

## **HIV DIAGNOSTICS: PAST, PRESENT AND FUTURE CHALLENGES**

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Diagnosis of HIV infection in health-care settings and screening of blood donors to prevent transmission of HIV through blood transfusion play key roles in fighting the HIV epidemic. During the past three decades, significant progress has been made to improve the sensitivity and specificity of HIV assays resulting in earlier diagnosis of HIV infection and greatly improved blood safety.

The first generation assays, developed in 1985 for diagnosis of HIV infections, used tissue culture derived viral antigens on the solid phase and polyclonal antibodies to human immunoglobulins conjugated to an enzyme for detection of HIV specific antibodies. During the early days of the HIV pandemic, these assays had a major role in reducing the number of new infections and improving the safety of the blood supply. The second-generation assays used HIV-1 and HIV-2 recombinant antigens instead of viral lysate resulting in improved assay specificity. The third generation assays replaced the anti-human IgG conjugate with recombinant antigens conjugated to an enzyme or hapten that could detect HIV specific antibodies bound to solid phase. The ability of these assays to detect IgM, in addition to IgG, resulted in reduction of the seroconversion window. Identification of HIV-1 group O and variable detection group O infections by assays using HIV-1 subtype B specific reagents led to the incorporation of recombinant group O antigens or peptides into HIV assays. At the same time, assays were also developed for detection of HIV-1 p24 antigen using antibodies to capture and detect HIV antigen. During the past decade, fourth generation assays have been developed that combine antigen and antibody detection in a single assay. As these assays have the ability to detect antigen and antibody simultaneously, detection of acute infections has improved significantly. Seroconversion panels have been effectively used to demonstrate performance of the fourth generation assays for detecting early, antigen positive, antibody negative bleeds. However, assays differ in their analytical sensitivity for p24 antigen which is correlated to their ability to reduce the window period. In addition, the genetic variability within HIV-1 groups and subtypes also impacts p24 antigen detection and represents a challenge for detection of acute infections. A recent evaluation showed a broad range of p24 antigen sensitivity for various HIV-1 subtypes between different fourth generation HIV assays.

One of the major challenges for HIV diagnosis continues to be genetic variation. HIV-1 is classified into four groups, M, N, O and P; each group arose from a separate transmission of SIV strains from nonhuman primates into humans. HIV-1 group M, which accounts for greater than 90% of HIV infections worldwide, is further classified into 9 subtypes and more than 50 inter-subtype circulating recombinant forms. Continued diversification and global redistribution of HIV groups, subtypes and recombinants make it imperative that serological and molecular assays be designed and evaluated to ensure reliable performance on all HIV infections.



## THE ROLE OF NRL IN THE EARLY DAYS OF HIV TESTING IN AUSTRALIA

Susan Best on behalf of NRL staff

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NRL was established in 1985, known at that time as the National HIV Reference Laboratory. Then, as now, there was a three-tier system for HIV testing in Australia. Screening was performed in blood transfusion laboratories and a number of public health and hospital laboratories. No HIV testing was conducted in private laboratories. Each state had at least one reference laboratory that performed confirmatory testing on specimens that were reactive on screening. Specimens that returned conflicting or equivocal results on confirmatory testing were referred to NRL. This testing was known as “tertiary reference testing”. Today this three-tier system for HIV testing still exists. In addition to reference testing, NRL’s roles in the early days of HIV testing were to:

- evaluate HIV test kits;
- maintain HIV testing statistics;
- monitor the specificity of test kits used to screen blood donations; and
- convene an annual national workshop.

As part of its confirmatory testing role, NRL developed and validated its own in-house HIV-1 Western blot (WB) and criteria for its interpretation. WB results that did not fulfil criteria for negative or positive were divided into four groups based on the HIV proteins to which reactivity was observed. Following extensive research it was determined that specimens showing group 1 or 2 reactivity could be reported negative whereas those with group 3 or 4 reactivity were considered indeterminate and repeat testing was required after six months. Laboratories from all around Australia collaborated in this activity by providing specimens for confirmatory and follow-up testing. NRL continues to use its in-house HIV-1 WB today.

This presentation will draw data from NRL Bulletins between 1985 and 1989 including test kit evaluations, numbers of samples tested and cumulative numbers of HIV positive individuals identified. The NRL Bulletin was an annual publication that reported results of NRL’s activities.

## **HIV: AN AFRICAN PERSPECTIVE**

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In 1991 the South African government implemented a strategy to measure the prevalence of the HIV endemic. As it was realized that HIV was a sexually transmitted disease the government implemented an antenatal survey which tested all pregnant woman that were enrolled in clinic visits. In the first year the prevalence was estimated to be 0.01% however it rapidly increased to 28% in 2004. In the general 16 to 65 year olds the prevalence according to UNAIDS is 18% of which the young adults 19 to 40 year olds have the highest prevalence and this is the population that is targeted for blood donation. The last three years has seen a plateau in the prevalence in the general population which may be due to the roll out of antiretroviral drug therapy.



## THE ROLE OF MOLECULAR DIAGNOSTICS IN THE HIV PROGRAM IN PAPUA NEW GUINEA

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### Introduction:

The diagnosis of HIV commenced in PNG in 1990s with the use of various rapid tests. With international collaboration and assistance from global fund for HIV/AIDS, tuberculosis and malaria (GFATM), Clinton Health Access Initiative (CHAI) and World Health Organisation (WHO), a programmatic national approach including diagnosis of HIV using a National HIV testing Algorithm at point of care was introduced in 2008 with Determine and Stat Pak following a validation process. Early infant diagnosis (EID) by dried blood spot (DBS) DNA PCR within prevention of parent to child transmission (PPTCT) program commenced around the same time with expansion to a second site. CD4 testing was introduced with the commencement of anti-retrovirus treatment. Different platforms were also examined for CD4 with the acceptance of BD FACSCount in Provincial Hospitals and PIMA for point of care testing. Viral load using standard PCR was introduced as a research project for HIV patients in Heduru Clinic and is ready to be implemented for clinical use using the Roche platform with improvement in turn-around time from two weeks to two days. The aim of this project was to improve turnaround time from the use of standard PCR to RT PCR for EID and viral load for patient care.

### Objectives:

1. Improve prevalence data of HIV in PNG through the roll out of HIV algorithm
2. EID to reduce transmission of HIV during pregnancy
3. CD 4 counts and Viral load for improved patient treatment and monitoring

### Methods:

National HIV testing algorithm includes Determine as the screening test for HIV, while Stat-Pak is used for confirmation of HIV at point of care. Standard PCR was initially introduced for the EID program and has been supplemented by the use of Roche Ampliprep Taqman Real Time PCR. The same PCR methods are used for HIV viral load.

### Results:

The introduction of HIV Rapid tests improved reporting for prevalence of HIV. From 2008-2012, HIV DNA from DBS testing of 2245 infants had a HIV positive rate of 24.4%. PCR was established in two sites while CD4 testing has been introduced to 37 sites within country. Recently viral load testing was validated by the use of Roche Ampliprep Taqman Real Time PCR.

### Conclusions:

Molecular diagnostics play an important role in EID program in PPTCT program and determining treatment to prolong lives of HIV/AIDS patients. More effective management of HIV/AIDS patients by guidance of CD4 counts has reduced admission of end-stage patients in hospitals in PNG. Further benefits include identification of HIV-resistant cases for alternate treatment protocols.

## **IMPLEMENTATION OF HIV POINT OF CARE DIAGNOSIS: FEASIBILITY AND INITIAL RESULTS**

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### **Introduction**

Vietnam is one of the few countries in the world piloting the Treatment 2.0 initiative launched by WHO and UNAIDS in 2011. One of the five pillars of this initiative is using HIV point of care (POC) diagnosis. Based on HIV test kit evaluation data from National Institution for Epidemiology and Hygiene (NIHE), a testing algorithm was developed for piloting which included Determine, ACON and DoubleCheckGold. With technical support from Australian NRL, National Institutions and WHO, health staff from 21 commune health centres (CHC) in two provinces Can Tho and Dien Bien were trained on HIV counselling and testing (HTC) using rapid test. This study was to evaluate the feasibility of using rapid testing at CHC and ascertain the performance of the testing algorithm.

### **Methodology**

Testing procedure was reviewed. Data on testing results were collected from patient results sheet. Personal information including patient's name, date of birth and address were not collected. Sensitivity and specificity of the rapid HIV testing algorithm were calculated. In addition, results of supervision and EQA performance were reviewed.

### **Results**

Testing procedure: HTC was provided by commune health staff. Samples were screened by Determine. Negative test results were returned to the clients straight away. The reactive samples were tested with the two confirmatory tests. All samples reactive with the Determine were sent to the confirmatory laboratory for confirmation. The results were then sent back to CHC to be reported to the client. For the purposes of testing algorithm validation and quality assurance, negative samples were collected for the first few months and were sent to the confirmatory laboratory (CL) for re-testing.

HTC results: The pilot started in August 2012. By the end of May 2013, a total of 3,516 people had received HIV tests at CHC including 2,452 pregnant women (PW), 490 MARPs, 367 partners of IDU or HIV positive people and 207 others. Male clients accounted for 85%. Ninety nine samples were reactive on the Determine and of these 54 were confirmed positive with the 2 rapid confirmatory tests. A total of 54 individuals were confirmed positive by the CL including six pregnant women. All of these patients were followed-up and majority of them were enrolled for care and treatment.

Testing algorithm: A total of 1,244 negative samples were sent to the CL. All were confirmed negative by Genscreen HIV1/2 V2. All 54 of the 99 samples that were positive with all three rapid test were also confirmed positive by CL. The results show 100% sensitivity and specificity of the testing algorithm. Although the number of positive sample was modest, the result was very promising.

Feasibility and quality assurance: All 21 sites participated in EQA provided by NIHE. All 20 sites received good results in two rounds except one site got one wrong result for one sample. Discussions with this site have occurred to address the issue. No technical issues occurred during implementation. The results of the pilot confirmed the capacity of commune health staff to implement HIV POC diagnosis.

