INFLUENZA HUMORAL IMMUNE RESPONSE

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Objective:
Complement Fixation (CF) is commonly used for diagnosis of influenza infection. Results correlate with clinical cases of influenza, but the test is complex, labour intensive, slow and interpreted subjectively. Enzyme Immunoassay (EIA) is preferred as assays are more sensitive, rapid and can be automated, but evaluation studies of influenza A and B IgG, IgM and IgA EIA show poor correlation with clinical infection. The difference in clinical value between CF and EIA assays may be due to: specific antibody detected (CF ribonucleoprotein antigen detects type-specific antibodies, EIA split virion antigen may detect type, sub-type and strain specific antibodies); more sensitive detection of vaccine immune responses by EIA; detection of different Ig isotypes, particularly IgG subclasses. CF detects complement fixing specific Ig (IgM, IgG1, IgG3 but not IgG4 or IgA). Anti-viral IgG is usually restricted to IgG1 and IgG3, with IgG3 appearing early during the course of infection. EIA’s detect specific IgM, IgA and IgG, and presumably all IgG subclasses.

Using CF and EIA, this study examined the humoral immune response to influenza immunisation, and clinical influenza infection, to determine the most useful marker (specific IgM, IgA, IgG or IgG3) to detect recent influenza infection, and to differentiate this from recent or past immunisation.

Materials and Methods:
To examine the humoral immune response to influenza immunisation, serial blood specimens were collected from nine volunteers before and after influenza immunisation in 2006. Diagnostic sera from patients with suspected respiratory infection which had been tested by CF were retrieved, clarified and tested by EIA. CF was performed on all sera (pre-and post-immunisation and diagnostic specimens) at ICPMR, Westmead Hospital using an in-house method with commercial antigen (Virion). Commercial ELISA (Genzyme Virotech) assays were used for Influenza A and Influenza B IgG, IgM and IgA, and a modified assay using a commercially available anti-human IgG3 conjugate in the Influenza A and B IgG assays.

Results:
Vaccinee specimens
Prior immunity (detectable immune response at day 0) was found, particularly for influenza A, for IgG, IgG3 and IgM, but CF reactivity although present at low level was below the clinical decision point. Detectable IgM was only found at day 0 in vaccinees who had been previously immunised.
At day 14 a CF response to immunisation was rare (one influenza A and one influenza B) and both occurred in first time vaccinees. IgM was the most common marker of response to immunisation for influenza A and B but responses for IgA, IgG3 and IgG also occurred; first time vaccinees responded more commonly. The duration of the immune response was examined at 24-33 days after immunisation; CF titres were unchanged, IgG and IgG3 responses generally remained but IgM and some IgA responses were reduced. The time period chosen was insufficient to thoroughly examine persistence of responses to immunisation.

Clinical specimens
Sera from patients with suspected influenza infection were selected for this study. The sera were mostly influenza A CF positive (19/35), as influenza A infection is more common; only 3/35 were influenza B CF positive. Many specimens were reactive for IgG and IgG3, with some also IgM reactive. IgA is not detected by CF and was rarely found in the sera selected for this study. CF dual response to influenza A and B was never detected, but was found for IgG and IgG3 and also found for IgM and IgA.
Agreement between influenza A CF and EIA assays was 50-70% and for influenza B was 49-89%. Influenza A IgG3, IgM and IgA appeared to be less sensitive than CF; these could be false negatives, or due to CF detection of IgG from past infection or immunisation as IgG and CF showed 77% agreement. Influenza A CF positive sera were negative for influenza B by CF but commonly positive for IgG and IgG3, suggesting lower specificity, or greater sensitivity of EIA in detecting influenza B past clinical infection or immunisation. Paired sera were collected from 6 patients; the date of onset was unknown, and the second specimen was collected between 7 and 56 days after the first. CF seroconversion indicating recent infection was detected for 2 patients, 1 for influenza B (also IgG and IgA seroconversion), and 1 for influenza A (IgG, IgG3 and IgM responses were already detectable in the first specimen).

The results obtained from this study provide valuable information on the humoral immune response to influenza which requires further investigation.
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The difference in clinical value between CF and EIA assays may be due to: specific antibody detected (CF ribonucleoprotein antigen detects type-specific antibodies, EIA split virion antigen may detect type, sub-type and strain specific antibodies); more sensitive detection of vaccine immune responses by EIA; detection of different Ig isotypes, particularly IgG subclasses. CF detects complement fixing specific Ig (IgM, IgG1, IgG3 but not IgG4 or IgA). Anti-viral IgG is usually restricted to IgG1 and IgG3, with IgG3 appearing early during the course of infection. EIA’s detect specific IgM, IgA and IgG, and presumably all IgG subclasses.

Using CF and EIA, this study examined the humoral immune response to influenza immunisation, and clinical influenza infection, to determine the most useful marker (specific IgM, IgA, IgG or IgG3) to detect recent influenza infection, and to differentiate this from recent or past immunisation.

Materials and Methods:
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A CF response to immunisation at day 14 was rare (one influenza A and one influenza B) and both occurred in first time vaccinees. IgM was the most common marker of response to immunisation for influenza A and B but responses for IgA, IgG3 and IgG also occurred; first time vaccinees responded more commonly. The duration of the immune response was examined at 24-33 days after immunisation. During this time period CF titres did not change, IgG and IgG3 responses generally remained but IgM and some IgA responses were reduced. Although one late specimen collected at day 55 also did not show a reduced immune response, the time period chosen was insufficient to thoroughly examine persistence of responses to immunisation.

Clinical specimens
Sera from patients with suspected influenza infection were selected for this study. As influenza A infection is more common, the sera were mostly influenza A CF positive (19/35), with only 3/35 influenza B CF positive. Many specimens were reactive for IgG and IgG3, with some also IgM reactive. IgA is not detected by CF and was rarely found in the sera selected for this study. Dual CF response to influenza A and B was never detected, but was common for IgG and IgG3 (suggesting EIA detection of past infection or vaccination) and also found for IgM and IgA.

Agreement between influenza A CF and EIA assays was 50-70% and for influenza B was 49-89%. Influenza A IgG3, IgM and IgA appeared to be less sensitive than CF; these could be false negatives, or indicate that CF is detecting IgG from past infection or immunisation as IgG and CF showed 77% agreement. For influenza B, IgG and IgG3 were more commonly detected than CF suggesting that the EIA, rather than being less specific, may be more sensitive and detecting past clinical infection or immunisation.

Paired sera were collected from 6 patients; the date of onset was unknown, and the second specimen was collected between 7 and 56 days after the first. CF seroconversion indicating recent infection was detected for 2 patients, 1 for influenza B (also IgG and IgA seroconversion), and 1 for influenza A (detectable IgG, IgG3 and IgM responses were already detectable in the first specimen).

The results obtained from this study provide valuable information on the humoral immune response to influenza which require further investigation.
BACKGROUND: The National Serology Reference Laboratory, Australia (NRL) coordinates a Quality Control (QC) programme for Epstein-Barr virus (EBV) testing in Australia. The EBV QC programme is conducted under contract to Panbio and provides laboratories with a mechanism to monitor the performance of their assays over time.

AIM: To analyse the QC data reported from Panbio EBV assays and to compare the precision of results between selected laboratories.

METHODS: Laboratories using Panbio EBV assays were invited by Panbio to join the EBV QC programme. Panbio provided laboratories with a QC sample of low positive reactivity, while the NRL provided each laboratory with access to the NRL’s internet-based program EDCNet for the entry and analysis of their QC sample test results.

It was recommended to laboratories to test the QC sample in each test run in addition to testing the controls supplied with the following Panbio assays:
- Panbio EBV EBNA IgG ELISA (EBNA IgG),
- Panbio EBV VCA IgG ELISA (VCA IgG),
- Panbio EBV VCA IgM ELISA (VCA IgM) and
- Panbio EBV VCA-p18 IgG ELISA (VCA-p18 IgG).

Results reported by laboratories between 01/07/2005 and 01/08/2006 for the EBV assays were examined for validity and errors and then analysed using EDCNet. Control charts were produced in EDCNet.

RESULTS: Twenty-seven laboratories were enrolled in the EBV QC programme. Twenty laboratories entered results into EDCNet between 01/07/2005 and 01/08/2006. Results for approximately 3,700 test runs were submitted.

During the review of data submitted in the specified date range, two assays showed considerable variation for certain reagent lots. Upon further investigation and communication with the manufacturer it was revealed that the specifications for the VCA IgG and VCA IgM assays had been modified. Following the modifications higher S/Co ratios with greater run to run variation were observed. For the two modified assays the data were split into two populations, and those reported after the modifications were analysed. For the unmodified assays the data analysed included all those reported over the specified date range.

The coefficient of variation (CV%) of results generated from testing QC sample Virotrol EBV Lot 04J151-1 were calculated for each laboratory:
- Seven laboratories using the EBNA IgG reported a total of 437 results. The CVs for these laboratories ranged between 13.95% and 41.67%.
- One laboratory used the VCA IgG and reported 5 results. The CV was 11.59%.
- Sixteen laboratories using the VCA IgM reported a total of 1040 results. The CVs for these laboratories ranged between 10.63% and 54.01%.
- Eight laboratories using the VCA-p18 IgG reported a total 738 results. The CVs for these laboratories ranged between 8.20% and 28.47%.

The results reported by two laboratories using VCA IgM were further analysed and compared. Results from laboratory A (n=145) were less variable (x=5.06, SD=1.39 and CV=27.47%) than results from laboratory B (n=103, x=3.75, SD=1.67 and CV=44.53%). These data were generated from four reagent lots in each laboratory. Laboratories A and B used one reagent lot (06004) in common for a comparable number of runs (n=50 and n=70, respectively). The precision of results from laboratory A was 14.32% compared with 32.04% for laboratory B using this reagent lot.

DISCUSSION/CONCLUSION: Diagnostic laboratories have used EDCNet to monitor the precision of EBV serology in ‘real time’ since August 2005. Irrespective of reagent lots, the precision of results observed for some laboratories was lower when compared with that of other laboratories testing the same QC sample in the same EBV assay. Laboratories with CVs greater than 25% are encouraged review their processes and to contact Panbio or the NRL for assistance with reducing variability of test results.
EVALUATION OF THE ROCHE COBAS AMPLISCREEN ASSAY FOR HIV-1 AND HCV

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Introduction  The COBAS AmpliScreen tests are in vitro assays for the direct qualitative detection of Human Immunodeficiency Virus Type 1 (HIV-1) or Hepatitis C Virus (HCV) RNA in human plasma from donations of whole blood and blood components for transfusion. The COBAS AmpliScreen utilises a generic sample preparation along with automated amplification and detection using PCR on the COBAS AMPLICOR Analyser.

