhance the detection of a broad range of infectious agents, particular those exhibiting extensive genetic variation.

P16.03

Development of Hepatitis D Virus Quantitative Real-time RT-PCR

Grace Chang, Geoff Higgins

Infectious Diseases Laboratories, SA Pathology, Adelaide SA

Introduction Hepatitis D is caused by the small (1.7kd) defective hepatitis D virus (HDV). HDV only infects Hepatitis B patients because it needs hepatitis B virus surface antigen for replication. This dual infection can lead to severe liver diseases. In this paper, we describe a quantitative real-time RT-PCR developed within SA Pathology for quantifying HDV RNA levels in patients with acute and chronic hepatitis and patients on antiviral therapy.

Materials and Methods 67 samples from 44 patients including 37 patients with antibodies to HDV and 8 patients suspected of HD but anti-HDV was negative. 60 samples from HIV, HCV, CMV, BKV and EBV infected patients and renal transplant patients (free of the above infections) were used for Specificity study. DNA oligo, clones and RNA transcripts were produced for drawing standard curve and extraction controls. Qualitative one-step nested RT-PCR method provided by Victorian Infectious Diseases Reference Laboratory was used as the reference assay. Superscript III RT-PCR Systems were used for both methods.

Results A. Analytical sensitivity (probit analysis) was 720cps/ml. **B.** Specificity: No false positive were detected in control samples. **C.** Linearity: Clone DNA was diluted to represent $7.8x10^2$ – $7.8x10^9$ cps/ml. It was linear throughout the dilution range with concentration deviation ranging from 1.6% to 24.2%. **D.** 57/67 (85%) samples gave concordant results (27 detected, 27 not detected, 3 borderline). 6 samples were detected only by Real-time RT-PCR; 1 detected only by nested RT-PCR and 1 detected by nested RT-PCR but borderline by the real-time RT-PCR. **E.** The highest viral load in samples tested was 5.7 million cps/ml. **F.** HDV was detected but below 720cps/ml in 7 samples.

Conclusion The quantitative real-time RT-PCR developed within SA Pathology was at least as sensitive as the one-step nested RT-PCR. It is quantitative, faster and needs less hands on-time when compared to the one-step nested RT-PCR.

P16.04

The Carboxyl Tail of the Mouse Cytomegalovirus (CMV) Seven Transmembrane Receptor (7TMR) Homologue pM78, Contains Positive and Negative Regulators of Constitutive Endocytosis

Nick Davis-Poynter[1], Joanna Fong[1], Helen Farrell[1]

[1]Sir Albert Sakzewski Virus Research Centre & Clinical Medical Virology Centre, Royal Children's Hospital & University of Queensland, Brisbane QLD

The 7TMR superfamily comprises a diverse array of cell-surface receptors that typically mediate G protein-coupled signalling in response to a variety of extracellular ligands. All sequenced beta- and gammaherpesvirus encode one or more 7TMR homologues, suggesting that they play important roles during the virus lifecycle. Regulation of cellular 7TMR signalling is normally tightly controlled, requiring ligand binding for activation of signalling, with subsequent desensitization/recycling mediated by endocytosis. In addition to several examples of constitutive (ligand independent) signalling, a number of the herpesvirus 7TMR exhibit constitutive endocytosis. Not only may this feature be important for the function of viral 7TMR, but they may also be useful probes for studying cellular regulation of 7TMR endocytosis and trafficking.

M78 of mouse CMV is a member of the "UL78 family" of 7TMR genes, conserved in all betaherpesviruses. Previous studies of pM78 and its counterpart in rat CMV (pR78) have demonstrated that they are required for efficient cell-cell spread in tissue culture and replication *in vivo*. Recently we have reported that pM78 is constitutively endocytosed via clathrin and lipid raft/caveolae-mediated pathways. More recently, we have shown that G protein-mediated signalling was not required for pM78 endocytosis. The aim of the study presented here was to examine the structural requirements for cell surface expression and endocytosis for pM78 by progressive truncation of the C-tail and point mutation of putative endocytic motifs. These studies demonstrated the importance of a di-leucine motif (aa 455-465) and a second region (aa 317-347) as positive signals for endocytosis and suggested that the intervening region may contain a negative regulator (aa 386-425). We are currently determining whether the endocytic function of pM78 plays a role during virus replication, by incorporation of selected mutations into recombinant MCMV.

P16.05

Investigation of the Role of Cell Surface Carbohydrates in Binding and Infection of Group A Human Enteroviruses

Sinéad M. Diviney[1], Sharon Sanders[1,2], John S. Mackenzie[1], Peter McMinn[3], David T. Williams[1]

[1]Australian Biosecurity CRC for Emerging Infectious Disease, Curtin University of Technology, Perth, Western Australia;
[2]Discipline of Microbiology and Immunology, School of Biomedical, Biomolecular and Chemical Sciences, the University of Western Australia, Perth, Western Australia;
[3]Discipline of Infectious Diseases and Immunology, University of Sydney, New South Wales.

The Group A human enterovirus (HEV-A) species is one of five groups within the *Enterovirus* genus of the *Picornaviridae* family and comprises sixteen genetically related virus serotypes (Coxsackie A virus (CAV) types 2-8, 10, 12, 14, 16 and Enterovirus (EV) types 71, 76, 89-91). Cellular receptors have a fundamental role in virus infection and are primary determinants of virus tissue tropism and pathogenesis. To date, there have been no published reports of cellular attachment receptors and entry mechanisms for the HEV-As. EV71, CAV type 2, 10 and 16, which are known to cause outbreaks of hand, foot and mouth disease and encephalitis in Asia, can bind to a wide range of cell types from several different