### **Conclusion**

Expanding the HTC model to CHC is feasible. It improved access to HTC services for MARPs and provided better linkage to care. The result of the HIV testing algorithm provided strong evidence to advocate that the Vietnam government should adopt POC HIV diagnosis at primary health care centres.

## **BUILDING THE EXTERNAL QUALITY ASSURANCE IN HIV TESTING IN RESOURCE LIMITED SETTING IN TANAH PAPUA**

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1 Clinton Health Access Initiatives, 2 Provincial Health Laboratories Jayapura

### **Introduction:**

Based on IBBS on general Population in 2006, Papua is reported as the fastest HIV spreading area in Indonesia with 2.34 % infected people in the population (over 10 times higher than the national rate). An early accurate laboratory diagnosis is a key component of HIV control and prevention, however, the availability of excellent HIV tests does not automatically guarantee reliable results. Provincial Health Laboratories Jayapura supported by donors have developed and conducted an Immunology anti-HIV External Quality Assurance Program to monitor and to improve the quality of HIV testing in Tanah Papua.

### **Objectives:**

To Monitor and improve the quality of HIV testing in Tanah Papua

### **Methods:**

Laboratories with anti-HIV testing capacity in Papua (hospitals, primary health care and VCT clinic laboratories) have been involved in this program since 2007. Initially, fifteen labs have joined in the program in 2007, the number rose to 31 in 2008 and continued in 2012 to become 50 laboratories which perform HIV testing that participated in this EQA. Five panel control tubes consisting of reactive and non-reactive anti-HIV serums were distributed to be tested concurrently by all the labs on their own anti-HIV routine testing procedure. The labs sent a written result report to PHL Jayapura in a defined time frame to be evaluated for the testing strategies and concordance of results.

### **Results:**

Up to now PHL Jayapura has sent the 10 cycles of HIV EQA throughout Tanah Papua which consist of Papua and West Papua Province. In 2007, fourteen labs have sent back the EQAS test result to the referral lab, of these, three labs were found using expired reagents, and the other labs showed a good performance. In 2008, seven of the 31 labs did not send back the test results to the referral lab, one lab was found to have used an expired reagent, and despite six of the labs showing inconsistent test results, the other labs demonstrated a good performance. Up to 2012 there were 16 out of 50 laboratories that did not send the results back and only two laboratories reported false results and 13 laboratories did not follow HIV national strategy for diagnosis correctly.

### **Conclusions:**

Based on results of The External Quality Evaluation Program, some laboratories with anti-HIV testing capacity in Jayapura have not exhibited a qualified performance yet, and it is necessary to continue providing this EQAS program for the labs on regularly basis to improve their quality and capacity in performing an HIV diagnostic test. Distributions of the panel due to geographical constraints is still one of the issues that need to be improved to minimize unreturned results. Beside that the field visit and on the job training for the laboratory with poor performance is needed to improve the quality of testing.

## **ESTABLISHMENT OF LABORATORY QUALITY SYSTEMS IN MYANMAR**

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NRL

### **Introduction:**

Accurate, reliable and consistent HIV test results are the cornerstone on which global, regional and national HIV responses are built. Without accurate, reliable and consistent HIV test results, there will be diminished confidence in the health system, increased likelihood of transmission of infection, inaccurate epidemiology leading to inefficient HIV responses, and poor patient care.

In the laboratory setting, Quality Management Systems (QMS) can be implemented to promote the accuracy and reliability of results by minimising error. QMS is an organised and systematic approach to the planning, management and operational activities of a laboratory, and comprises elements which are fundamental to ensuring accurate and reliable results. Without a functioning QMS, the likelihood of laboratory errors is high.

### **Methods/Discussion:**

This presentation will discuss NRL's laboratory strengthening program in Myanmar, which is funded by the Australian Agency for International Development (AusAID) under the Regional HIV/AIDS Capacity Building Program.

The two-year program is focused on supporting the National Health Laboratory in Yangon (NHL) in achieving its goal of obtaining accreditation, by enhancing NHL's QMS as well as its capacity as the Central Reference Laboratory for HIV in Myanmar. The program also supports four central level laboratories in Yangon, Mandalay and Nay-Pyi-Taw by establishing the fundamentals of their QMS.

This presentation will discuss the progress made by all five laboratories since the start of the program in 2012, the challenges faced, and plans to ensure sustainability of the program when it ends in 2014.



## **DIASORIN Q-LAMP: REAL-TIME MOLECULAR DIAGNOSTICS *BUT NOT AS YOU KNOW IT***

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DiaSorin Ireland, DiaSorin UK, DiaSorin Australia

Loop-mediated isothermal amplification (LAMP) is an isothermal method for rapid and highly specific amplification of nucleic acid through the recognition of multiple DNA/RNA target regions and employs a strand displacement polymerase.

Until now, LAMP has been viewed as a qualitative application for end-point analysis of a single analyte, and has been mainly confined to research laboratories. DiaSorin SpA has modified basic LAMP technology into an advanced form, Q-LAMP; which has the quantitative, real-time and multiplex capabilities required by today's modern molecular diagnostic laboratory.

Results are generated in 60 minutes or less. Single tube, single temperature RNA amplification, qualitative and quantitative applications for infectious disease and onco-haematology are all features of a low-cost flexible molecular system; which is a breakthrough in isothermal technology.



## LOW VIRAL LOAD HIV-1 GENOTYPIC RESISTANCE ASSAY USING VIROSEQ KIT

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### Introduction

Current antiretroviral therapy (ART) regimes are responsible for turning HIV-1 into a chronic manageable disease, however, drug resistance remains a problem. When patients are administered HIV therapy, the goal is to achieve viral suppression below the limit of clinical significance. To assist in achieving this goal, HIV-1 genotypic resistance assay (GRA) is routinely performed to identify point mutations which are known to be associated with HIV-1 drug resistance. The Abbott Viroseq HIV-1 Genotyping System requires a minimum HIV-1 viral load of 2000 copies/mL in order to perform the assay. Therefore, clinicians suspecting drug resistance in their patients must wait for the HIV-1 viral load to increase to 2000 copies/mL before a GRA can be attempted at Pathology Queensland.

By implementing an alternative extraction protocol (QIAGEN QIAamp Viral RNA Mini kit) instead of the extraction method used in the Abbott Viroseq HIV-1 Genotyping System, a purer extract can be eluted. This allows the sensitivity of the Abbott Viroseq assay to be increased well below the recommended cut off of 2000 copies/mL.

By increasing the sensitivity of the HIV-1 GRA assay to below 2000 copies/mL, clinicians may be able to pick up potential drug failures/drug resistances earlier in patients, therefore resulting in improved patient management and care.

### Method

- ❖ HIV-1 viral load was determined using the Abbott RealTime HIV-1 m2000 platform
- ❖ 1mL of plasma was microcentrifuged at 24000 x g for 1 hour
- ❖ Supernatant was carefully removed (without touching/disturbing the viral pellet) leaving behind 140uL of plasma
- ❖ The QIAGEN QIAamp Viral RNA Mini kit extraction protocol was followed
- ❖ RT-PCR / PCR and Sequencing was performed on the QIAGEN extract using the Abbott Viroseq HIV-1 Genotyping Systems protocols
- ❖ Sequences were performed on the ABI 3730XL
- ❖ Sixteen (16) HIV-1 samples of various known viral loads below 2000 copies/mL with previous HIV-1 genotypic resistance profile were extracted using the QIAamp Viral RNA Mini kit and amplified/sequenced using the Viroseq Kit
- ❖ Five (5) HIV-1 samples of various known viral loads below 2000 copies/mL (with no previous HIV-1 genotypic resistance profile) were extracted using the QIAamp Viral RNA Mini kit and amplified/sequenced using the Viroseq Kit
- ❖ Quality of sequences were observed and comparisons were made with previous HIV-1 genotypic resistance profiles (where available)

### Discussion

The quality of the sequences for all patients tested were good, obtaining an average QV of  $\geq 40$ . Samples which had a previous HIV-1 genotypic resistance profile were compared. All samples showed highly concordant results producing the same subtype, and expected "other mutations". All samples that had a significant mutation in the previous sample were still detected. There was no evidence that the modification to the Viroseq method reduced the effectiveness of the assay to detect significant mutations.

Five (out of sixteen) patients that had a previous GRA profile, demonstrated a significant mutation (causing resistance) that was not previously detected. All five patients were on antiretroviral therapy and had a persistently low but detectable HIV-1 viral load. This indicates that the patient developed a significant mutation whilst on therapy, which may explain why the HIV-1 viral load was not full suppressed.

Furthermore, significant mutations (causing resistance) were detected in two (out of five) patients with no previous GRA data.

With less RNA being analysed, random errors due to the processes of PCR must also be considered. It is possible that resistant mutations are present in a minority population of the virus species cannot be detected; however, our finding suggests that significant mutations can still be detected early in low viral load patients. This provides the treating clinician with the opportunity to perform GRA's on patients with persistently low but detectable HIV-1 viral loads where ART failure is suspected. By detecting potential development of drug resistance earlier in the course of treatment, without needing to wait for the HIV-1 viral load to increase above 2000 copies/mL, clinicians will be able to change the patient's therapy earlier, thus resulting in better patient care.

# EVALUATION OF THE GEN PROBE PANTHER APTIMA HUMAN PAPILLOMAVIRUS ASSAY, A COMPARISON WITH DIGENE HYBRID CAPTURE II

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## Introduction:

Invasive cervical cancer (ICC) is one of the most prevalent female cancers worldwide. High risk human papillomavirus (HPVs) types are associated with ICC and various nucleic acid tests are used as indicators of disease which are superior in sensitivity to cytology but inferior in clinical specificity. At SA Pathology we use Digene hybrid capture II assay (HC2) to detect HPV DNA by hybridisation of RNA probes specific for 13 oncogenic types. A drawback of the HC2 assay is the known cross-reaction with non-ocogenic types reducing test specificity. It has been reported that detection of E6/E7 oncogene expression is a more specific predictor of cervical cancer risk than HC2 assay (Clad et al., J. Clin. Micro., 2011; Ratnam et al, J. Clin. Micro., 2011).