Methods  A total of 22 blood (serum) samples were collected from cadavers. All samples previously tested negative for both HIV-1 and HCV RNA. The 22 samples were spiked with a known HIV-1 antibody and HIV-1 RNA positive sample and subsequently assayed on the AmpliScreen HIV-1 test including the Roche inhibitor control. The inclusion of the Roche inhibitor control permits detection of false negative results due to inhibitory factors or inadequate or failure of amplification by PCR. This procedure was also repeated using a known HCV antibody and HCV RNA positive sample. A total of 19 specimens: 9 specimens for HIV-1 and 10 specimens for HCV were tested on their corresponding AmpliScreen assay to investigate RNA degradation. Specimens were sampled daily and stored at +4 degrees C, as a series of plasma aliquots from a known HIV-1 positive donor and from a HCV positive donor. A third panel using 15 samples was introduced to measure the rate of degradation of HIV-1 RNA over time. Single plasma was collected from two HIV seropositive RNA donors, one with a high viral load and the other with a low viral load. Collections were stored at +4 degrees C, sampled daily and assayed on both the HIV-1 quantitative viral load assay and AmpliScreen HIV-1 test as per standard processing protocol. All tests included two pedigree external quality assurance samples (NRL 208 and 115) and were completed by the same operator and same reagent batch lot to minimise intra-laboratory influences.

Results  All samples were tested by the standard protocol for diagnostic serum samples from living patients. All 22 cadaveric samples gave optical density (OD) for AIH (HIV-1 sequence) and ACH (HCV wildtype sequence) consistent with strong and un-inhibited samples. The Roche Multiprep internal control (AIH) was detectable in samples with strong amplification of HIV-1 or HCV wild type sequence. A total of 9 and 10 samples were harvested from the clot/cell interface over a total of 10 and 17 days respectively. The results showed no significant difference reported in the AmpliScreen results. For the third panel, the high viral load donor: (5.82 log (10) copies HIV RNA mL) and the second donor, low viral load: (3.38 log (10) copies HCV RNA mL) a total of 9 and 6 samples were harvested from the clot/cell interface over a total of 16 and 9 days, respectively. Results from both these panels showed significant difference reported in the calculated HIV viral load with a calculated range (log10) of 1.65 and 0.65 respectively. All AmpliScreen results for these panels gave optical density (OD) for AIH (HIV-1 sequence) consistent with strong samples.

Discussion  Cadaveric samples did not appear to be inhibitory to either HIV-1 or HCV PCR tests within the collection timeframes. It was also evident no degradation was witnessed of HIV-1 or HCV RNA in serum sample over 10 and 17 days respectively post collection. There was apparent degradation of HIV in the plasma samples over the two donor post collections. Results show a marked decrease in HIV-1 viral load from day 3, post collection. Overall log (10) results show a delta change of approximately 2 and 1 log10 difference respectively. It is noted these specimens were collected from a living donor. No discrepancies were observed in AmpliScreen HIV-1 results in comparison to the degraded plasma samples within the post collection study.
EMERGING DISEASE THREATS TO AUSTRALIA – DIAGNOSTIC ISSUES

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Serology remains an important tool in the diagnosis and study of infectious disease. The pathology industry relies on commercial manufacturers for the supply of kits and reagents. A range of factors governs the development and manufacture of diagnostic kits and the performance of such kits. These include the application (e.g., diagnosis, screening and blood bank), sensitivity and specificity of the assay, prevalence of disease, quality, validity and availability of reference samples and the dynamics of the milieu of infectious agents within a given territory. These factors will be discussed using specific examples to illustrate key points. First, the impact of the coincidence of factors such as sensitivity, specificity and disease prevalence on the performance of diagnostic assays will be discuss against a background of assay specifications. Second, the impact of the importation of a new agent on a well established assay system will be reviewed using the example of the potential threat from chickangunya. Finally, the question of the value of broad or narrow test specificity in the design of an assay will be reviewed with respect to the threat of avian influenza.
DEVELOPMENT OF A PROTOTYPE RAPID POINT OF CARE IMMUNOASSAY FOR THE DETECTION OF *TREPONEMA PALLIDUM*-SPECIFIC IGM.

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Ian Potter Hepatitis Research Laboratories, Burnet Institute, Melbourne, Australia and Select Vaccines Ltd, Melbourne, Australia

**Introduction:** Syphilis remains a worldwide public health problem. The World Health Organisation (WHO) estimates that there are 12 million new cases of syphilis each year, with more than 90% occurring in developing nations, and increasing numbers in Russia and much of Eastern Europe. In many developing countries congenital syphilis remains a leading cause of still births and deaths among neonates. In countries where syphilis is less common such as North America, Western Europe and Australia rates of infection are increasing, particularly in men who have sex with men.

A wide range of diagnostic tests exist to diagnose both active and past syphilis infection, however many of these tests are of limited value in primary healthcare settings, especially in developing countries. Rapid point-of-care (RPOC) tests for the detection of antibodies against *Treponema pallidum* have been widely available for a number of years, most in the form of immunochromatographic strips where antibody–antigen reactions appear as a coloured line. These tests require little or no equipment; can be stored at room temperature for at least 6-12 months; have a limited number of steps; are simple to use; and give a result in under 20 minutes. Evaluation of these tests by WHO and other researchers suggest they are at least as sensitive and specific as the *T. pallidum* particle agglutination test. However, existing RPOC tests have the disadvantage that they detect all classes of anti-treponemal antibodies, or in some cases IgG alone. There are no RPOC tests that can detect IgM class antibodies to *T. pallidum* alone and none that can distinguish between infections that are active or those that have been treated in the past. Hence these tests have limited value in areas where syphilis is endemic or in some high risk groups, such as men who have sex with men.

**Aim:** To develop and evaluate a simple, rapid point of care assay to detect *T. pallidum* specific IgM.

**Methods:** A simple, lateral flow format was developed that allows the detection of *T. pallidum* specific IgM, without the interference of specific IgG. This assay, the SLT Syphilis M assay, takes the form of an immunochromatographic strip where the anti-IgM–*T. pallidum* antigen reaction is visualised as a pink/purple line. A procedural control has been included in the test to indicate that the assay has been performed correctly. The test time is \( \leq 15 \) minutes and requires the use of 20 \( \mu \)L of serum or plasma.

A preliminary in-house evaluation was conducted to assess sensitivity and specificity of the SLT Syphilis M rapid assay. To assess sensitivity, 49 sera positive for anti-treponemal antibody (total), 13 of which were IgM positive by a commercial ELISA, were tested by the rapid SLT Syphilis M prototype. These sera consisted of a commercially available Syphilis Mixed Titre Performance Panel (Boston Biomedical Inc, USA PSS202) and *T. pallidum* positive sera donated by a number of recognised pathology laboratories located in Australia. The specificity of the assay was assessed using 50 plasma samples from Australian blood donors.

**Results:** The SLT Syphilis M prototype rapid assay was able to detect 12 out of 13 anti-*T. pallidum* IgM positive sera. Only two of the remaining 36 total antibody positive samples were positive on the rapid assay. Of the 50 blood donor samples one was positive by the rapid SLT Syphilis M assay.

**Conclusion:** The sensitivity of the SLT Syphilis M prototype rapid assay was found to be 92% (12/13) for patients with both *T. pallidum* IgM and IgG. The specificity in patients with only *T. pallidum* IgG was 94% (34/36) and in a *T. pallidum* negative blood donor population was found to be 98% (49/50). The SLT Syphilis M prototype rapid assay represents the first rapid point of care assay for the detection of *T. pallidum* specific IgM, and should be a valuable tool for the improved control of syphilis worldwide. Work is continuing to expand the sensitivity and specificity data with additional samples from diverse geographic regions.
FIRST DOCUMENTED CASE OF TRANSFUSION TRANSMITTED DENGUE VIRUS INFECTION

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Introduction

Since 1994, dengue fever has become a notifiable disease in Hong Kong. For each case notified, site investigations, entomological and active case findings are initiated for prompt control of any further spread of the infection. With a local outbreak of dengue fever in 2002, joint investigation among the Department of Health (DH), the Hong Kong Red Cross Blood Transfusion Service (BTS) and hospital clinicians led to the discovery of the first documented case of transfusion transmitted dengue virus infection in human, which is reported in this abstract.

Case Summary

The donor was a 17 years old male. He had donated blood twice with the last donation made at a donor center on 17 July 2002, which was processed into red cells, platelet concentrates and plasma. The platelet concentrates and red cells were issued to hospitals on 21 and 23 July 2002 respectively.

The donor recalled that he had symptoms of fever, skin rash and retro-orbital pain one day after blood donation on 17 July. However, he did not notify the BTS at that time. Since his symptoms were suggestive of dengue fever, the DH took his blood sample for laboratory testing and the result confirmed that he had recently contracted dengue infection. Upon notification by the DH, the BTS immediately conducted a look-back to trace the recipients who had received his blood. The BTS also traced the archive sample of his last donation and dengue virus (type 1) was detected by reverse transcriptase-polymerase chain reaction (RT-PCR)\(^1\).

These red cells were transfused to a 72 years old lady who was admitted into Queen Mary Hospital in August 2002 with anaemic symptoms (i.e. malaise, dizziness). Results of laboratory investigation including bone marrow examination were consistent with megaloblastic anaemia. She received the blood transfusion and developed a fever of 38.4-38.6°C on day 3 after transfusion. The fever was associated with moderate neutropenia, severe thrombocytopenia and hypotension. The hypotension responded to fluid resuscitation and antibiotic therapy was given for the possible septicaemia. The fever subsided after 4 days. Blood culture was negative. Despite the resolution of the fever, the total white cell, absolute neutrophil and platelet counts continued to fall. During the neutropenic period, her condition was complicated by urinary tract infection which had responded to a course of clarithromycin. She received a course of rehabilitation and was discharged from hospital. She was contacted and counseled by her doctor-in-charge about the possible risk of dengue infection on 7 October 2002 (44 days after transfusion). A blood sample was taken for testing. It was positive for dengue IgM antibodies and negative for dengue reverse transcriptase-polymerase chain reaction (RT-PCR). She had no recent history of travel or mosquito bite prior to admission to hospital in August. She did not live in the area where the local outbreak of dengue fever was reported. The hospital laboratory was able to trace her blood sample taken on the day immediately before blood transfusion, day 2 and 9 after transfusion. The samples taken before and 2 days after transfusion were negative for dengue antibodies and RT-PCR, while that taken 9 days after transfusion was positive for dengue RT-PCR (type 1).
Conclusion

In the case described above, the donor was at the asymptomatic viraemia stage, i.e. the window period, at the time of blood donation. While it was normal that asymptomatic individuals might pass the donor screening procedure, it was the BTS routine practice to ask all donors to inform the BTS whenever acute infections occur within 2 weeks of blood donation. In this case, the donor had not reported to BTS when he developed the febrile illness one day after blood donation.

