Human Herpes simplex virus types 1, 2 (HSV1 and HSV2) and Varicella Zoster virus (VZV) infections are the most common viral infections detected in viral diagnostic laboratories. The aim of this study was to evaluate a newly introduced LightCycler multiplex real-time PCR (LCmPCR) kit (Roche, Australia) for the detection and differentiation of HSV1, 2 and VZV, against our existing nested multiplex PCR (nmPCR) which detects HSV1, HSV2, VZV, CMV, EBV and Enterovirus, and our one-step multiplex PCR (mPCR), which detects HSV1, HSV2, VZV and Adenovirus.

A total of 228 specimens were assessed, 43 using all three LmPCR, mPCR and nmPCR with identical results for the 12/43 positive and 31/43 negative specimens. We tested 124 clinical specimens using the LCmPCR and mPCR in parallel, and detected 9/124 HSV1 (7.26%), 15/124 HSV2 (12.1%) and 13/124 VZV (11.4%), with 100% correlation between the two assays. We tested 147 specimens using the LCmPCR and nmPCR in parallel, and detected 7/147 HSV1 (4.76%), 6/147 HSV2 (4.08%) and 7/147 VZV (4.76%) with 100% correlation between these two assays. LCmPCR showed very good specificity. The sensitivity of LCmPCR is the same as the one-step mPCR, but less sensitive than two-step nmPCR on limit of detection testing using dilutions of DNA extracted from cell culture material.

The LCmPCR offers specificity, sensitivity combined with rapid (two hour) quantitative detection and differentiation of HSV1, HSV2 and VZV. Although nmPCR is a more sensitive method, allowing detection of more virus species, it takes two-working days to obtain results. LCmPCR is particularly helpful for rapid turnaround and for guiding therapy.

P16.16

Towards Development of a MassTag PCR Assay for the Syndromic Detection of Pathogens that can cause Encephalitis

David T. Williams[1], David Menegola[1], Thomas Briese[2], Rafal Tokarz[2], Gerald B. Harnett[3], John S. Mackenzie[1], David W. Smith[3], W. Ian Lipkin[2]

[1]Australian Biosecurity CRC for Emerging Infectious Disease, Curtin University of Technology, Perth, Western Australia;
[2]Center for Infection and Immunity, Mailman School of Public Health, Columbia University, New York, USA;
[3]PathWest Laboratory Medicine WA, Perth, Western Australia.

MassTag PCR is a novel technology for the rapid, sensitive and simultaneous detection of multiple gene sequences. This technique, developed at the Center for Infection and Immunity (CII) at Columbia University, utilises a library of unique molecular tags, each unique in its molecular weight. MassTags are conjugated to oligonucleotide primers using a UV-cleavable linker that enables separation of primer and tag. Different primers are labelled each with a different molecular weight tag and are used to amplify target nucleic acids in a multiplex RT-PCR. After removing unincorporated primers, tags are released by UV irradiation and analysed by mass spectrometry. Thus, amplification of the gene target produces a unique dual signal in mass spectrometric analysis that allows its identification. MassTag PCR offers an inexpensive and sensitive diagnostic platform suitable for high-throughput testing that can be adapted to suit diagnostic needs (e.g., syndrome-, vector-based). To date, MassTag PCR panels have been developed for the *detection of respiratory and tick-borne pathogens, and viruses* that cause haemorrhagic fever. An additional MassTag PCR assay is being developed to identify microbial agents that cause neurological disease in a North American/European diagnostic setting. In collaboration with the CII and PathWest Laboratory Medicine, we have begun modifying and developing this assay to address pathogens relevant to the Australasian region. Initial research has involved extensive bioinformatics analyses for the design of primer sets that are specific only for the cognate target pathogen and that will not cross-hybridise with existing primers comprising the panels. This has been followed by in vitro evaluation of multiplex panels using untagged primers and agarose gel analysis. Preliminary research experiences and progress towards development of this assay will be presented.

P16.17

Lyssaviruses and Southern African Bats

Wanda Markotter, Louis Nel

University of Pretoria, Department of Microbiology and Plant Pathology, 0002 Pretoria, South Africa

Rabies virus, the genotype 1 (gt1) lyssavirus, is found throughout Africa and worldwide – Australia being a notable exception – harboring only the Australian bat lyssavirus (gt7). Three additional lyssavirus genotypes are exclusive to Africa. These are Lagos bat virus (LBV, gt2), Mokola virus (MOKV, gt3) and Duvenhage virus (DUVV, gt4). Both LBV and DUVV have been associated with bat reservoirs - LBV appears to primarily circulate in frugivorous bats and DUVV in insectivorous bats. However, little is known about the activity and distribution of these viruses and few isolates have been made over past decades. Recently, we have embarked on passive as well as active surveillance for these bat-associated rabies-related lyssaviruses. We tested samples from frugivorous and insectivorous bats (brain, serum, oral swabs) collected during our field excursions or obtained from museums, bat interest groups and veterinary diagnostic laboratories, for the presence of lyssaviruses. Brain samples were tested for viral antigen (fluorescent antibody test, FAT) and oral swabs for viral RNA (nested RT-PCR). Serum samples were tested for lyssavirus antibodies using a rapid fluorescent focus inhibition test (RFFIT).

Six new isolates of LBV have been recovered from frugivorous bat species and characterized with regard to nucleoprotein sequences. These isolations followed a period of 13 years in which LBV had not been encountered in South Africa, demonstrating active endemic cycles and the value of targeted surveillance for such viruses. In addition, oral swabs collected from bats that were positive by FAT, also tested positive for the presence of viral RNA, confirming the likely involvement of saliva in virus transmission. Regarding serology we have, in tandem with related studies, confirmed surprisingly high rates of seropositivity among healthy animals. Our findings re-emphasize a lack of surveillance and understanding of the true prevalence and epidemiology of lyssaviruses throughout Africa.

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Posters – Biosafety

Thursday 9 July 3.30pm
Exhibition Hall (Pavilion Hall 1)

P17.01

Commencement of the Security Sensitive Biological Agents Regulatory Scheme

Letitia Toms[1], Gary Lum[1]

[1] Office of Health Protection, Australian Government Department of Health and Ageing, Canberra ACT

The outcome of the Council of Australian Governments review of hazardous biological materials is the implementation of the Security Sensitive Biological Agents (SSBA) Regulatory Scheme on 30 January 2009. The legislation underpinning the scheme is the *National Health Security Act 2007*, which was passed by Parliament