Panther Aptima HPV (AHPV) is a fully automated, random access assay that detects E6/E7 mRNA of 14 high-risk oncogenic HPV subtypes. Liquid cervical (ThinPrep; Surepath) and brush specimens are suitable. The assay uses specific RNA-capture technology, transcription mediated amplification (TMA) and specific probe detection.

## Objectives:

To compare the HC2 and AHPV assays to determine if the AHPV assay is a suitable replacement for the HC2 assay.

## Methods:

We have compared HC2 and AHPV assay on 840 ThinPrep samples requesting HPV assay, collected from patients with previous high grade abnormalities (collected from August 2011 to August 2012). HC2 assay testing was performed prospectively and 564 samples were tested retrospectively and 276 prospectively in the AHPV assay.

## Results:

Of the 840 samples tested (91.7%) were concordant between the two assays (see table). Ten percent were positive and 81% negative by both assays.

		AHPV			
		Detected	Invalid	Negative	Total
HC2	Detected	86	0	59	145
	Invalid	0	0	0	0
	Negative	8	2	685	695
	Total	94	2	744	840

Eight percent (67) of samples produced discordant results the majority of which (59) were detected by HC2 but negative by the AHPV assay. Fifty seven HC2+/AHPV- samples had either low grade (LGSIL) or normal cytology; one sample had high grade (HGSIL) (histologically CIN1) and one sample had normal cytology but a histology result of CIN2. Of the 8 HC2-/AHPV+ samples results; 6 had normal/LG cytology and 2 had HG/CIN2.

## Conclusions:

Our results are consistent with published reports which show AHPV has higher specificity for disease than HC2 (Clad et al., J. Clin. Micro., 2011; Ratnam et al, J. Clin. Micro., 2011). Further, that a HC2+/AHPV- result is frequently from cross reaction with low risk HPV types. We also find that AHPV is more time efficient than HC2 testing in our laboratory

## QIAGEN BLOOD BORNE VIRUS (BBV) TESTING IN AUSTRALIA: A NEW ALTERNATIVE

### Plachot C

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**Introduction/Objectives:** Viral load quantification for blood borne virus (BBV) is standard of care in the developed countries for diagnostics and monitoring of antiviral therapy. Further to the uptake of the BBV solution in Europe, QIAGEN now has TGA registration for its *artus* BBV assays consisting of HIV-1, HCV and HBV. This presentation will report the performance characteristics of QIAGEN's BBV assays.

**Methods:** The QIASymphony SP/AS platform (QS) along with the Rotor- Gene Q (RGQ) real-time PCR machine is QIAGEN's solution to fully automated sample preparation, assay set-up and detection. To evaluate performance characteristics, the QS-RGQ solution was tested using predefined positive sample material (WHO or Acrometrix standard) for each of the three viral pathogens. For comparative studies, HIV-1, HBV and HCV viral loads were quantified against highly diversified specimen panels in parallel to Abbott *Realtime* and Roche COBAS AmpliPrep/COBAS TaqMan v2.0 platforms and tests.

**Results:** The limit of detection for HIV-1, HCV and HBV from human plasma using the combination QIASymphony SP/AS with the corresponding *artus* RGQ PCR assay shows sensitive detection of viral nucleic acids: 34 copies/ml, 21 IU/ml, and 10 IU/ml, respectively. No occurrence of cross-contamination was observed using highly concentrated positive material in a 96-sample checkerboard pattern. Bland–Altman analysis showed strong correlation between the Roche, Abbott and QIAGEN systems across a wide range of sub-types.

**Discussion/Conclusion:** Now available in Australia, the *artus* HIV-1, HBV and HCV assays' performance characteristics compare well to other testing solutions currently used in Australia. From a hardware perspective, the QIAGEN QS-RGQ workflow offers automation as well as the flexibility of an open platform to accommodate home-brew assays.



## TROPICAL MEDICINE IN THE TOP END

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### **Abstract:**

The Top End of the Northern Australia shares proximity, climate and culture with Indonesia, SE Asia, and the SW Pacific. The current clinical and diagnostic aspects of local tropical diseases in the Top End, and imported diseases will be reviewed, and will focus on aspects of the BBV's, and other significant regional diseases including dengue, influenza, chikungunya, and tuberculosis.

This review will also include an update on the clinical and diagnostic aspects of diseases that fall in the current WHO list of neglected tropical diseases in the region.

Aspects of two diseases in particular will be highlighted:

HTLV 1 infection is a subject of renewed interest due to (i) recent studies of the clinical disease associations of the virus with respiratory disease and geo-helminth infection, in addition to the rarely seen and well described blood disorders and myelopathies; (ii) Improved understanding of the epidemiology of transmission, which is leading to innovative disease control programs, such as altering breast feeding regimes in endemic zones; and (iii) significantly improved viral diagnostics, consisting of initial screening by the Abbott Architect assay, confirmation by Western Blot, and the development of local viral load and pro-viral DNA assays. These advances all have direct local application for BBV management and designing future viral control programs.

Melioidosis caused by the soil bacterium *Burkholderia pseudomallei*, is an important pathogen in tropical Australia, and SE Asia, and current clinical and diagnostic issues will be reviewed.

Both these diseases are also illustrative of the issues routine diagnostic labs face from new technologies, and changing regulatory environments. There is a paradigm shift currently underway in microbiology laboratories due to the introduction and implementation of new, novel and rapid identification and susceptibility technologies, including point of care diagnostics (POCT), and challenges lie ahead for the ongoing quality control of new technologies.



## **GONE VIRAL: INFECTIOUS DISEASES, REGULATORY APPROACHES AND COMPLICATIONS**

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Therapeutic Goods Administration

### **Abstract**

The safety, quality and efficacy of therapeutic goods marketed in Australia is the responsibility of the Therapeutic Goods Administration (TGA) through application of the Therapeutic Goods Act 1989, Therapeutic Goods Regulations 1990 and associated subordinate legislation.

The TGA's approach to therapeutic goods manufactured using human, animal or cell culture derived materials aims to ensure that such products pose only a minimal, acceptable risk of adventitious infectious agent contamination to the patient.

An acceptable level of infectious disease risk in therapeutic goods is ensured through:

- Sourcing appropriate raw materials;
- Testing of raw materials and process intermediates for infectious agents; using the appropriate In Vitro Diagnostic Kits (IVDs)
- Validation of manufacturing process to inactivate/remove infectious agents (pathogen reduction technologies).

The level of infectious disease risk is now often so low, for example with plasma derived products, the estimation of infectious disease risk often relies on mathematical modelling rather than actual observation of prevalence of infectious agents or incidence of infection. Blood donors and plasma pools are still screened for conventional infectious agents like HIV, HBV, HCV, HAV and Parvovirus B19 using serological marker tests and/or nucleic acid based tests.

As some pathogenic entities such as transmissible spongiform encephalopathies (TSEs) caused by prions, and are not effectively captured by mass screening tests, the TGA ensures that medicines, medical devices and biologicals do not contain material of animal or human origin from countries where there is a known risk of TSE except where such use can be scientifically justified.

As risks with 'traditional' infectious disease agents have abated, often other emerging and re-emerging infectious agents have attracted attention and newer therapeutic products with a higher risk profile have emerged.

Globalisation of travel, commerce and environmental changes are factors that have increased the risk of delivering potential infections rapidly ahead of an understanding of their epidemiology.

This presentation will explore these and other related issues.



## MURRAY VALLEY ENCEPHALITIS AND KUNJIN VIRUSES – THE VICTORIAN EXPERIENCE

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### Introduction

Murray Valley encephalitis (MVE) and Kunjin (KUN) viruses are mosquito transmitted flaviviruses that infect humans and animals. MVE is endemic in much of Northern Australia, while occasional MVE outbreaks occur in south-eastern Australia. The Victorian Arbovirus Task Force (VATF), established by the Victorian Government in 1987, use three monitoring methods to try to predict the reappearance of MVE virus in south-eastern Australia: serological testing of sentinel chicken flocks together with monitoring of vector mosquito species, rainfall (Forbes hypothesis) and barometric pressure (Nicholls hypothesis). Although one or more methods have suggested an increased risk of MVE or Kunjin outbreaks, notably in 2011, these did not eventuate. However the absence of clinical cases does not exclude silent circulation as only 1/800 infections generally result in symptomatic disease and the risk to human populations in Victoria remains unclear. To detect whether there had been transmission of MVE and KUN viruses to humans over the last 11 years, three serosurveys were carried out: the first was in 2002, VATF asked VIDRL to retrospectively test specimens collected as part of a Victorian Q Fever serosurvey, the second was in 2008 after 3 out of 10 sentinel chicken flocks showed reactivity to MVE for the first time in 30 years and the third was in 2011 following seroconversion of chicken flocks in the contact of environmental conditions favourable to MVE spread.

### Objectives:

To determine if there had been undetected subclinical infection of MVE and Kunjin viruses since the last major outbreak in 1974.

### Methods:

Sera were tested using an in-house epitope blocking EIA for total antibody and an in-house immunofluorescence IgM assay to determine the seroprevalence for MVE and KUN viruses in the 3 serosurveys.