We concluded that the recipient of the donor’s red cells contracted dengue through transfusion because of the following findings. First, the patient’s pre-transfusion sample was negative for both dengue antibodies and RT-PCR. Her blood was still negative for dengue two days after blood transfusion. Second, she developed fever on day 3 after transfusion. The clinical features (fever, hypotension, thrombocytopenia, neutropenia) were typical of dengue fever and 9 days after transfusion, dengue viruses were found in her blood. Third, RT-PCR showed that the dengue viruses found in the donor and recipient blood samples were both type 1.

We believe this is the world’s first case of documented transfusion transmitted dengue fever. Dengue fever is not endemic in Hong Kong and with the growing incidence of dengue fever on a global scale, transfusion transmitted dengue might not be a rarity especially in areas endemic of dengue fever. There could have been under-diagnosis as dengue fever is generally not considered during the investigation of post-transfusion febrile reaction, and the readily reversible clinical course when managed in hospital setting.

Reference

NEW DEVELOPMENTS IN CONGENITAL TESTING AND IMPLICATIONS FOR PATIENT MANAGEMENT

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One of the challenges obstetricians face in the management of their patients is to ensure a good clinical outcome for the pregnancy, i.e. the birth of a healthy baby. Congenital Rubella Syndrome has been largely eradicated in countries with adequate vaccination programs for Rubella, resulting in sufficient herd immunity. Since there are no vaccines currently available for Cytomegalovirus (CMV) or Toxoplasma (Toxo), congenital CMV and Toxo infections continue to remain complications to pregnancy. The monitoring and diagnosis of maternal and congenital CMV and Toxo infection and evaluation of the possible risks to the foetus and newborn infant remain a significant challenge to the diagnostic laboratory. However, the development of the CMV* and Toxo* immunoassays (including IgG avidity) on the ARCHITECT® high throughput analyzer coupled with improved diagnostic algorithms, will permit modern laboratories to better assist clinicians in combating these diseases and mitigate their impact.

*In development
COMPARISON OF THE CHIRON PROCLEIX® TIGRIS® AND ROCHE COBAS S 201 NUCLEIC ACID TESTING SYSTEMS FOR SIMULTANEOUS DETECTION OF HIV/HCV RNA AND HBV DNA

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Background
Since July 2002 the Australian Red Cross Blood Service (ARCBS) has performed HIV-1 and HCV nucleic acid testing (NAT) for the Hong Kong Red Cross Blood Transfusion Service (HKRCBTS). The high HBV prevalence in Hong Kong, along with the development of ‘multiplex’ assays incorporating simultaneous HIV, HCV and HBV nucleic acid detection, motivated the current study to:

1. Estimate the prevalence of HBV DNA positive / HBsAg negative (Yield) donors in the Hong Kong blood donor population.
2. Evaluate the operational performance of two ‘multiplex’ NAT assays and their respective testing platforms.

Materials and Methods
The HBV NAT yield rate was estimated from 10,397 HKRCBTS blood donor samples concurrently tested by ARCBS on Chiron’s PROCLEIX® ULTRIO® assay as individual donor samples using the fully automated TIGRIS® platform, and on Roche’s Cobas TaqScreen Multiplex (MPX) test in pools of 6 (PDT6) using the modular automated s 201 platform. Reactive samples were assigned a final HIV, HCV and HBV status based on pre-defined viral confirmatory algorithms. Analytical sensitivity was assessed by probit analysis of diluted international standards. Operational performance was compared based on multiple factors, including daily workflow analysis, invalid sample rates and failed run rates.

Results
There were 72 HBV DNA positive samples detected in this study which were also HBsAg positive (Abbott PRISM®). Seventy one were detected by the ULTRIO® assay and 72 by the TaqScreen MPX test. Each system independently detected 2 HBV NAT yield samples for a combined HBV NAT yield rate of 4 in 10,397 (0.04%). The TaqScreen MPX test detected one additional reactive sample that remains unresolved. The 95% detection limits for HIV-1, HBV and HCV were 42.2, 12.2 and 2.0 IU/mL respectively for the PROCLEIX® ULTRIO® assay and 50.5, 8.4 and 6.0 IU/mL for the Cobas TaqScreen MPX test using individual donor testing (IDT) on both systems. The invalid test and failed run rates were 0.05% and 2.92% for the TIGRIS® system and 2.39% and 5.53% for the Cobas s 201.

Conclusions
1. Based on this study, the estimated HBV NAT yield rate in Hong Kong blood donors is 0.04%. Although implementing either assay alone would be expected to detect only half (0.02%) of these yield donors, the consensus outcome is an incremental increase in blood safety for the HKRCBTS.
2. There appears to be no difference in clinical sensitivity for HBV in Hong Kong blood donors when testing in pools of 6 on the MPX assay and IDT on the ULTRIO® assay.
3. When testing in IDT on both systems there was no significant difference between the 95% detection limits for HIV-1 and HBV however the PROCLEIX® ULTRIO® assay had a significantly (p<0.05) lower 95% detection limit for HCV.
4. Workflow analysis demonstrated that testing completion times for daily workload of 200 donor samples (IDT vs. PDT6) did not differ markedly between the two systems.
5. Based on a lower invalid sample and failed run rate, the TIGRIS® system demonstrated better overall operational performance.
AN ALTERNATIVE FROM BIO-RAD TO FULFIL HIGH VOLUME BLOOD BANK REQUIREMENTS

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Introduction:
Bio-Rad has introduced a high end automated solution to fit today’s blood bank needs.
It offers a tailor-made and completely validated solution in terms of safety, traceability and cost-effectiveness for the system and reagents in blood bank screening.

Description:
With a unique triple liquid detection, Elite provides capacitance, continuous pressure monitoring and colorimetric verification of all samples and reagents to combine the highest levels of safety and productivity available today.
The Elite has been introduced to the market two years ago and already successful installations have started in both USA and Europe.
The Elite has been used for more than a year in US diagnostic laboratories.
In Europe, our latest installation is the Finnish Red Cross (FRC) where five systems have been validated and installed.

Results:
The Elite installation of the Finnish Red Cross streamlines the complete workflow management from the pre-analytical stage, primary sample pipetting, microplate processing to validated sample reports to Progesa software.
The Bio-Rad solution has complete audit trails, tracks sample Ids from start to finish, and shows a completely sortable list by range of dates, by events, including who, what, when?
Of course, the Elite solution is fully compliant with the ISO 9001, CE-IVD and GMP regulations.
The pre-analytical stage currently used consists of the OLA 2500 HS system which sorts samples and loads automatically Elite racks, saving time.
Sample racks are then loaded on the Elite which fully automates the microplate processing taking into account new and regular donors, tracking reactive samples for further processing, interfacing Progesa and interfacing our QC Expert Data Management software.

Discussion:
Elite is designed to suit high throughput laboratories with a flexible reagent mix, and to streamline blood bank workflow with the ability to link multiple Elite and Evolis.

Bio-Rad offers a complete solution with the Elite instrumentation associated with the validated “Ultra” line reagents in accordance with the essential requirements in terms of safety, traceability, and productivity.

Bio-Rad is now offering a real alternative to current systems to fulfil high volume Blood Bank requirements.
EVALUATION OF THE DIESSE CHORUS EPSTEIN-BARR VIRUS ASSAYS

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Introduction: Serology testing for Epstein - Barr virus (EBV) is commonly requested in diagnostic pathology. Currently, most laboratories in Australia perform EBV serology using microtitre enzyme immunoassays as few automated options are available. In our laboratory, samples are batched and tested twice weekly, resulting in less than desired turnaround times. The Diesse Chorus (distributed by Laboratory Diagnostics) and the diaSorin Liaison (distributed by Immuno) are currently the only two automated assays available in Australia. Testing samples by the Chorus can be performed daily as minimum calibration and controls are required due to the use of disposable, separate, self-contained testing devices.

Methods: A total of 288 serum or plasma samples obtained from individuals who were acutely infected with EBV (n=69) or blood donors (n=140) were characterised using the Trinity Biotech EIA, Oxoid Infectious mononucleosis heterophile antibody assay, and/or blood film and the Euroimmune Western Blot assay. These samples were used to estimate the sensitivity and specificity of the Chorus EBV Viral Capsid Antigen (VCA) IgM, VCA IgG and EBNA IgG assays. A total of 79 samples known to contain potentially cross-reacting antibodies were also tested in the Chorus EBV VCA IgM assay to determine that assay’s level of cross-reactivity. Precision was estimated by testing patient serum known to have low reactivity (S/Co <5) repeatedly in each Chorus EBV assay. A commercially-available EBV multimarker quality control sample was also tested with each test run and the inter-run precision also calculated.

Results: The sensitivity of the Chorus VCA IgM (n=69) was estimated to be 100% (CI: 93-100%) and the specificity (n=140) was 97-99% (CI: 92-100%) (depending on whether equivocal results were treated as positive or negative). Of the 79 potentially cross-reacting samples, 4/79 (ASOT, Cardiolipin, Rubella and Mycoplasma IgM reactive samples) were found to be reactive by Chorus EBV IgM (S/Co range 2.8-5.8). These samples were negative by the Trinity Biotech VCA IgM EIA assay. No cross-reaction was found with the 6 Parvovirus IgM reactive samples tested.

The sensitivity of the Chorus VCA IgG (n=100) was 97-99% (CI: 91-100%) (depending on whether equivocal results were treated as positive or negative) and the specificity (n=40) was ≥ 85% (CI: 70-94%). The sensitivity of the Chorus EBNA IgG (n=100) was 91-92% (CI: 83-96%) (depending on whether equivocal results were treated as positive or negative) and the specificity (n=40) was ≥ 85% (CI: ≥ 70-94%).

The precision measured as a coefficient of variation (CV) for each Chorus EBV assay was:
- Chorus EBV VCA IgM: 17.4% (Low level patient sample; n=30) and 37.0% (Commercial QC; n=25).
- Chorus EBV VCA IgG: 5.9% (Low level patient sample; n=22) and 21.0% (Commercial QC; n=27).
- Chorus EBV EBNA IgG: 6.6% (Low level patient sample; n=22) and 14.3% (Commercial QC; n=27).

Conclusion: The results of the study show that the Chorus EBV assays had sensitivity of greater than 90% and precision which is acceptable for routine diagnostic EBV testing and screening when the limitations of each assay are taken into account. The Chorus offers the advantages of automation and improved turnaround times.
"Harm reduction among injecting drug users in Asia: evidence for effectiveness."