Sera were grouped according to postcode of residence. Seropositives were analysed by age and sex

### Results:

In 2002, 529 specimens from 6 different regions around Victoria were tested. Twenty three of 529 (4.3%, 95%CI 2.8-6.4) were positive for MVE and 24/529 (4.5%, 95%CI 2.9-6.7) were positive for KUN total antibody (Ab). For MVE, prevalence in the Murray River region compared to other regions was 6.2% and 1.8% respectively ( $p=0.01$ ). Similarly for KUN 6.5% and 1.8% respectively ( $p=0.01$ ). Sex and age data were also studied. For MVE, males were more likely to be Ab positive than females (4.4% vs 0.8%,  $p=0.063$ ), for KUN (4.4% vs 1.6%,  $p=0.185$ ). Age was strongly associated with antibody positivity for MVE and to a lesser extent for KUN. As the last outbreak of MVE in Victoria was in 1974, positive results were analysed according to individuals <40 years of age vs > 40 years, MVE seropositivity was significantly different in the two age groups ( $p=0.049$ ), but KUN seropositivity was not ( $p=0.38$ ). In 2008, 121 samples from the Kerang-Mildura district were tested. Only 1/121 (0.8%, 95%CI 0.02-4.6) was MVE Ab positive. Kunjin Ab testing was not performed.

In 2011, 1,115 specimens were tested. Twenty four of 1115 (2.1%, 95%CI 1.3 – 3.0) were positive for MVE total Ab, but all were negative for MVE IgM. Three positive MVE patients were less than 37 years of age, indicating infection since 1974. Thirty four of 1116 (3.0%, 95%CI 2.0 – 4.0) were positive for KUN total Ab, 3 were positive for KUN IgM.

### Conclusion:

Seroprevalence studies suggest minimal exposure to either MVE or Kunjin viruses in Victoria in recent decades, despite models predicting otherwise. People living near the Murray River and males were more likely to be positive. Age was associated more strongly with MVE seropositivity than with KUN.

The absence of predicted disease circulation raises important questions about how existing models are used to predict MVE virus outbreaks. In an outbreak situation a large proportion of the Victorian population remain at risk of infection.

## MAINTAINING A SAFE BLOOD SUPPLY IN A HIGH ENDEMIC COUNTRY

Vermeulen M

South African National Blood Service

### **Abstract**

Since the inception of the South African blood transfusion service (SABTS) in the 1950's all blood has been donated on a voluntary non remunerated basis. In 1985 South Africa implemented screening of all blood donations for anti-HIV along with the rest of the world and measured the prevalence annually. In 1998 it became clear that the HIV prevalence in blood donors was increasing at the same exponential rate as in the antenatal population and therefore the general population however it was 100 fold lower at 0.28%. This caused concern for the safety of the blood supply therefore in 1998 a risk policy was implemented that used a triage of ethnicity, gender and number of donations to classify whether components would be used. Black donors were not recruited however if they presented to donate a unit of blood, they were accepted, donated and then after collection the blood was destroyed and not used for transfusion. This risk policy had the desired affect and the HIV prevalence in blood donors decreased by 10 fold to 0.03%. This risk policy was neither sustainable as over 80% of the population is of the black race group nor politically acceptable and in 2004 the now called South African National Blood Service (SANBS) changed the risk policy to only include number and frequency of donations to classify risk but at the same time implemented individual donation nucleic acid testing to screen all blood donations for HIV RNA, HCV RNA and HBV DNA.

At the time of donation each blood donor has a confidential one on one interview with a medical personnel to ensure that the blood donor does not practice any high risk behaviour. Once accepted as eligible to donate blood the blood is collected and components are made depending on number and frequency of donations. Fresh frozen plasma (FFP) is made from all donations and subjected to a quarantine system whereby if the donor returns to donate again and all screening tests are negative the index FFP is released for issue to a patient. Pooled platelets are only made from donations where the blood donor has donated more than 4 donations in the previous 2 year period. Red blood cells are made from donations where the blood donor has donated 1 or more donation in the previous 12 months. This risk strategy along with ID NAT screening was extremely successful with no HIV transmissions by blood transfusion in 5 years compared to 2 transmission events per annum for the five years prior to ID NAT screening. In 2012 red blood cells from new donors was also used for transfusion. This presentation will show the details of prevalence, incidence and residual risk before and after the change in risk policy.



# FOLLOW-UP AND INTERPRETATION OF HIV REACTIVITY IN BLOOD DONORS: A CASE STUDY THAT HIGHLIGHTS THE POTENTIAL COMPLEXITY AND NEED FOR CAUTION.

Kiely P<sup>1</sup>, Catton M<sup>2</sup>, Brown D<sup>1</sup>, Chibo D<sup>2</sup>, Karapanagiotidis T<sup>2</sup> and Spelman D<sup>3</sup>.

1. Australian Red Cross Blood Service, Melbourne 2. Victorian Infectious Diseases Reference Laboratory, Melbourne  
3. The Alfred Hospital, Melbourne

## Introduction:

The Australian Red Cross Blood Service (Blood Service) screens all donations for anti-HIV-1/2 and HIV-1 RNA. Donations that are reactive on the primary and secondary anti-HIV-1/2 screening immunoassays are referred to the Victorian Infectious Diseases Reference Laboratory (VIDRL) for anti-HIV confirmatory testing.

## Objectives:

To present a case study that demonstrates the potential complexity of interpreting HIV screening and confirmatory test results in blood donors

## Methods:

The Blood Service screens all donations for anti-HIV-1/2 using the PRISM HIV O Plus chemiluminescent immunoassay (Abbott Diagnostics, Delkenheim, Germany) and donations that test repeatedly reactive are further tested on the Genscreen Ultra HIV Ag-Ab EIA (Bio-Rad, Redmond, WA). Donations that test reactive on both immunoassays are referred to VIDRL for anti-HIV Western blot testing (MP Diagnostics 2.2, Genelabs, Singapore). In addition all donations are screened for HIV-1 RNA, HCV RNA and HBV DNA using the PROCLEIX ULTRIO (HIV-1/HCV/HBV multiplex) assay (Ultrio) on the PROCLEIX TIGRIS automated platform (Gen-Probe/Novartis Diagnostics, San Diego/Emeryville, CA). Samples initially reactive on the Ultrio assay were 'discriminated' to identify the specific virus using the PROCLEIX HIV-1, HCV and HBV discriminatory assays. HIV-1 RNA testing was also performed at VIDRL using either the Abbott Real Time HIV-1 RT-PCR assay or Roche Ampliprep/Taqman HIV-1 Test version 2.0 (Roche Diagnostics, Indianapolis, IN).

## Results:

Date	Blood Service results				VIDRL results	Western blot <sup>4</sup>	Abbott Real Time	Roche <sup>5</sup>
	PRISM HIV O Plus	Genscreen HIV Ultra Ag-Ab	NAT <sup>1</sup>					
			Ultrio <sup>2</sup>	dHIV <sup>3</sup>				
17/03/12	REA	REA	NR	NT	REA	IND Gr 4	ND	NT
12/04/12	REA	REA	NR	NR	REA	IND Gr 4	NT	ND
10/05/12	REA	REA	NT	REA	REA	IND Gr 4	ND	NT
12/06/12	REA	REA	NR	NR	REA	IND Gr 4	NT	ND
21/06/12	REA	REA	NR	REA (2/20) <sup>6</sup>	NT	NT	NT	ND (9/9) <sup>7</sup>

REA = reactive; NR = non-reactive; NT = not tested; ND = Not detected; IND Gr 4 = indeterminate group 4

<sup>1</sup> NAT = nucleic acid testing; <sup>2</sup> Ultrio = PROCLEIX ULTRIO multiplex; <sup>3</sup> dHIV = PROCLEIX HIV-1 discriminatory assay.

<sup>4</sup> MP Diagnostics 2.2; <sup>5</sup> Roche Ampliprep/Taqman HIV-1 Test version 2.0.

<sup>6</sup> 2 of 20 replicates were reactive; <sup>7</sup> 9 of 9 replicates were non-reactive.

Three subsequent samples were taken from the donor (16/08/12, 29/11/12 and 12/03/13) and tested by VIDRL. Serology remained indeterminate for all three samples and HIV-1 RNA was not detected by the Roche Ampliprep/Taqman and Abbott Real Time HIV-1 RT-PCR assays. In addition HIV-1 proviral DNA was not detected when performed on the buffy coat of 3 samples collected on 12/06/12, 21/06/12 and 12/03/13.

## Conclusions:

Based on the screening tests performed at the Blood Service on the index donation and during the first three months of follow-up, the PRISM and Genscreen assays were reactive with relatively high sample to cutoff ratios and the Ultrio HIV-1 discriminatory assay was reactive on 2 samples (albeit only 2 of 20 replicates for one of the samples). However, for the confirmatory testing performed at VIDRL, over the 12 months of follow-up the indeterminate Western blot did not progress to a positive profile, HIV-1 RNA was not detected on any samples by the Roche and Abbott assays, and HIV-1 proviral DNA was not detected. After the 12-month follow-up period VIDRL concluded that the results were not consistent with HIV infection. This case highlights the need for caution and sufficient followup of blood donors with unusual HIV reactivity that cannot be confirmed by laboratory testing.

## **NRL EQAS: OBSERVATIONS FROM THE NAT BLOOD SCREENING PROGRAM 2012**

**Cabuang LM**, Vincini GA, Best SJ

NRL, Australia

### **Introduction:**

NRL has conducted a nucleic acid testing (NAT) external quality assessment scheme (EQAS) (NATA4315) for blood screening laboratories since 2000. A total of 93 laboratories from 27 countries participated in the 2012 program. NATA4315 allows us to identify aberrant test results and improves our understanding of testing strategies. Analysis of results provides valuable comparative data on the performance of assays as well as informing participants of their performance relative to others using the same assay (known as a peer group)

### **Objectives:**

1. To review the results from NATA4315 to determine reproducibility of assays when testing low concentrations of HCV RNA and HBV DNA.
2. To review the NAT testing strategies implemented by laboratories.