Professor Robert Power

Evidence-based harm reduction programs, especially centred on needle and syringe exchange and drug substitution treatment, are effective in changing high risk behaviour amongst drug users and curbing the spread of HIV infection. However, comprehensive integrated and innovative community-level responses are needed to sustain long-term impact and to achieve necessary coverage. This presentation will draw on examples from the Asia region to explore both the successes and challenges for the harm reduction paradigm.
Twenty-five years after the first recognition of clinical AIDS, HIV infection continues to be one of the greatest epidemics of our time. Over 70 million people have become infected with HIV, of whom nearly 30 million have already died. As a global pandemic, HIV is extremely heterogeneous and dynamic in nature and is comprised of numerous sub-epidemics within different geographical locations and populations each with their own distinctive features such as risk factors for transmission, clinical presentation, and viral subtypes in circulation. While the incidence of HIV has stabilized in much of the developed world, it continues to increase dramatically in resource limited countries. Africa continues to bear the greatest burden of this disease. Despite having 10% of the world’s population, sub-Saharan Africa is home to two-thirds of all people living with AIDS and 80% of all the deaths due to AIDS. In sub-Saharan Africa life expectancy is now 47 years instead of an expected 62 years without AIDS. With an associated mortality rate > 90% and with the current lack of vaccine, it is clear from these estimates that the HIV pandemic will continue to escalate worldwide and have an enormous impact on public health over the next several decades. Although there is uncertainty about the future spread of HIV and its ultimate global dimensions, short-term projections predict that the incidence of HIV will increase by 25% this year alone with 45 million new infections occurring over the next six years. Women will also account for more than half of the 45 million people newly infected worldwide, with an even higher proportion in developing countries. Social determinants of female vulnerability to HIV-1 include gender disparities, poverty, cultural and sexual norms, lack of education, and violence. Women are also more susceptible to HIV-1 due to hormonal changes, vaginal microbial ecology and physiology, and a higher prevalence of STDs. In sub-Saharan Africa, females constitute 60% of those infected with HIV-1 and 75% of individuals infected between the ages of 15 and 24. Women also comprise half of the adults living with HIV-1 in the Caribbean, and one third in Latin America, with a higher burden in young women. In addition to the direct impact that HIV-1 infection has on these women, there is also a high risk of infant infection during pregnancy and a resulting plethora of consequences for the family. Prevention strategies must address the wide range of gender inequalities that promote dissemination of HIV-1, and include interventions to prevent mother to infant transmission.

While treatment has made a tremendous impact on the survival of infected patients, interventions to prevent subsequent transmission have not been fully successful in part due to the some of the unique virologic dynamics of HIV transmission. Recent investigations have been able to quantify the probability of sexual transmission at various stages of HIV providing new insights into biological mechanisms of HIV transmission. In our recent studies from Uganda, we have been able to calculate that the overall probability of transmission per sexual act among HIV-discordant couples. Among 235 monogamous discordant couples within the same population, we were able to match transmission through molecular phylogeny of the transmitted virus with that of the index partner. Among these linked partners the probability of HIV transmission was greatest during the first several months of HIV infection and was responsible for over 43% of all transmissions. Probability rates subsequently declined during latent infection from 0.008/coital act during the acute seroconversion period to 0.0015/coital act six to 15 months after seroconversion, to 0.0007/act among HIV prevalent index cases and rose slightly to 0.0028/act in the one to two years prior to index case death. Within this model early and late stage infection, high viral load, genital ulcer disease, and young age of the index HIV-infected case were significantly associated with higher rates of transmission. Although we could not document the exact rate of transmission during the first several weeks of infection, it is likely that the probability of transmission may be as high as 0.02/coital act. Because of the high association with the appearance of genital ulcers during acute HIV infection, plus the high viral load and the lack of an effective immune response, it can be concluded that acute HIV is responsible for the vast majority of sexual transmissions, and that interventions targeting this highly infectious stage of HIV are critical in the efforts to curb the relentless spread of HIV throughout the world.
AN EVALUATION OF A RAPID ASSAY (CLEARVIEW IM) AND AN AUTOMATED SYSTEM (LIAISON) WITH 2 MICROPLATE ENZYME IMMUNOASSAYS FOR THE DIAGNOSIS OF PRIMARY EBV INFECTION.

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Introduction: Several evaluations of commercial diagnostic assays for EBV (Epstein Barr virus) antibodies have been published and presented in recent years. Most of these studies have involved testing stored serum samples and assessing the sensitivity and specificity on the agreement between kits. Using sets of sera collected from patients with primary EBV we have previously shown that EBV-VCA (Viral capsid antigen) IgM has a poor specificity when taken in isolation but the combination of negative EBNA-1 (EBV nuclear antigen) IgG and Low (<70%) VCA IgG avidity had a sensitivity and specificity of 100% for establishing recent (<6 weeks) primary EBV infection. Among the causes of these false positive IgM reactions Infection with Parvovirus B19 has been identified. This study compares the performance of the assays for IgM and EBNA IgG in clinically defined groups rather than an evaluation based on the degree of concordance of the assays.

Methods: Using the criteria of VCA IgG avidity and EBNA IgG status, sera from patients submitted to this laboratory for investigation of illness were classified into recent EBV infection, past EBV infection, and EBV seronegative as well as recent parvovirus B19 infection. These sera were used to evaluate an automated system (Liaison Dia Sorin), a rapid cassette test for IgM heterophile antibodies Clearview IM and 2 traditional microplate EIA tests (Pan Bio and Trinity diagnostics.) Of the 20 patients with recent EBV infection 3 were age <8 years

Results: Pan Bio and Trinity assays detected IgM in all 20 patients with recent EBV. Liaison IgM was negative in 1 (age 17) and Clearview IgM was negative in 3 patients (2 aged <8). In the Past infection group (n=38) Trinity and Clearview IgM were all negative while Liaison gave 4 and Pan Bio 6 positives. Among the Parvovirus IgM positive samples with high EBV-VCA avidity (n=20) no Pan Bio or Clearview IgM assays were positive while 5 different samples were positive in either Trinity (1) or Liaison (4).

In the seronegative group there was complete agreement between the 3 IgG EBNA assays however in the recent EBV infection group both Trinity (1) and Liaison (2) detected positives while in the past infection group 2 different sera were found negative in either of Pan Bio or Liaison.

Conclusion. Based on a limited sample set of sera Liaison provides an automated system for measuring EBV-VCA IgM and EBNA IgG antibodies with similar accuracy to microplate assays. Recent Parvovirus infection was confirmed as a cause of false positive EBV-VCA IgM antibody but appears to have been eliminated as a cause of false positives in the Pan Bio assay. Clearview IM appears a convenient and specific test for diagnosing primary EBV with the inherent problem of heterophile assays in that it may be negative in a proportion of young children
Human papillomavirus DNA testing methodology

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Cervical cancer is one of the few human cancers with well-defined link to a virus, i.e. human papillomavirus (HPV). Since HPV cannot be efficiently propagated in-vitro, conventional viral culture techniques have not been used for its detection. Therefore, molecular biological detection methods have been used for HPV detection and genotyping. Utilizing DNA homology of papillomaviruses, there are now well over 100 types recognized. Main assays utilized for detection of HPV in laboratories today include signal and target amplification assays. As HPV detection and typing results can now offer information useful in various clinical situations and patient treatment, a number of methods are becoming commercially available. This presentation will highlight methods being utilized in various laboratories across the globe for detection of HPV.
A recent editorial in The New England Journal of Medicine described the necessary association of HPV to cervical cancer and the role the addition of HPV DNA testing may play in cervical cancer screening and prevention. The editorial stated, "The discovery that persistent, oncogenic HPV infection is necessary for the development of cervical cancer is revolutionizing our approaches to screening and prevention, because an obvious corollary is that the absence of infection means that the risk of cancer is negligible."

For over 40 years, counties have been relying on cytology to detect and screen women for the presence of cervical disease. With increased knowledge about the natural history of HPV, many researchers are now evaluating the utility of HPV DNA testing for Cervical Cancer screening.

HPV DNA testing adds high positive and negative predictive values in not only identifying the women with current disease but also impending risks of progression to cervical cancer within next 5 to 15 years when correlated with the advanced age of the subject. In recent years a number of primary screening studies have been undertaken to assess the utility of the HC2 HPV assay as a primary screening tool.

A Meta Analysis of a number of European and North American studies show that HPV DNA testing has a sensitivity for the detection of High grade disease of 98.6%, while cytology was 53%. Practical and clinical issues related to the rationale necessary when assessing the use of HPV DNA tests for cervical cancer screening will be outlined.

The use of HPV in screening programs represents a major advancement in the fight against cervical cancer, offers women and physicians an objective screening measure of a woman's risk for cervical cancer. It is also the first virus DNA test to be evaluated for wide scale use as a screening test for cancer, and represents a significant paradigm shift from the historical use of cytology.
Introduction: As a National HIV Reference Laboratory, we use a combination of HIV antibody and antigen assays for testing. These assays include Abbott Architect HIV Ag/Ab Combo and/or AxSYM Ag/Ab Combo for screening, Fujirebio Serodia Particle Agglutination (PA) assay and VIDAS HIV DUO as supplementary tests, MP Diagnostics HIV Blot 2.2 (HIV Blot) for confirmation. Biomerieux will soon cease production of VIDAS HIV DUO in year 2006 and is offering VIDAS HIV DUO Ultra as an alternative.

Objective: To evaluate the performance of VIDAS HIV DUO Ultra to replace VIDAS HIV DUO. VIDAS HIV DUO Ultra is an enzyme-linked fluorescence assay enabling the simultaneous detection of p24 antigen and HIV IgM and IgG using a double-sandwich EIA.

Materials and Method: Reference assays used in this evaluation are Architect or AxSYM screening, Fujirebio PA, and MP Diagnostics HIV Blot 2.2. One hundred and nine samples were used in this evaluation. These samples were divided into 5 groups: Group A: Confirmed negative samples. Group B: Confirmed positive samples. Group C: Screening reactive, confirmed antibody negative (HIV Blot). Group D: Sero-conversion samples. Group E: Commercial HIV-1 sero-conversion panel (PR954, purchased from Boston Biomedica Inc, USA). The results from VIDAS HIV DUO Ultra were compared to the results obtained from the reference assays.

Results: Group A (True negative, N=22): All samples in this group tested negative by VIDAS HIV DUO Ultra. The specificity was 100%. Group B (True positive, N=44): All samples tested positive for antibody on VIDAS HIV DUO Ultra. None of these samples was positive for antigen. The sensitivity was 100%. Group C (Screening reactive, HIV Blot confirmed negative, N=28): VIDAS HIV DUO Ultra detected 5 of the 28 samples as antigen positive and none of these samples tested antibody positive. These 5 samples were confirmed positive by Murex HIV Antigen mAb (an antigen test). The remaining 23 samples tested negative by Murex HIV antigen mAb. Group D (sero-conversion routine clinical samples, N=8, 4 pairs): The first samples of the pairs were Screening reactive, PA positive (except for one case), and HIV Blot indeterminate. VIDAS HIV Duo detected one antigen positive (PA negative) and 3 antibody positive. One pair (311005/5709 and 101105/5711) was detected to be antigen positive by VIDAS HIV DUO Ultra. However, it failed to detect HIV antibodies in 101105/5711 although HIV antibodies were detected both by PA and HIV Blot. Group E (Sero-conversion panel PR 954, N=7): VIDAS HIV DUO Ultra detected antigen positivity in the last 2 samples of the panel (PR954-06 & 07). These two samples tested reactive by Screening and HIV Proviral DNA PCR and negative by Cambridge Biotech western blot. For assay precision, the inter-assay coefficient of variation (CV) for positive sample is 5.133% and the intra-assay CV for moderate and high positive samples are 4.403% and 0.256%, respectively.

Discussion and Conclusion: The performance of VIDAS HIV DUO Ultra is satisfactory by achieving 100% on specificity and sensitivity in the true negative and positive groups. It picked up 5 antigen positive samples that were negative in the HIV Blot. In paired routine sero-conversion samples, it successfully detected HIV antigen and antibodies in the samples from patients with early sero-conversion status. The excellent sensitivity and specificity of VIDAS HIV DUO Ultra and its ability to detect antigen positive samples will be useful in early detection of HIV infection and is a satisfactory replacement for VIDAS HIV DUO in the National HIV Reference Laboratory.
There are four species of malaria that infect man, with mortality mainly attributable to Plasmodium falciparum, the parasite responsible for cerebral malaria and severe malaria in pregnancy. In certain regions, P.vivax is a major cause of morbidity, and has the troubling characteristic of causing recurrent symptoms months after the initial bite from an infected mosquito.

More than 100 years ago the lifecycle of malaria was described, with obvious implications for designing strategies that could be used to control the disease, but these have had only partial success. Reduction in mosquito breeding sites by environmental engineering, and the use of residual insecticides for indoor spraying had major effects on disease transmission in many parts of the world, but in areas of highest transmission such as sub Saharan African, malaria was never controlled.

There has been a resurgence of malaria in the last 20 years with the breakdown of malaria control programmes, particularly in the Indian subcontinent, and Sri Lanka. Concerns about the use of residual insecticides greatly restricted the use of DDT, even though the environmental impact was largely attributable to agricultural rather than medical use.

A major boost to the field of malaria research and control has been provided by support from international agencies, partially based on recent analyses suggesting that major economic benefits and economic development can occur through control of malaria, in addition to the benefits for the health of individuals. International efforts have been expanded by philanthropy, particularly the Bill and Melinda Gates Foundation, and the Global Fund for malaria, AIDS and tuberculosis. Vaccine trials demonstrating partial efficacy have been a great stimulus to the field and there is considerable interest in testing combinations of vaccine candidates in areas of high endemicity.

Research funding has increased our knowledge about the Plasmodium parasite, but has not provided new agents to prevent severe consequences of malaria such as anaemia, or the pathology that results from the unique ability of the P.falciparum parasite to sequester in deep tissues of the host.

The presentation will discuss historical methods for malaria control, current challenges, and an insight into recent discoveries of the pathogenesis of severe disease.
Introduction: The RCPA Serology QAP introduced the Retroviral Module in 2006, after numerous requests from participants over the last few years. This module comprises six surveys for HIV and two surveys for HTLV. Currently, there are 47 participants enrolled in this module.

Objective: To introduce a retroviral module including HIV and HTLV, review survey performances for the first four surveys of the year and address problems or inconsistencies in results.

Method: Review the components of the Retroviral Module and survey performances.

Serology QAP reports focus on the qualitative data for assessment. Assigned results are determined by a consensus of ≥80% of participating laboratories but the report also identifies other inconsistencies in results and data including: omission of kit details, use of expired kits, occurrence of transcription errors, inconsistent or incomplete data, inappropriate interpretative comment selection and non identification of clerical errors.

Reports also provide statistical analysis on the quantitative data for user groups of greater than ten participants. The median and normalised IQR (interquartile range) are used as they are robust statistics and measure the centre and spread of the data (respectively), similar to the mean and standard deviation, but have the advantage of not being influenced by the presence of outliers in the data.

Results: Results for all surveys so far have shown good concordance of results. Analysis of results by robust statistics for Abbott AxSYM HIV Ag/Ab Combo (the largest user group), has shown a few participants with outlying results, although all except one participant returned results that agreed with the consensus.

Conclusion/Discussion: The information provided in the Retroviral Report enables participants to review their results and evaluate their performance. The detailed analysis of results helps participants to check the validity of assay results and identify procedural and technical problems through comparison of data returned by other participants using the same methodology. Multiple surveys throughout the year with corresponding documentation of corrective actions facilitate trend analysis for ongoing monitoring of laboratory performance in both the HIV and HTLV components of the Retroviral Module.
**PHYLOGENETIC ANALYSIS OF HEPATITIS C INFECTION IN SOUTH AUSTRALIA**

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**Background and Objectives:** Hepatitis C virus (HCV) infection is one of the most significant health problems in the world. Approximately 3% of the world population is infected with HCV. The seriousness of this disease is the high percentage of chronic infection (85%) and the high risk of developing hepatocellular carcinoma. The association between HCV genotypes and both the responsiveness to anti-viral treatment and the degree of clinical progression have made genotyping an important tool in patient management. Based on the revised classification, 6 genotypes, and more than 70 different subtypes of the virus have been identified. More than 14,000 HCV cases (0.94%) have been reported in South Australia (data from STD clinic SA). Genotyping is offered by IMVS to HCV infected patients to assist with treatment and provide epidemiological data. This study primarily used direct sequencing of the C/E1 region for the determination of HCV genotype distribution in South Australia.

**Methods:** Between May 2004 and December 2005, 864 blood samples with detectable HCV RNA were sequenced. For RNA detection, a 494bp fragment was amplified on the Light Cycler (or nested PCR for older samples) using C/E1 primers. In the cases where C/E1 PCR failed to amplify, an alternative PCR using highly conserved 5’UTR primers (298bp) was performed. Sequencing of HCV RNA was performed using ABI Prism dye terminator kit and DNA sequencer. Commercial program Kodon was used for phylogenetic analysis, and Bayer HCV InnoLiPA Hybridisation assay was used to resolve some difficult sequences.

**Results:** 1. Of the 864 samples sequenced, 830 were able to be analysed. Of which, the C/E1 primers were able to amplify 750 samples (90%). 80 samples were only amplified with 5’UTR primers. 2. In total, there were 423 genotype 1 (51%), 331 genotype 3 (39.9%), 36 genotype 2 (4.3%), 26 genotype 6 (3.1%), 13 genotype 4 (1.6%) and 1 genotype 5 (0.1%). 3. Genotype 6 was primarily associated with patients of Asian origin, and country of origin of other genotypes will be discussed. 4. Subtyping was not possible for the 80 samples typed with 5’UTR primers. The results of the 750 subtyping using C/E1 primers were: 1a (313), 1b (78), 2a (8), 2b (11), 2c (10), 3a (295), 3k (1), 4a (8), 5a (1), 6a (15), 6d (8), 6g (1) and 6h (1). 5. 12 sequences suspected of mixed infection were selected for the InnoLiPA assay. Of which, 2 were potentially mixed (1a/1b and 2a/2c).

**Conclusions:** High degree of diversity was detected in the South Australian HCV population. This could be a reflection of multiculturalism in South Australia. Although no mixed infections were detected by direct sequencing, mixed infections may exist in South Australian population.
Introduction: The bacterial genus Leptospira is antigenically diverse, with over 200 serovars differentiated by the agglutinating epitopes of the surface lipopolysaccharide. Serovars are considered as falling into about 25 serogroups on the basis of antigenic similarity. The microscopic agglutination test (MAT) for leptospirosis is a simple technique used throughout the world, and employs cultures of live leptospiral bacteria as test antigens. The MAT is considered to be broadly serogroup-specific; however, cross-reaction between serovars within a serogroup is not always observed, and cross-reaction between serogroups sometimes occurs. Patterns of cross-reactivity can affect the serological diagnosis of leptospirosis.

Objectives: To investigate the possibility of regional differences in cross-reactions in the leptospirosis MAT.

Methods: Titres reported in 2006 by participants in an international proficiency testing scheme for the MAT were analysed to study some cross-reactions between leptospiral serovars, within and between serogroups. Each laboratory employs its own panel of serovars as test antigens, so each serovar is used by only some of the participating laboratories. Results were analysed from laboratories testing a rabbit antiserum to L. borgpetersenii serovar Hardjo type bovis (in serogroup Sejroe) with test antigens serovar Sejroe (also in serogroup Sejroe) and L. interrogans serovar Canicola (in serogroup Canicola). Likewise, results were analysed from laboratories testing a rabbit antiserum to L. interrogans serovar Hebdomadis (in serogroup Hebdomadis) using L. borgpetersenii serovar Sejroe (in serogroup Sejroe) as test antigen. Positive titres were defined as ≥80.

Results: Thirty-four of 50 laboratories reported positive results from testing antiserum to serovar Hardjo with serovar Sejroe: 26/32 (81%) from Europe, 6/6 (100%) from Asia and the Pacific, but only 2/12 (17%) from the Americas.

Cross reactions when testing antiserum to Hardjo with serovar Canicola also showed some indication of regional variation, for example 6/7 positive in North America but only 8/20 in South and Central America. In Europe, 26/42 laboratories reported positive results, including 7/8 German laboratories.

Eight of 39 laboratories reported positive results when testing antiserum to serovar Hebdomadis with serovar Sejroe; of these, 6/22 were found positive by laboratories in Europe, but only 2/17 outside Europe. Seven of 8 German laboratories reported negative results.

Conclusion: Cross-reactions in the MAT, within and between serogroups, sometimes appear to vary geographically. This may reflect antigenic variation among cultures used as test antigens. Laboratories often use cultures supplied by leptospirosis reference laboratories in their own geographic regions, and this may create clusters of laboratories reporting similar serological results.
PERFORMANCE OF THE ABBOTT ARCHITECT HIV Ag/Ab ASSAY IN A LOW RISK POPULATION WITH COMPARISONS TO THE ABBOTT AXSYM COMBO ASSAY

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Introduction: SNP laboratory services a population at low risk for Human Immune Deficiency Virus (HIV) infection. Combined HIV Antigen / Antibody testing has been used over the last 2 years for routine HIV screening in our laboratory, initially using the Abbott AxSYM combo assay, and since July 2005 the Abbott Architect combo assay.