### **Methods:**

The 2012 NATA4315 program consisted of three 15-member panels. Each sample in the panel contained HIV-1 RNA, HCV RNA and/or HBV DNA positive plasma from blood donors, or normal human plasma, confirmed negative for the presence of HIV/HCV RNA and HBV DNA. Each analyte was provided at different concentrations that were calibrated against the WHO International Standard for HIV (97/650), HBV (97/750) and HCV (06/100). Throughout the year, participants were provided with samples containing low concentrations of HCV RNA (40 IU/mL, ten replicates) or HBV DNA (40 IU/mL, nine replicates). They were requested to test the samples in the same manner as their donor samples. The results were submitted to NRL using OASYS, an internet-based proficiency test application (Oneworld Accuracy, Canada). The results were then compared with internally-generated reference results as well as with those obtained by the peer group.

### **Results:**

For the three test events, a total of 11,374 results were generated from 21 assays; 15 assays were used to screen samples and the remaining six assays were used in discriminatory testing. Irrespective of assay, 1409 results were submitted for the sample containing 40 IU/mL of HCV RNA, with a detection rate of 99.50%. Irrespective of assay, 1230 results were submitted for the sample containing 40 IU/mL of HBV DNA, with a detection rate of 99.35%. Five participants submitted results for in-house developed assays. Of the samples analysed, the in-house developed assays detected low concentrations of HBV DNA and HCV RNA at an equivalent rate to that of the commercially available assays. In the analysis of testing strategies, one participant performed discriminatory testing on assays intended for viral load quantification, which resulted in three of the nine HBV DNA positive replicates being falsely reactive for HIV RNA. Further analysis of aberrant results revealed that some of the errors identified were due to data entry.

### **Conclusions:**

NATA4315 provided participants with valuable information regarding their performance within a particular test event. In this particular program, multiple replicates of the same sample were provided over the three test events, allowing for a more in-depth analysis of results obtained from peer groups and assays, not just of individual participants' results. Overall, the performances of all the assays used by the participants were similar.

## **BLOODGROUP: SHARING KNOWLEDGE, EXPERTISE AND OPPORTUNITIES**

Pham T

NRL

### **Abstract:**

BloodGroup is a new initiative from NRL in 2013. BloodGroup will bring together individuals and organisations working in the field of Transfusion Transmitted Infections (TTI) who all have common goals and challenges. BloodGroup seeks to foster collaboration, share knowledge, expertise and opportunities to advance the field of TTI testing.

### **BloodGroup** aims to:

- Support consistent blood screening for TTI
- Promote sustainability of laboratory services
- Improve quality of testing through accreditation and standardisation
- Facilitate strong collaboration and communication amongst members
- Create a forum for the exchange of ideas and advice



## **SAMPLE TO ANSWER PCR – IMPROVING EFFICIENCY: USER’S PERSPECTIVE**

Tony Field

Healthscope Pathology, Melbourne

### **Abstract:**

Traditionally, PCR requires extraction and purification of DNA or RNA from patient material. This step is essential to remove inhibitory substances and to concentrate the nucleic acids. However, nucleic acid extraction leads to increased operation costs and processing time from sample to result.

The Simplexa Bordetella pertussis & parapertussis, Flu A, B & RSV and HSV1/2 & VZV PCR reagents manufactured by Focus Diagnostics utilises an extraction free real time PCR protocol – in which pathogens can be detected directly from patient samples. Aside from having a small footprint, the hardware system supports up to 4 fluorescent channels enabling the use of multiplexed targets.

Initial comparative evaluations of the Simplexa system resulted in comparable specificity and sensitivity across all targets when compared to pre-existing assays. As extraction is no longer needed, deployment of this platform into our laboratory resulted in improvement to our workflow; with additional benefits including cost reduction, staffing flexibility and decreased turn-around time.

As evident in our laboratory, the molecular diagnostics sector grows at a rate of 20% annually and operates under demanding conditions; samples are received en masse, clinicians expect accurate results with short processing time, whilst operation costs are kept at a minimum. Finding the right balance to satisfy all these criteria whilst maintaining a tight budget seems almost an impossible task. In our case, we have utilised and taken advantage of a direct PCR platform to improve workflow and enhance efficiency – adapting to the mounting pressure of the industry.



## DEVELOPMENT OF A HUMAN T-CELL LYMPHOTROPIC VIRUS TYPE-I VIRAL LOAD ASSAY

Dick.S, Wilson.K

NRL, Melbourne, Australia

### Introduction:

Human T-cell Lymphotropic virus type I (HTLV-I) infects an estimated 15 to 20 million people world-wide<sup>1</sup>. In endemic populations, such as among indigenous Australians in Central Australia, infection is generally acquired in early childhood through the ingestion of infected lymphocytes in breast milk<sup>1</sup>. Consequently, the maternal HTLV-I proviral load (PVL) is a strong predictor of the risk of transmission to infants<sup>2</sup>. Although most HTLV-I carriers remain asymptomatic, significant complications ultimately arise in up to 10% of cases.

The Australo-Melanesian variant of HTLV-I, subtype c, which is found in Papua New Guinea, the Solomon Islands and Australia, demonstrates a highly divergent genetic sequence, exhibiting only 92% sequence identity to the other known HTLV-I subtypes<sup>3,4</sup>. The large sequence variation between the Australian HTLV-I isolates and the prototype virus used to manufacture commercial assays may result in a significant decrease in assay sensitivity, when testing specimens from individuals infected with the Australo-Melanesian strain.

### Objective:

To develop a quantitative, real time PCR (qPCR) assay, to detect HTLV-I provirus, specific for the current circulating strains of HTLV-I.

### Methods:

A Basic Length Alignment Search (BLAST) was conducted to compare as many HTLV-I subtypes as possible, with the aim of finding a highly conserved region amongst the various subtypes. A selection of HTLV-I serology positive specimens from Australian indigenous and non-indigenous individuals, were used to amplify a 760 bp fragment of the gag gene for sequencing and to select a suitable region for assay development. Alignment data was used to identify an 88 bp sequence of the gag region as a potential target for assay primers and probe. The region selected was highly conserved between all sequences available in the database and our sequencing, allowing various subtypes to be detected in the one assay.

Quantification of HTLV-I PVL was achieved by creating a 10 fold dilution series of SP cells, which are known to contain 1 copy of the HTLV-I genome and 2 copies of the albumin gene. The dilution series was run in parallel with specimens throughout the extraction and qPCR, accounting for variations of efficiencies throughout all assay processes. A standard curve for both HTLV-I and the albumin gene was generated using the cycle threshold (Ct) value for each member of the dilution series. The Ct values of individual specimens were read off the standard curves and the corresponding Ct values used to deduce the number of HTLV-I provirus and albumin copies in a specimen. These values were then expressed as the number of provirus copies per number of leukocytes in the specimen. In addition to samples, quantification QCs with identical viral loads to the standards were also included in each test run, along with controls for HTLV-I, HTLV-II, no-template controls and non-amplification controls.

### Results:

Approximately 350 clinical specimens consisting predominantly of buffy coats, but also whole blood and dry blood spots were tested on the HTLV-I PVL assay. The clinical specimens consisted of HTLV-I serology positive, indeterminate and negative specimens. The PCR assay results demonstrated good concordance with the serology results. Quantifiable provirus was detected in some HTLV-I serology indeterminate specimens, this was not unexpected as NRL's HTLV Western blot interpretation criteria are conservative. The quantitative QC's indicated good viral load concordance with the standards

### Conclusion:

The assay has the ability to be a reliable method to quantify HTLV-I provirus in the common subtypes encountered in laboratory testing. The assay will require a period of validation to determine its sensitivity and effective dynamic range, specificity and reproducibility. The PVL result generated will serve as a strong predictor of the risk of transmission and disease progression

# EVALUATION OF LIGHTMIX® PARASTICAL GASTROENTERITIS KIT FOR THE DETECTION OF COMMON GASTROINTESTINAL PARASITES IN CLINICAL STOOL SAMPLES

Colin Pham, Darren Jardine, Linda Joyce

St. Vincent's Hospital, Melbourne

## Introduction:

The laboratory diagnosis of gastrointestinal parasites has traditionally been performed by microscopy. Faecal concentration and microscopy will recover most parasites however, this procedure is time consuming and the training required for screening and identifying parasites highly specialised. Enzyme Immunoassays (EIA) have higher sensitivity than microscopy; however, they are specific for certain parasites only. The emergence of RT-Multiplex PCR has enabled the detection of multiple pathogens and mitigates the subjective nature of identifying parasites by morphology. The Lightmix® Parasitological Kit has the potential to improve sensitivity and specificity rates and provide rapid and accurate diagnosis.

## Objectives:

To evaluate the performance of Lightmix Parasitological Gastroenteritis Kit for the detection of *Giardia intestinalis*, *Cryptosporidium parvum*, *Dientamoeba fragilis*, *Blastocystis hominis* and *Entamoeba histolytica*, and to determine its suitability for use in a diagnostic laboratory.

## Methods:

Sample characterisation:

A total 113 stool samples, including both positive and negative samples, were tested. Samples were processed for routine culture and parasite examination according to the testing algorithm developed by St. Vincent's Pathology. Positive samples were characterised as follows:

- *G. intestinalis* and *C. parvum* antigen determined by a reactive Prospect Giardia/Cryptosporidium EIA and confirmed by Remel Xpect ICT.
- *D. fragilis*, *E. histolytica/dispar*, *B. hominis* are identified by morphology in the Wheatley's Trichrome stain smear

Stool samples were retrieved from storage at -20°C, thawed and extracted on the Roche MagNA Pure 96, utilising the Universal Pathogens Small Volume protocol. Amplification and detection was performed using the LightMix Parasitological Gastroenteritis Kit on the LightCycler 480® Instrument as per manufacturer's instructions.

## Results:

The Lightmix® Parasitological Kit detected 6 additional parasite positive samples when compared with the current methods (one *G. intestinalis*, two *D. fragilis* and three *B. hominis*)

Of the ten specimens positive by microscopy for *E. histolytica/dispar*, only two were positive for *E. histolytica* DNA by PCR. This is a consistent finding with other investigators who have observed low positive predictive values of identification by microscopy which cannot distinguish between the pathogenic *E. histolytica* and the non-pathogenic *E. dispar* [1, 2].

One specimen was positive for *C. parvum* by EIA and ICT, but was not detected by PCR. This sample was also positive for *G. intestinalis*. This is most likely due to competition for reagents between the *G. intestinalis* and *C. parvum* assays.

Abbott and QIAGEN systems across a wide range of sub-types.

## Conclusion:

LightMix® Kit Parasitological Gastroenteritis assay offers rapid accurate screening for common parasites however, microscopy still plays a key role in less common parasite identification, especially in specific patient groups, where parasites other than those covered by this multiplex, are suspected. Appropriate algorithms need to be developed to make use of emerging molecular methods

## References:

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2. Nazeer JT, El Sayed Khalifa K, von Thien H, El-Sibaei MM, Abdel-Hamid MY, Tawfik RA, Tannich E: Use of multiplex real-time PCR for detection of common diarrhea causing protozoan parasites in Egypt. *Parasitol Res* 2013, 112:595-601.

## **COMPARISON OF THREE COMMERCIAL ASSAYS FOR THE QUANTITATIVE DETECTION OF BK VIRUS IN RENAL TRANSPLANT PATIENTS**

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Centre for Infectious Diseases and Microbiology, Institute for Clinical Microbiology & Medical Research (ICPMR), Westmead, NSW 2145

### **Introduction:**

BK virus associated nephropathy occurs in up to 10% of kidney transplant recipients, resulting in premature graft loss in many affected patients. Routine monitoring of BK virus levels in transplant recipients is recommended as graft loss can be reduced by altering immunosuppression levels in patients exhibiting BK viraemia. This study examined three commercial assays for sensitivity, specificity, cost and ease of use to find the assay best suited for detection of BK viraemia in renal patients.

### **Methods:**

123 EDTA and plasma samples were collected from renal patients during May 2013. DNA was extracted from plasma using the NucliSENS easyMAG (BioMerieux) and the samples were tested using both the Liaison lam BKV Q-Lamp Quantitative assay (DiaSorin), the Artus BK Virus PCR kit (Qiagen) and an in-house qualitative assay for polyomavirus. Samples positive in any of these methods were further tested using the Affigene BKV trender (Cepheid) which uses DNA extracted from EDTA blood. Discrepant results were forwarded to St Vincents Hospital for analysis using an in house BK/JC virus specific assay.

### **Results:**

The results to date for the in-house qualitative assay and all three commercial quantitative assays correlated for 109 samples (14 positive, 95 negative). Some discrepancies were noted in the quantitative value given for each method. 6 samples were found to be weak positives and were not detected in all assays. The results between assays were discrepant for 8 samples. Further characterisation of these samples is ongoing.

### **Discussion:**

Accurate and timely detection of BK viraemia is essential for effective management of renal transplant patients. The correct choice of assay for quantitative detection is vital for this process. The results for the three commercial assays examined in this study correlated for the majority of samples but varied in regard to their cost and the quantitative result reported for samples. While further analysis of the discrepant results is still ongoing, it appears that some assays may have reduced specificity and/ or sensitivity which could negatively impact patient management.



## PCR: SPECIALIST MOLECULAR OR POC?

Christel Armstrong

Diagnostic Technology

### **Abstract:**

PCR has long been a technique used in Diagnostic Laboratories for the highly sensitive and specific detection of pathogens, with a relatively quick time to results (when compared with traditional microbiology culture and identification techniques). From Kary Mullis' discovery of PCR in 1983 to today's techniques used in laboratories, there has been a rapid change in how molecular detection of organisms, is determined, along with laboratories centralizing this once complicated technique. Batch analysers allow economies of scale and walk away automation, however, is it these exact efficiencies for large metropolitan laboratories which disadvantage smaller regional or remote diagnostic laboratories.

Cepheid is a leading molecular diagnostics company that is dedicated to improving healthcare by developing, manufacturing, and marketing accurate yet easy-to-use molecular systems and tests, with the GeneXpert instrument and associated assays amongst one of the most widely used RT PCR instruments in today's Clinical Laboratories. The GeneXpert is a Real Time PCR Instrument with on board extraction and amplification, a one step process, with minimal hands on time, walk away automation. The simplicity of the system, along with the quick turnaround time of the assays is a solution for regional and remote communities within Australia. The company's High Burden Developing Countries program offers M. tuberculosis testing in remote communities in countries such as Uganda, Tanzania and Papua New Guinea utilising the GeneXpert technology in mobile TB clinics and laboratories fuelled by solar power. In Australia, the Test, Treat ANd GO (TTANGO) trial conducted by the Kirby Institute and Burnett Institute, is a randomised controlled trial of Chlamydia and Gonorrhoea point of care testing in remote Aboriginal communities in Queensland and Western Australia, the overall aim of the trial is to measure the effectiveness, cost-effectiveness and cultural and operational acceptability of POC testing for CT and NG infections in remote Aboriginal communities.

With technology continuing to advance at a rapid rate, the implementation of such systems is crucial for regional and remote areas which often suffer the consequences of centralised testing, which poses the question: is PCR testing still a specialist laboratory test?



## **SYPHILIS IN THE NEW MILLENIUM**

Leslie DE

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### **Abstract:**

Syphilis, the great imitator, is back with a vengeance in Australia. After decades of declining incidence in developed countries worldwide, since 2000 there has been a major resurgence, predominantly affecting men who have sex with men (MSM), especially HIV-infected MSM. However, over the same time there have been significant advances in our understanding of *Treponema pallidum*.

Since the *T pallidum* genome was sequenced by Fraser et al in 1998, recombinant immunodominant protein antigens have been produced, greatly improving the sensitivity and specificity of diagnostic EIA assays, and several *T pallidum* specific genetic targets have been identified, allowing PCR development for greatly improved direct detection of spirochaetes in primary and secondary lesions. Simple typing and macrolide resistance detection PCR assays have also been trialled. This in turn has assisted our understanding of serological responses in all stages of syphilis, improved detection of re-infections and confirmed that penicillin is still the only reliable treatment for all stages of syphilis.

This talk will review the epidemiology of syphilis in Victoria since 2000 and highlight the association with HIV. Dogma regarding diagnostic tests and testing algorithms will be discussed in the light of modern assays, and future directions and needs for ongoing R&D and quality assurance briefly explored.



## **SYPHILIS, YAWS & LYME BORRELIOSIS**

Dr BJ Hudson

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### **Abstract:**

Syphilis, yaws and lyme borreliosis have particular issues that confound interpretation of their serology results. Whilst most laboratory scientists and medical practitioners are comfortable with the known issues surrounding syphilis serology, serology for yaws and lyme borreliosis presents special issues that may raise new or unusual problems.

Yaws is one of the endemic non-venereal treponematoses caused by *Treponema pallidum* subspecies *pertenue*, a spirochete that is almost identical to the causative agent of the venereal treponematoses, syphilis (*Treponema pallidum* subspecies *pallidum*). Other endemic treponematoses include bejel (*Treponema pallidum* ss *endemicum*) and pinta (*Treponema carateum*). Yaws is the most common occurring mainly in poor communities in warm, humid tropical areas of Africa, Asia, Latin America and Western Pacific with the less common being Bejel (Sahel region of Africa and Arabian Peninsula) and pinta (Latin America). These infections have no non-human reservoir. Mass eradication campaigns using long-acting penicillin injections in the 1950s-60s reduced global numbers by 95% but yaws reappeared in the 1980s. Renewed attempts at eradication saw WHO eradication initiatives launched in 2012 and success stories such as eradication in India (no new cases since 2004). While diagnosis is mostly clinical, serology is important to confirm or exclude transmission. Typically RPR tests on children aged 1-5 years are performed in serosurveys, with negative results excluding the diagnosis and transmission. Specific treponemal tests usually are not done, but have been developed for field tests. Serology behaves similarly to syphilis - specific tests can stay positive for life. The main problem in developed countries is interpretation of positive specific treponemal tests in sera sent for diagnosis or for antenatal screening. With global population movements, it is likely that more persons in developed countries who have had an endemic treponematoses will present with positive specific treponemal tests. An approach to these patients is described.

Lyme borreliosis (LB), controversial from the time that mothers in Connecticut USA queried the diagnosis of JRA in children who had reported strange bullseye lesions following tickbite to 2013 in Australia where existence of a local spirochete cause is hotly debated. While LB is still a clinical diagnosis when a typical skin lesion occurs at the site of tickbite, other clinical manifestations, in the absence of such skin lesions, are problematic. Specific PCR on skin lesions, synovial fluid and cerebrospinal fluid can be confirmatory but PCR still lacks adequate sensitivity. Culture of the organism remains the gold standard test but is not available in many centres. Positive cultures reported in the literature are overwhelmingly from skin lesions. Accordingly, most cases are diagnosed by serology. Screening tests are typically EIA or IFA, commercial or in-house. IgM and combined IgG and IgM assays commonly yield false positive results, so separate IgM and IgG assays are preferred as screening tests. Traditionally, two-tier testing has been recommended whereby positive screening tests are followed with western blots, with various and controversial interpretative criteria (e.g. CDC vs MiQ 12 plus VlsE). This approach has been questioned with the availability of newer generation EIAs that incorporate the Vmp-like sequence, expressed (VlsE) protein or an immunodominant, largely conserved 25-mer oligopeptide (C6 peptide) corresponding to the sixth invariable region within VlsE. The pros and cons of dropping the two tier approach will be discussed.

## EVALUATION OF THREE COMMERCIAL EIA KITS FOR THE DETECTION OF LYME BORRELIOSIS ANTIBODIES IN HUMAN SERA COMPARED TO WESTERN IMMUNOBLOTS

David Dickeson<sup>1</sup>, Nandita Kataria<sup>2</sup> & Dipty Gounder<sup>1</sup>

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<sup>2</sup> Student, Veterinary School, University of Sydney.