Objectives: To review the performance of the HIV Architect assay over the last 12 months, and to compare its performance with the previously used AxSYM Combo Assay, and the AxSYM antibody only assay.

Methods: Routine samples submitted for HIV testing were analysed using the Abbott AxSYM Combo assay from January 2005 to July 2005, and then on the Abbott Architect to the present time. Prior to 2005 the AxSYM HIV1/2gO assay was used. All initially reactive samples were repeated in duplicate on a previously stored sample, and repeat ably reactive samples were forwarded to QHPS laboratories for follow up by Western Blot and p24 antigen testing.

Results: The repeatable reactive rate was very similar between the three assays. For Architect Combo it was 0.28% (225 samples), for AxSYM Combo 0.29% (93 samples), and AxSYM HIV 1/2 0.28% (175) in 12 months preceding introduction of Combo assays. Some of these samples were repeat specimens on the same patients. All reactive samples were forwarded to QHPS for confirmatory testing. For the Architect Combo assay, 61 samples were confirmed positive by Western Blot. A further 6 samples were p24 antigen only positive, and 6 were Indeterminate group 4 and p24 positive, giving a positive rate of 0.092% in the samples tested. This represented 32% of our repeat reactive samples. Of the 152 false positive samples, 116 were Western blot non-reactive, p24 antigen non-reactive. These included 82 with S/CO < 2.0, 26 with S/CO between 2.0 and 10.0, and 8 samples with S/CO > 10.0. Of this last group, 4 samples had S/CO >300.0 on repeat collections. A further 40 samples were returned as indeterminate (6 group 1, 7 group 2 and 27 group 3). These results give a specificity of 99.81%.

For the AxSYM Combo assay, 39 were confirmed positive by Western Blot, a further sample was p24 antigen only positive, and 3 samples indeterminate group 4 and p24 positive, giving a positive rate of 0.136% during this period. Of the 50 false positive results, 38 were Western Blot negative, p24 negative. Thirty one (31) of these had S/CO <2.0 and 7 had S/CO between 2.0 and 4.6. During the first year of use, the Architect Combo assay identified 6 patients as reactive who were only p24 antigen positive, and would have been negative in the HIV 1/2 antibody assay. In the previous 6 months the AxSYM Combo assay identified 3 similar patients who were only p24 antigen positive. A number of the repeatable reactive samples were run on both the AxSYM and Architect Combo assay, all of the confirmed reactive samples were reactive in both assays.

Of the reactive samples on the Architect Combo Assay that were not confirmed by Western Blot or p24, as true HIV positive; 40 % of these initial repeatable reactive were non-reactive on the AxSYM Combo assay and negative by Western Blot. A further 20% of repeat reactive Architect samples, were negative by AxSYM and returned as indeterminate groups 1,2 or 3 on Western Blot.

Conclusion: The performance of the Architect HIV Ag/Ab Combo assay has more than met our expectations. The specificity seen has been as expected. The percentage of repeatable reactive specimens by the three screening assay formats was the same in the population tested over time, but it appears that repeat reactive results on the Architect Combo assay comes from a different group of specimens to that seen on the AxSYM Combo assay. This difference is probably due to the different antibody formats used in the two assays. The majority of the false positive results had initial S/CO of <10.0 on the Architect, although two samples had levels > 300.0 on repeat collections.
Post-Evaluation of Rapid HIV Kits in The Korean Market by an Anti-HIV EQAS Panel

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Background: Rapid tests to detect Human Immunodeficiency Virus (HIV) antibodies are widely used and are in increasingly greater demand due to their convenience and ease of access. The present study aims to provide information about both HIV Rapid kits by the anti-HIV External Quality Assessment Schemes (EQAS) panel of the Korea National Institute of Health (KNIH) and the panel for Rapid HIV test of the U.S., Centers for Disease Control and Prevention (CDC).

Materials and Methods: Each KNIH Anti-HIV EQAS panel from 2003 to 2005 consisted of four or five samples of plasma obtained from blood donors with a strong positive or negative reaction to HIV. We delivered each panel to public health centers and analyzed their HIV test results. We also, compared the reactivity of the five Rapid HIV kits currently used in the Korean market with that of a reference.

Results: The analytic sensitivity and specificity of the Rapid HIV kits for the KNIH Anti-HIV EQAS in 2005 were 99.3% and 99.1%, respectively; in 2004, 98.82% and 97.06%; and in 2003, 94.74% and 95.11%. Five Rapid HIV kits from the CDC panel showed consistently positive reactivity for strong positive samples in all kits, but some showed erratic reactivity for weakly positive samples.

Conclusion: This is the first report on post-evaluation of Rapid HIV kits in the Korean market by an anti-HIV EQAS panel. We estimated that the quality of performance of the Rapid HIV tests had been improved each year but should be interpreted with caution for weakly positive samples.
EVALUATION OF TWO COMMERCIAL ELISA ASSAYS FOR DETECTION OF IgA ANTIBODIES AGAINST BORDETELLA PERTUSSIS

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Introduction:
Whooping cough or pertussis is a highly contagious respiratory tract infection caused by the gram negative bacillus Bordatella pertussis. It causes severe disease in young children with paroxysmal spasms of severe coughing with whooping and vomiting that last for many weeks. It is also emerging as a common cause of disease in adults.

Diagnosis can be made by culture or nucleic acid detection from respiratory samples, or by specific serological methods. The presence of IgA antibody to B. pertussis is an indicator of recent infection. We describe a comparison of two commercial ELISA assays for detection of specific IgA antibody; the Panbio B. pertussis IgA and the Savyon SeroPertussis IgA/IgM kits.

Objectives:
The study was precipitated by the high numbers of equivocal and weak reactive results detected in patients who clinically did not have pertussis.

The Panbio assay uses a whole cell antigen, whilst the Savyon assay uses an antigen enriched with Pertussis Toxin (PT) and Filamentous Haemagglutinin (FHA). It was decided to compare these two assays using fresh and stored serum run in parallel.

Results:
Upon initial investigation we selected 50 Reactive, 19 equivocal and 51 Negative samples tested by the Panbio assay (120 samples). Of these:
- 47 were Non-reactive by both assays and 24 Reactive by both assays.
- 41 samples found to be Equivocal or Reactive on the Panbio assay were Non-reactive by the Savyon assay.
- Another 4 samples Non-reactive by Panbio were Equivocal (2) or Reactive (2) by the Savyon assay.

These results were deemed to be significant and further investigation using stored ante-natal serum was tested (53 samples). Of these:
- 39 were Non-reactive by both assays and 2 Reactive by both
- 8 Reactive and 2 Equivocal samples run by Panbio were Non-reactive by the Savyon assay

Discussion:
Based on this data, the Panbio assay is likely to be more sensitive, but the Savyon is clearly more specific. Whilst a clinical evaluation is required for accurate interpretation of the discordant results on clinical requests, our decision to change to the Savyon kit was based on client needs for a more specific assay. The selection of an assay for determination of IgA antibody to B. pertussis appears to be a choice between the clinical needs of the laboratory, based on the sensitivities and specificities of the assays.
This Old English saying reminds us that there are many things that can go wrong in any complex process. The issuing of a correct result of a diagnostic assay on a patient sample is a good example of a complex process, and there are many points between the initiation of an order for a test and the issue of a correct result where the system can fail. This talk will break the process into its components and discuss the ways in which these components can be monitored.

The laboratory has primary responsibility for the analytical phase of the process, and many systems are in place to ensure that the result obtained for the specimen entering the laboratory is as accurate as possible. These processes include the validation of the assay used (Method Quality Control), validation of the run (Assay Quality Control), monitoring of laboratory proficiency (External Quality Assurance) and monitoring of individual performance (Individual Competency Assurance). These measures may all have a place in a given setting, but their use is determined by the type of testing being done.

Considerable attention is paid to ensure the quality of the analytical phase, but a major proportion of errors occur in either the pre- or post-analytical stages of the process. Pre-analytical errors include the wrong patient being sampled, the wrong specimen taken, inadequate specimen, contaminated specimen, specimen too old, wrongly or inadequately-labelled specimen and wrong assays assigned. Post-analytical errors include wrong result released, excessive delay in the issue of a result, wrong patient name and wrong receiving doctor for the result. Each of these represents a ‘key incident’ for the laboratory, and should be recorded and investigated as part of a good Quality System. The RCPA QAP is introducing a Key Incident Monitoring and Management System to quality assure these non-analytical aspects, and to enable laboratories to benchmark themselves against others.

There are many points in the process where errors may occur, and analytical accuracy is only one of them. A laboratory with a true commitment to quality will be looking at all aspects of the quality chain, and be using all available tools to identify and minimise errors.
Investigation of a non-confirming Hepatitis B surface antigen (HBsAg) positive sample.

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We reported on a true positive Hepatitis B surface antigen (HBsAg) result which failed to confirm on the Abbott AxSYM immunoassay analyser. All HBsAg samples found to be initially reactive on the AxSYM are confirmed by neutralisation with specific antibody against HBsAg (anti-HBs) using the Abbott AxSYM HBsAg Confirmatory assay.

This sample was obtained from a 75 year old male with renal disease and abnormal liver function tests. It was Reactive on the AxSYM HBsAg assay with a S/CO ratio of 104.31.

In the AxSYM HBsAg Confirmatory assay, the sample is incubated with anti-HBs and normal serum in two different reaction vessels. If the anti-HBs reduces the S/CO ratio by ≥ 50%, the sample is considered reactive. This sample could not be neutralised by the Abbott AxSYM HBsAg Confirmatory assay, even after diluting the sample to reduce the concentration of HBsAg in the serum. The presence of HBV in the sample was confirmed by PCR for HBV DNA.

The sample was tested for HBsAg and confirmed by their respective Confirmatory assay performed on a number of other platforms such as BioMerieux VIDAS, Abbott Architect but not confirmed on the Abbott PRISM. The Bayer ADVIA Centaur screening assay failed to detect the HBsAg. Interestingly, when the sample was neutralised with the antibody from the Architect Confirmatory assay and run on the AxSYM, the sample was neutralised and confirmed as positive. This indicates that the anti-HBs in the AxSYM confirmatory assay is unable to bind to the HBsAg in this patient.

The sample was forwarded to VIDRL for sequencing of the surface antigen gene. There were multiple mutations at Q101H, S113A, S117R, P120Q, Y134S, P135L, E164G, V190A, I226N. Whist none of these mutations are in the region aa 139 to147 (vaccine escape mutants), mutations between aa 121 and 149 will cause a change in antigenicity.