### Introduction:

The aim was to evaluate two commercial enzyme immunoassay (EIA) Lyme Borreliosis IgG kits namely the NovaLisa Borrelia burgdorferi IgG ELISA (recombinant) and the Euroimmun Anti-Borrelia (whole cell lysate) plus VlsE ELISA IgG compared to the screening kit currently utilised namely the MarDx Diagnostics (Trinity Biotech) Borrelia burgdorferi EIA IgG and IgM whole cell lysate and an in-house western immunoblot IgG using whole cell lysates of B.burgdorferi and B.afzelii. 267 previously tested and stored serum samples were tested in the EIAs and 226 of these were also tested in western immunoblots. These specimens also included a specificity panel (leptospirosis, syphilis, H. pylori, EBV, ANA and rheumatoid factor positives), four dilution series and one sample repeated ten times. The recombinant antigen NovaLisa IgG had an overall agreement of only 60% with the whole cell antigen total antibody MarDx while the whole cell plus VlsE Euroimmun IgG showed a higher agreement of 72%. This was expected as the comparison with western immunoblot using the CDC criteria for positive IgG blots of five specific bands gave MarDx only a 45% agreement, NovaLisa 88% agreement and Euroimmun 69% agreement. The dilution series indicated that Euroimmun IgG was slightly more sensitive (one doubling dilution) for two specimens and NovaLisa IgG more sensitive for one specimen so they could not be separated by this method. The specificity panel showed NovaLisa IgG was the most specific at 96% with MarDx 88% and Euroimmun 83%. Repeatability gave coefficients of variation of 5% for Euroimmun and 15% for NovaLisa but this may have indicated operator variation at the time of testing. With a greater specificity compared to western immunoblot and known cross reactive specimens, the NovaLisa IgG recombinant EIA was considered the most accurate kit to use as a screening test for Lyme borreliosis.

Further reading: CW Ang, DW Notermans, M Hommes, AM Simoons-Smit, T Herremans.

Large differences between test strategies for the detection of anti-Borrelia antibodies are revealed by the comparing eight ELISAs and five immunoblots. Eur J Clin Microbiol Infect Dis 2011;30:1027-32

## REVIEW OF RPR TITRES

Nilukshi Arachchi and Wayne Dimech

NRL, Melbourne, Australia

### Introduction:

The rapid plasma reagin (RPR) assay is a non-treponemal test used to detect antibodies to antigens comprising of cardiolipin, lecithin and cholesterol. The reagin test targets the antibodies against these lipoidal materials which are released by the cells when they are damaged by the *Treponema pallidum*. RPR testing can be used for qualitative screening or as a quantitative test. Quantitative RPR tests are expressed in doubling dilution titres and are mainly used to monitor the progress of disease over time and response to therapy. RPR titres decline with effective treatment whereas specific antibodies to *T. pallidum* generally remain detectable life-long.

NRL External Quality Assessment Scheme (EQAS) participants use a variety of commercially available RPR assays. Reading RPR results is generally considered subjective and the intensity of the reaction may vary from run to run and/or assay to assay. Traditionally, titres that are greater than one doubling dilution apart are considered significantly different. In this study, the RPR titre results submitted for syphilis EQAS over a three year period were reviewed.

### Objectives:

The aims of the review were to analyse the consistency of titres reported by participants using the same assay (known as a “peer group”) and titres reported by participants testing the same sample using different assays.

### Methods:

The positive quantitative results, expressed as doubling dilution titres, obtained from each sample in the EQAS test events from 2010 to 2012 were reviewed. Any false positive results and titres less than 1:2 were disregarded. The results were analysed using the following methods:

1. Titre results were converted to whole numbers (i.e. 1:2 = 1, 1:4 = 2, 1:8 = 3 etc.).
2. The median and mode of the converted results were calculated for each sample/assay combination.
3. A difference of greater than one between the median or mode of each sample’s converted results was considered a significant difference.
4. The median and mode of converted titre results obtained from a sample that was tested three or more times in multiple assays were compared using the same criteria of significance.

### Results:

There were 526 titre results from the 9 test events reviewed. The within-assay median and mode of the converted results were no greater than one doubling dilution different from each other for all assays reviewed. Of the 526 titre results, 48 (9.1%) were significantly different from the median/mode of the converted results calculated for that sample/assay combination. Of these 48 samples, the converted result was different to the mode in 28 (5.3%) cases and different to the median in 9 (1.7%) cases. Nine samples were tested three or more times on multiple assays. Of those nine samples, the median or mode of the converted titre result for each sample/assay combination was significantly different in only two cases. In both cases the results were from the same assay.

### Conclusion:

The review of titre results reported for samples in the NRL syphilis EQAS program demonstrated variability of titre results between assays but good within-assay consistency. Titres reported multiple times for the same sample using different assays demonstrated concordance except for two samples. On both occasions the discordant results were reported using the same assay. Although the reading and interpretation of RPR test results is considered subjective, our findings conclude that participants of the NRL syphilis EQAS consistently report RPR titre results that were concordant with each other.

## **IMPROVEMENT OF STABILITY AND MINIMIZATION OF VARIABILITY FOR HEPATITIS B SURFACE ANTIGEN IN PELISPY MULTIMARKER SEROLOGICAL CONTROLS**

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### **Introduction:**

The lot-to-lot variation of Hepatitis B Surface Antigen (HBsAg) reactivity at the time of manufacture and a decrease of HBsAg reactivity over time in PeliSpy Sero Controls (PSSCs) have been observed. A systematic investigation has begun to identify the root cause of the lot-to-lot variation of HBsAg reactivity. The serum diluent matrix utilized during the manufacture of PSSCs showed a potential correlation between a change in matrix lot and its corresponding change in the performance of HBsAg. It is hypothesized that various level of anti-HBs which compromises the reactivity of HBsAg analyte in PSSCs might be the reason for which various range of initially observed HBsAg activity depending on the specific serum matrix lot used. Furthermore, the presence of anti-HBs in PSSCs is hypothesized to affect the long-term stability of HBsAg reactivity.

### **Objectives:**

To prove a direct relationship between the level of anti-HBs in serum matrix and HBsAg activity in PSSCs, the concentration response and time-dependent studies are performed.

### **Methods:**

The level of anti-HBs and HBsAg is measured by the Ausab and HBsAg Qualitative CMIA assay, respectively, on the Abbott Architect platform. The unit used for anti-HBs (Ausab) and HBsAg is mIU/mL and S/CO, respectively.

### **Results:**

Multiple lots of individual serum diluent matrix prior to pooling in the manufacture process are obtained from Europe and US. The range for anti-HBs across a dozen different blood units was 0.0 – 4.1 mIU/mL. As expected the level of anti-HBs in US blood units was higher than one in Europe. To examine the effect of anti-HBs on HBsAg performance, a various amount (0 - 74 mIU/mL) of anti-HBs was spiked into the PSSC Type 38 and HBsAg activity was measured at Day 1 and Day 8 post-spiking. The greater inhibition of HBsAg activity was observed as anti-HBs concentration increases. Also, there was a greater decrease of HBsAg over time. Two independent matrix lots were cleaned up by rProteinG agarose to remove anti-HBs. Then, HBsAg concentration historically used in Type 36 was spiked into the cleaned up diluent matrix containing 0.0 mIU/mL and the stability of HBsAg was monitored for 12 wks. A sustained HBsAg activity in a cleaned up matrix was observed. In contrast, the HBsAg activity dropped to 1.3 S/CO from 2.6 S/CO in 2 wks when the original uncleaned matrix was used as a diluent. In addition, it has been confirmed that the raw material components used to manufacture all types of PSSC do contain undetectable level of anti-HBs.

### **Discussion:**

It is proven that the root cause of lot-to-lot variation for HBsAg activity and decrease in activity over time in PSSCs is the diluent matrix which comes with various levels of anti-HBs to begin with. Current manufacture of all types of PSSCs utilizes serum diluent matrix containing undetectable levels of anti-HBs and uses positive analyte (HBsAg, anti-HCV, anti-HIV, anti-HTLV, anti-HBc, or anti-CMV) raw materials containing very low level of anti-HBs (i.e., < 1.0 mIU/mL) when it applies

### **Conclusions:**

It is proven that the root cause of lot-to-lot variation for HBsAg activity and decrease in activity over time in PSSCs is the diluent matrix which comes with various levels of anti-HBs to begin with. Current manufacture of all types of PSSCs utilizes serum diluent matrix containing undetectable levels of anti-HBs and uses positive analyte (HBsAg, anti-HCV, anti-HIV, anti-HTLV, anti-HBc, or anti-CMV) raw materials containing very low level of anti-HBs (i.e., < 1.0 mIU/mL) when it applies.

# INVESTIGATION OF RESULTS OBTAINED FOR AN EXTERNAL QC SAMPLE USED FOR MONITORING INFECTIOUS DISEASE SCREENING AT THE AUSTRALIAN RED CROSS BLOOD SERVICE

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## **Abstract:**

The Australian Red Cross Blood Service Infectious Diseases Screening (IDS) Laboratories screen blood donors for Transfusion Transmitted Infection (TTI) serological markers using the Abbott PRISM Chemiluminescent (ChLIA) platform / assays. As part of a comprehensive QC program the Blood Service uses PeliSpy Sero Control Type 36 (T36) (AcroMetrix by Life Technologies) supplied by the NRL as an external QC sample (Go, No-Go control). T36 is a multi-marker QC sample for the detection of hepatitis B surface antigen (HBsAg), antibodies to hepatitis B core antigen (anti-HBc), antibodies to hepatitis C virus (anti-HCV), antibodies to human immunodeficiency virus types 1 and 2 (anti-HIV-1/2) and antibodies to human T-cell lymphotropic virus types I and II (anti-HTLV-I/II). QC result monitoring is performed using the NRL's web based QC program EDCNet.