These results demonstrate that a HBsAg containing multiple mutations in the ‘a determinate’ of the surface antigen are unable to be neutralised by the Abbott AxSYM and Prism Confirmatory assays as well as failing to be detected by the Bayer ADVIA Centaur HBsAg Assay.
ASSESSMENT OF THE PERFORMANCE CHARACTERISTICS OF AN HIV VIRAL LOAD ASSAY PERFORMED FROM DRIED FLUID SPOTS.

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INTRODUCTION: The NRL, in collaboration with WHO, examined the performance of assays which offer a potential solution to providing HIV viral load estimates in resource-limited settings. The Primagen Dried Fluid Spot (DFS) filter paper can simplify transportation and reduce shipping costs to facilitate patient management in resource-limited settings. Once the DFS is dried it is non-infectious and stable, so testing can occur in either the local country, or if resources for testing are limited, can be sent via mail to qualified laboratories anywhere in the world. Primagen’s DFS technology offers a solution to the logistical and cost challenges of transporting samples for viral load testing. To estimate HIV viral load, samples spotted onto Primagen filter papers were tested in the Roche COBAS AmpliPrep/TaqMan 48 HIV-1 assay.

METHODS: The performance of Primagen Dried Fluid Spot technology as a sample type for the Roche COBAS AmpliPrep/TaqMan48 HIV-1 (DFS/Ampliprep/TaqMan HIV-1) assay was evaluated by NRL in collaboration with Primagen and the Department of Essential Health Technologies, World Health Organization. The NRL supplied the specimens and was responsible for the preparation of the DFS. After preparation the impregnated filter papers were transported to Primagen where the testing using the Roche AmpliPrep and HIV-1 TaqMan reagents, was performed.

Several performance characteristics of the DFS/Ampliprep/TaqMan HIV-1 assay were determined:

- The reproducibility was assessed by testing multiple DFS that had been prepared from a single sample with a viral load of approximately 1,000 copies/mL, over a number of different runs.
- The sensitivity of the DFS/Ampliprep/TaqMan HIV-1 assay was examined by testing DFS prepared from 57 specimens positive for various subtypes of HIV-1. Thirty-four DFS were prepared from plasma drawn from individuals infected with HIV-1 and 23 were prepared from cultures of HIV-1 spiked into normal human plasma.
- Linearity was determined by testing five DFS prepared from each member of a six-member dilution series. Regression analysis was used to estimate linearity.
- The specificity of the system was estimated in 115 DFS prepared from anti-HIV negative specimens.

RESULTS: All DFS gave valid results. The coefficient of variation of the results of replicate testing of one QC sample was <7%. Eight of the 34 DFS produced from plasma samples were below the assay’s limit of detection. All DFS from cultured isolates of HIV-1 gave results above the assay’s limit of detection. Regression analysis gave an r² value of 0.99. DFS from all of the sero-negative samples gave results below the assay’s limit of detection.

CONCLUSION: Using Primagen Dried Fluid Spot technology as the sample type for the Roche COBAS AmpliPrep/TaqMan48 HIV-1 Test has shown linearity, reproducibility and acceptable specificity. The preparation of the DFS requires minimal training and access to laboratory infrastructure. With their ease of use and overall performance characteristics, when coupled with the Roche TaqMan HIV-1 assay, the Primagen DFS may provide a less expensive, simpler alternative for monitoring response to therapy.
QUALITY MANAGEMENT SYSTEMS IN THE REGULATION OF IN-VITRO DIAGNOSTIC DEVICES

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The Therapeutic Goods Administration (TGA) is working to implement a new regulatory framework for in-vitro diagnostic devices (IVDs). The goal of the new IVD framework is to contribute to high quality diagnostic testing in Australia by ensuring that the IVDs available for supply are safe and perform as intended. The proposed framework imposes certain obligations on the IVD manufacturer; in particular it will be the manufacturer’s responsibility to provide evidence that the IVD they manufacture is safe and performs as intended.

The quality management standard ISO 13485:2003, Medical devices – Quality management systems – Requirements for regulatory purposes will be used as a benchmark in quality management for the manufacture of IVDs under the proposed IVD framework. This standard is a tool that is used by the IVD manufacturer to demonstrate that they are applying a quality management approach to the design and production of their IVD. It is also used by regulatory bodies to gain formal commitment from a manufacturer that required improvements or corrections to their quality management approach will be implemented.

This presentation looks at how the use of a quality management approach from IVD inception, design and production, through to the release of the diagnostic result will ensure the goal of quality diagnostic testing in Australia.
LONG TERM FOLLOWUP OF DONORS WITH BIOLOGICAL FALSE REACTIVITY ON THE PRISM HTLV-I/HTLV-II CHEMILUMINESCENT IMMUNOASSAY (ChLIA).

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Background: The management of donors who give biological false reactivity (BFR) on screening immunoassays (IAs) remains an ongoing issue for blood services. Donations from these donors must be discarded and donors who repeatedly give BFR results are informed and counselled. The purpose of this study was to followup donors who gave an index BFR result on the PRISM HTLV-I/HTLV-II ChLIA, determine if such donors continued to give BFR results at subsequent donations, and to examine the implications of this data for donor management.

Materials and Methods: At the Australian Red Cross Blood Service all donations are screened for anti-HTLV by the PRISM HTLV-I/HTLV-II ChLIA. Samples that test repeatedly reactive on the PRISM assay are further tested by an alternate immunoassay (Abbott-Murex HTLV I + II EIA). Results for samples that are negative on the alternate IA are defined as BFR and the donor is considered negative for anti-HTLV. Samples that are reactive on the alternate IA are further tested by immunoblot and/or HTLV DNA to clarify the donor’s HTLV status. All donors who gave an index anti-HTLV BFR result between 7 May 1997 and 31 December 2004 were included in this study. All subsequent donations from these donors were identified. Donors who gave a reactive result on the alternate IA were excluded from the study (regardless of the immunoblot result).

Results: During the study period a total of 333 donors gave an index anti-HTLV BFR result. Of these, 227 gave 1 or more subsequent donations and these donors were divided into four groups as follows: (i) 82/227 (35.8%) of these donors gave only negative results at subsequent donations with a mean of 6 (range 1 – 31) negative donations per donor over 25.4 months (3.0 – 90.0); (ii) 50/227 (21.8%) donors gave only BFR results at subsequent donations with a mean of 2 (1-11) over 11.8 (0.25 – 56.0) months; (iii) 45/227 (19.7%) gave one or more subsequent BFR results then gave only negative results with over half (26/42) becoming negative after a single subsequent BFR result and most (35/45) becoming negative after 3 subsequent BFR results; (iv) 52/227 (22.7%) gave intermittent BFR and negative results with approximately half (25/52) giving only 1 subsequent BFR result before giving a series of negative results (mean of 8 negative donations per donor).

Discussion: These results indicate that up to half of all donors who gave an index anti-HTLV BFR result and one or more subsequent donations either gave no further BFR results, only 1 or 2 additional BFR results following which all subsequent donations tested negative, or gave intermittent BFR results with most donations testing negative. Currently donors who consistently give anti-HTLV BFR results are not notified and are eligible to continue donating as their donations can still be used for fractionated plasma products. However, assuming the PRISM HIV O Plus and HCV ChLIAs showed similar results to the HTLV-I/HTLV-II ChLIA, these findings would support the current ARCBS policy of notifying donors only if they give 2 anti-HIV or anti-HCV BFR results within a 12 month period.
THE SUBJECTIVE INTERPRETATION OF SIMPLE/RAPID HIV TESTS: IMPLICATIONS FOR QUALITY.

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Background: The demand for immediate access to knowledge of HIV serostatus has led to increased use of testing strategies utilising simple/rapid (S/R) HIV diagnostic tests at the point-of-care. This has resulted in non-laboratory health staff being trained to perform the tests at Voluntary Counselling and Testing centres (VCT). The move towards testing personnel having minimal training has led to concerns over the adequacy of training and implications for the quality of test results. In a pilot study we investigated the efficacy of using photographed results for the assessment of interpretation of 5 S/R tests.

Materials and Methods: A panel of 5 samples, consisting of dilutions that approached the limit of detection for each of the 5 tests, plus positive and negative controls, were tested and the results photographed. These were sent to laboratories enrolled in NRL External Quality Assessment Scheme (EQAS). Interpretations by 88 Australian (including New Zealand) and 66 international laboratories were returned. Participants were grouped according to both general experience (prior experience with a simple/rapid test) and specific experience (specific to the selected tests). Interpretations discordant with the reference interpretation were analysed by Kruskal-Wallis and Chi-square.

Results: The majority of photographed results were interpreted accurately. However, accuracy of interpretation differed between the five tests (p<0.0001). The results also indicated that both inexperienced and experienced readers could interpret clearly positive and negative results accurately, but that difficulty arose with weakly reactive samples. Laboratory technicians with specific experience were found to interpret with greater accuracy the Abbott Determine™ HIV-1/2 (p=0.034), Trinity Capillus™ HIV-1/HIV-2 (p=0.022) and the Fujirebio Serodia® HIV (p=0.017) tests, compared with less experienced technicians. For those participants with general experience a significant difference in the accuracy of interpretation was found both overall (p=0.032) and for Trinity Uni-Gold™ (p=0.001).

Discussion: We have demonstrated that it may be feasible to establish an EQAS for S/R tests through the use of photographs to monitor the accuracy of interpretation. Both general and specific experience were found to influence the accuracy in interpretation of simple/rapid HIV tests. The present study also elucidates concerns over the interpretation of weakly reactive samples. A greater problem may exist when these results are transferred to poorly trained or inexperienced personnel and the effect this may have on the quality of results reported from S/R HIV tests. This problem may be exacerbated in VCT centres where training of personnel in performance of the tests may be minimal.
Currently, the only In vitro diagnostic devices (IVDs) undergoing extensive evaluations in Australia are those used for the detection of Human Immunodeficiency Virus (HIV) and Hepatitis C Virus (HCV). A new risk-based regulatory system aligned with international best practices is being introduced for all IVDs available for diagnostic testing. The new regulatory framework will encompass a rules-based risk classification system. There will be four risk classes, and the class of the IVD is prescribed by the overall impact on personal risk and public health. Assays with low personal and public health risk are designated Class 1 IVDs, while Class 4 IVDs have a high public health risk. Class 4 IVDs include screening tests to ensure safety of the blood supply and tests for the diagnosis of serious life-threatening transmissible diseases.

When the new IVD framework is implemented, all commercially supplied and in-house assays will be subject to regulation by the TGA, and will undergo a degree of premarket evaluation commensurate with the risk posed by that particular IVD. In-house assays are regarded as any IVDs that are developed de novo; created from or modified from a published source; modified or adapted from any other source; or modified from their intended purpose. In-house IVDs also include commercial assays that are marked for research only purposes but are used by laboratories for a diagnostic application. Extension, by a laboratory, of the intended purpose of a commercial assay, results in that assay becoming “in-house”. A laboratory that produces in-house assays takes on the regulatory responsibilities of an in-house manufacturer.