In mid 2011 the Blood Service commenced using a new batch of T36 (035717: exp:31Mar2013). Fixed Control limits were set for each marker from data reported in EDCNet over approximately a 1 month period. For example, Prism HBsAg: Fixed mean: S/CO = 1.86; Fixed Upper Limit: S/CO = 2.40; Fixed Lower Limit: S/CO = 1.32, calculated from data reported in EDCNet from 10 Laboratories, n = 1168 and 7 reagent batches. By mid 2012, the Blood Service observed a decrease in reactivity of T36:035717 on the PRISM HBsAg assay across all Blood Service laboratories (Brisbane, Melbourne, Sydney, Adelaide and Perth). This decrease in HBsAg reactivity in T36:035717 was further compounded by the use of what appeared to be several "low running" HBsAg reagent batches, to the point where results were often very close to the assay defined cut-off for reactivity (S/CO $\geq$ 1.00). Liaison with the NRL resulted in T36:035717 being replaced with a new batch of T36 (201305: exp:31Jan2014) which the Blood Service commenced using in August 2012. Initial results obtained using T36:201305 were very similar to those initially observed with T36:035717. However, like T36:035717, several months into use, the Blood Service observed a similar decrease in reactivity of T36:201305 with the PRISM HBsAg assay across all Blood Service IDS Laboratories, again compounded by the use of what appeared to be several "low running" PRISM HBsAg reagent batches. Multiple laboratories reported results below the cut-off value for reactivity (S/CO $<$ 1.00).

In order to understand what was causing this decrease in PRISM HBsAg reactivity in the T36 QC samples, the Blood Service initiated investigations into the operational performance of the PRISM instruments, and conducted numerous discussions with Abbott Diagnostics and the NRL (who in turn liaised with the manufacturer of the QC material AcroMetrix / Life Technologies).

Due to the fact that negative T36 HBsAg external QC results were delaying the release of test results by the IDS laboratories, which in turn could have a potential impact on the supply of blood within Australia, the Blood Service notified the TGA of this issue via a "Sponsors/Manufacturers Medical Device Incident Report". Meanwhile, the Blood Service and the NRL determined that a new batch of T36 be requested from AcroMetrix/ Life Technologies (still under manufacture) and fast-tracked.

Investigations by the Blood Service, Abbott Diagnostics, NRL, AcroMetrix, Life Technologies Corporation, Life Technologies Australia and the TGA, determined that the presence of varying amounts of HBs antibodies in the diluents matrix used in the manufacture of these batches of T36 resulted in decreased reactivity of the HBs antigen in the Prism HBsAg assay. These batches of T36 were recalled in February 2013, and subsequent batches of T36 provided by AcroMetrix / Life Technologies through the NRL are performing without incident.

This incident highlights the importance of good communication and information sharing within a QC peer group, as well as between the participant organisation, the QC material provider / manufacturer, assay manufacturers, and regulatory bodies. This, coupled with participation in a strong and robust QC program is essential in maintaining quality in our IDS testing processes, ensuring the safety of the Australian Blood Supply.

## **Acknowledgments:**

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## USE OF THE PELISPY T36 AS A PRECISION CONTROL

Lycett L

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### **Abstract:**

ARCBS laboratories have historically used the Pelispy T36 control on the Abbott PRISM as a Stop/Go control to validate release of results. Any result  $> 1.02$  is considered acceptable.

Results are entered onto EDCNet retrospectively (up to a week later) to assess acceptability against peer group data.

This system appears to be based on the premise that serology (PRISM) QC does not lend itself to the statistical approach taken by the Clinical Biochemists - perhaps, in part, due the inherent reagent batch to batch variability of serology assays.

In doing so, it relegates the PRISM Pelispy QC to a low order of QC power - analogous to the card agglutination tests.

As a part of our TQM program the Melbourne lab has been exploring ways to extract better QC value from the T36 - more in keeping with a large sophisticated multichannel immuno-analyser.

By using reagent lot specific data we have eliminated the "hot and cold" effect which tends to skew target ranges based on multiple lots of reagent.

Applying this reagent lot specific data to Levey Jennings charts with a single S.D. + 3 S.D. rule we have been able to systematically monitor bias, precision and trending in an analogous manner to the clinical biochemists.

As part of real time validation of assays - outliers are repeated and if the result is still not within the acceptable range results are withheld until the problem is resolved.

Based on nine months of using this system across 3 in-house PRISM's we have noted charting characteristics typical of clinical chemistry assays with outliers facilitating the identification and troubleshooting of significant problems.

During this period we have also been liaising with NRL to identify features of our system that could be incorporated into EDCNet version 2.

In summary, by using this system, we have developed higher levels of confidence in the day to day performance of our PRISM's.



## **HIV-1, HBV AND HCV VIRAL LOAD NUCLEIC ACID TESTING EXTERNAL QUALITY ASSESSMENT SCHEMES, 2012**

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### **Introduction:**

NRL provides Quality Assurance (QA) programs to laboratories that test for blood-borne infectious diseases by serology and/or nucleic acid testing (NAT). One of the components of NRL QA is External Quality Assessment Schemes (EQAS). Participation in EQAS gives participants a means of assessing independently their laboratory performance and comparing their results with others in their peer group. It also offers a means of examining the performances of different assays. The objective of this study was to examine the performance of the NAT assays used by the participants of NRL EQAS for HIV-1, HCV and HBV viral load (VL) in 2012.

### **Methods:**

Each HIV-1, HBV and HCV VL EQAS consisted of three five-member panels. These panels were composed of well-characterised plasma samples of known (but undisclosed) concentrations and were designed to examine a number of aims including the linearity of detection in ten-fold serially diluted samples and inter-run reproducibility of the assays used by participants. The results of testing were submitted to NRL using the internet application OASYS (Oneworld Accuracy, Canada). The log<sub>10</sub> transformed results reported by participants using the same assay were grouped and analysed for inter-run reproducibility and linearity of detection as determined by the panel aims.

### **Results:**

Analysis showed that the results of inter-run reproducibility for duplicate samples provided over the calendar year differed by no more than 0.22 log<sub>10</sub> for HIV-1 VL, no more than 0.35 log<sub>10</sub> for HBV VL and no more than 0.27 log<sub>10</sub> for HCV VL. The results of testing ten-fold dilution series were analysed by peer group. The mean of each dilution was a plot and a line/curve of best fit which was interpolated with a linear relationship demonstrated for each analyte over the various assays.

### **Conclusion:**

Results reported by the participants for HIV-1, HBV and HCV VL EQAS demonstrate that commercially manufactured assays performed in the manner described in each manufacturer's instructions for use and results derived from in-house methods were comparable to commercially available assays.



## **EXTERNAL QUALITY ASSESSMENT FOR HIV PROVIRAL-DNA TROPISM TESTING FOR AN INTERNATIONAL CLINICAL STUDY - THE MARAVIROC SWITCH COLLABORATIVE STUDY (MARCH)**

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### **Introduction:**

Results in an External Quality Assessment Scheme (EQAS) were harnessed to assure that multiple laboratories testing HIV co-receptor tropism for an international clinical study – MARCH – were performing to an agreed level of competency and reporting consistent test outcomes. MARCH is a maraviroc switch study in virologically suppressed subjects on stable protease inhibitor-based therapy. HIV-1 tropism is determined using population-based sequencing of proviral-DNA in aviraemic patients. Before initiating the clinical trial, a 3-phase proviral-DNA tropism EQAS was implemented across the candidate international testing laboratories.

### **Methods:**

The EQAS for MARCH was conceived to involve two pre-study phases [Phases 1 and 2] and an on-study phase [Phase 3]. Phase 1 required chromatogram interpretation (n=10); samples deemed to be of poor sequence quality were included (n=2); tropism was determined by reference laboratory testing; competency was defined as 100% concordance with reference laboratory output. Phase 2 required triplicate testing of 20 DNA samples from HIV-positive volunteers [VL<50cp/mL (n=18); at least ten were CXCR4-tropic on prior phenotypic testing] where the lowest FPR [false positive rate] of any replicate defined tropism of a sample as determined by online algorithm Geno2Pheno [<http://coreceptor.bioinf.mpi-inf.mpg.de/index.php>]. The Geno2Pheno algorithm derives the FPR from the V3 sequence of each replicate. In Phase 3 two samples were derived from clones (and therefore did not contain viral quasispecies) and eight were randomly selected from those that had been used in Phase 2.

### **Results:**

In Phase 1 the reference tropism was reported by all laboratories for all samples of acceptable sequence quality however most laboratories also reported results from sequence of substandard quality. The Phase 2 results highlighted the potential for variability between laboratories' test outcome using samples derived from clinical material. Follow-up of Phase 2 results showed automated sequence analysis can increase concordance between laboratories. As outcomes of the Phase 2 results the FPR was reviewed from 20% to 10%; competency criteria when testing clinical material were adjusted to allow one CXCR4 sample and two CCR5 samples to be misclassified; and a requirement to amplify a PCR product from at least 80% of clinically derived samples. For Phase 3, 11/12 laboratories were competent; one laboratory miscalled two CXCR4 samples as CCR5. Their processes are undergoing further investigation.

### **Conclusion:**

This international EQAS revealed concordance when laboratories tested chromatograms but inter-laboratory variability in tropism determination from proviral-DNA. This variability would have been missed had a single or duplicate sequencing approach been used. These outcomes highlight the necessity to quality assure laboratory groups testing for clinical studies to ensure competency and consistency across test outcomes and further, the need to implement EQAS prior to commencement of the study. Parameters of this EQAS were flexible in response to outcomes; any suboptimal performance was addressed and rectified by supplemental testing and coaching by an expert panel. In the end consistent outcomes were achieved, competent testing promoted, and inter-laboratory networks were strengthened.