For laboratories that produce Classes 1 – 3 in-house assays, fulfilment of regulatory requirements will be demonstrated by compliance with the relevant National Pathology Accreditation Council (NPAAC) standards and certification to the International Standards, ISO 15189 or ISO 17025 for Quality Management Systems. In simple terms NATA accreditation will provide satisfactory evidence of compliance with regulatory requirements, therefore the impact of the planned changes for Class 1 – 3 in-house assays in most cases will be minimal. Laboratories producing in-house Class 4 IVDs will be subject to the same regulatory requirements as applied to commercial manufacturers of IVDs.

This poster presents a summary of the new IVD framework and its impacts on the laboratory, outlines the responsibilities of sponsors and manufacturers, and highlights how increased quality of diagnostic products supplied to the Australian market is achieved through closer regulatory scrutiny of the manufacturing process.
WORKING STANDARDS FOR ANTI-HIV

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Introduction. The Division of Retrovirology at the NIBSC produces a number of different biological working standards and reference materials for HIV diagnostic (serological) anti-HIV assays and nucleic acid-based amplification techniques (NAT). In response to the needs of the UK Blood Transfusion Service (UKBTS), the first NIBSC British Working Standard for Anti HIV-1 (BWS) was produced in 1996. UKBTS guidelines state that in each series of tests a positive result should be obtained with the UK anti-HIV-1 working standard. The BWS was formulated to produce a weak positive reaction with most 3rd Generation EIAs, allowing it to be used as a run control or 'go/no go' quality control reagent. In the intervening years we have produced a more dilute BWS for use with more sensitive assays and have introduced working reagents for anti-HIV-2 and anti-HTLV-1. These serological reagents are supplied to blood transfusion laboratories, blood fractionators and diagnostic, research and reference laboratories to monitor test performance and for batch pre-acceptance testing. These working reagents fall within the In Vitro Diagnostics Directive (IVDD) are therefore produced in conformance with ISO 13485 and are CE-marked.

Methods. The BWS is prepared from a masterpool containing 8 confirmed anti-HIV-1-positive plasma donations. Aliquots of masterpool are diluted 1:200 in phosphate buffered saline containing 5% bovine serum albumin and 0.05% Bronidox as a preservative. New batches are tested in three independent assays and release criteria state that the mean OD/CO must fall within ±20% of the frozen baseline value. Reagents are distributed to UKBTS laboratories and several international laboratories. All laboratories are identified by a code number and are requested to submit data, where possible via E mail, to NIBSC on a monthly basis. Quarterly reports are generated for users, showing inter-laboratory comparisons, response/cut-off ratios and precision plots.

At NIBSC, weekly anti-HIV screening assays of plasma pools and blood products destined for use as therapeutic medicines include the BWS as an independent QC reagent. In order for the assay to be valid, the BWS must give an OD/CO value within ±3 SD of the mean value for the previous 12 months of data.

Results. In total 13 batches of BWS has been produced since 1996. We currently produce approximately 3000 vials per batch which are despatched over a 14 month period. Of these batches, 8 have been produced in conformance with the IVDD. During this time no batch has been rejected due to OD/CO ratios falling outside the acceptable limits.

Over 12500 results were returned from 35 laboratories using the BWS between September 05 and February 06. Eleven different commercial assay systems, consisting of a combination of 3" and 4" generation EIA's, were used. The most widely used assay during this period was the Prism Ab-Ag Combo, with 13 labs reporting data from this assay. In general, precision plots showed tight clustering of laboratories with CV’s ranging form 10.3 to 15%. Box plots showed different degrees of variation in kit performance, with the Vironostika Uniform assay showing the greatest variation.

Discussion

The collation and analysis of data over the past 10 years has clearly demonstrated that the NIBSC working standards play a valuable role as quality control materials. Through the regular submission of data to NIBSC and its analysis, we are able to keep laboratories informed regarding their performance and rapidly identify any aberrant results and their possible causes. This also enables NIBSC to keep abreast of the range of diagnostic assays used by the UKBTS and to ensure that we produce QC reagents that meet the needs of our customers.
An International External Quality Assessment Scheme for Hepatitis C Virus Genotyping.

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Background and Objectives

Genetic testing can exhibit variable results. In 2005 the National Serology Reference Laboratory, Australia (NRL) commenced an external quality assessment scheme (EQAS) for genotyping of Hepatitis C virus (HCV). The broad objectives of the EQAS were to assess whether participants were able to obtain genotyping results that were concordant with those of the reference laboratory and to establish whether the choice of genotyping method influenced the genotyping results obtained. The specific aims of the 2005 EQAS were to establish whether participants were able to identify correctly commonly occurring HCV genotypes and subtypes and identify samples that were duplicated within and between EQAS panels.

Methods

The EQAS was offered as an international programme that provided a challenge format of five samples, three times a year. To address the aims the 2005 HCV Genotyping EQAS, participants were supplied with three identical panels that each contained four different samples (one sample was included in duplicate and samples were identified differently in each panel). All stock samples were assigned a genotype/subtype by nucleic acid sequencing of the HCV core gene. Each stock sample was assigned the genotype/subtype of the NCBI reference sequence to which it displayed the greatest homology. All stock samples were diluted in BaseMatrix (Boston BioMedica) to a final concentration of approximately 10,000 IU/mL. All panel members were prepared and dispensed into aliquots at the same time. The samples were stored at –70°C before distribution.

Results

Twenty one laboratories from three countries (Australia, New Zealand and Japan) participated in the HCV Genotyping EQAS in 2005. The participants used commercially-produced and in-house assembled assay systems that targeted the 5’utr, core and env genes. Of the 21 participants, 16 (76%) reported results for all three EQAS panels. Of these 16 participants, 14 (88%) reported the correct genotype for each sample in all three EQAS panels. The incorrect results arose from errors in result transcription. One participant demonstrated difficulties in obtaining reproducible results in all three EQAS distributions using a core/env-based sequencing technique.

Conclusion

The majority of participants demonstrated an ability to deliver reproducible genotyping results for samples having commonly occurring genotypes. However the EQAS also demonstrated that incorrect genotyping results (that could influence significantly a clinician’s decision regarding patient therapy) can arise through simple errors such as inaccurate transcription. The EQAS also highlighted the difficulties that can arise in controlling the quality of assembled assay systems.
**Bland Altman Method Comparison Analysis Demonstrates the Equivalence of Dried Venous Blood to Serum for Quantifying Measles IgG.**

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**Background:**
Seroepidemiological surveys are frequently used to evaluate population based immunisation programs. Collection, storage and transport of serum from areas with poor infrastructure may present logistic difficulties. Dried blood can be collected and transported at room temperature, and is not subject to the regulations of the International Air Transport Authority. Dried blood may thus offer advantages over serum for seroepidemiological studies in resource poor settings.

**Aim:** We aimed to determine if dried venous blood (DVB) samples were an acceptable alternative to serum for the quantification of measles specific immunoglobulin G (IgG).

**Method:** Paired serum and DVB samples were collected from 99 suspected cases of measles and 1152 school aged children surveyed by a 3 stage random cluster sample in Victoria, Australia. The DVB and serum samples were tested using the Dade Behring Anti-Measles-Virus/IgG immunoassay. The optical densities (ODs) of the DVB, prepared under different elution conditions, were analysed for agreement with the ODs of the paired serum samples using a method developed by Bland and Altman.

**Results:** The simplified Bland Altman analysis revealed systematic bias in the OD measurement of highly reactive samples. An adjustment factor, calculated by the square root of the ratio of the regression coefficients, was applied to the OD of DVB to allow quantification of the titre of specific measles IgG. Further Bland Altman analysis of the adjusted DVB ODs and serum ODs showed that adjusted DVB OD values were not significantly different to the serum OD values.

**Conclusion:** Bland Altman analysis of the serum and adjusted dried venous blood ODs established the equivalence of these measurements. Adjusted DVB OD values could be used to calculate measles antibody titres in accordance with the manufacturers protocol. These results demonstrate the potential of DVB samples to be used in place of serum for the investigations of measles immunity.
STRONGYLOIDES SEROLOGY – AN EVALUATION OF A COMMERCIAL ELISA ASSAY

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Introduction: Strongyloidiasis is caused by the intestinal nematode Strongyloides stercoralis. This parasite has global distribution infecting millions of people. The highest prevalence in the world is in Australian remote and rural aboriginal communities. Infection is often asymptomatic and can remain undetected for decades. The filariform larvae penetrate the skin from contaminated soil. Adult worms produce eggs which hatch into rhabditiform larvae. These larvae are passed in the stool or can cause autoinfection by penetrating the intestinal mucosa or the perianal skin. Autoinfection may explain the possibility of persistent infections and hyperinfections in immunodepressed individuals.

Diagnosis of strongyloidiasis is problematic as the parasite load is low and larval output is intermittent. Faecal concentration and culture on agar plates improves sensitivity however antibody detection is considered the most sensitive. Antibody tests cannot differentiate past from current infection. Serologic monitoring may be useful in the follow-up of immunocompetent treated patients. Antibody levels are said to decrease markedly within 6 months after successful chemotherapy. ELISA assays are most sensitive but subject to cross reactivity with other parasites. With commercial kits not previously available, in-house ELISA assays, based on S.ratti antigens have been utilised. A commercial ELISA (IVD Research Inc. USA) incorporating S.stercoralis antigen for the detection of IgG antibodies is now available.

Objective: To evaluate the IVD assay as an alternative to referring samples to an external laboratory.

Method: The IVD Assay was evaluated utilising a panel of 135 sera. This included 41 specificity sera and 94 sera (83 patients) classified by an in-house Strongyloides (S.ratti antigen) assay performed at The Institute of Clinical Pathology and Medical Research (ICPMR), Westmead, Sydney. Specificity samples were positive by either serology or faecal examination for other parasitic infections. Blood donors represented the “normal” population.

Results: Based on 94 classified sera, the assay demonstrated sensitivity of 92% and specificity of 80%. Assay specificity calculated using the specificity panel was 90% (4/41). Combined specificity (classified negatives and specificity panel) is 86%. Eight patients had serial samples collected before and after therapy. The mean time interval to the second collection was 5.4 months (1month – 1.3 years). In both assays 7/8 patients with paired sera demonstrated a significant decrease in antibody level for the second sample. Inter-run reproducibility for the positive control of one kit batch (4 runs) was 1.4%. Intra run reproducibility calculated on 2 patients (8 replicates each) was 8.5%. There were 15/95 (16%) IVD results that were discordant when compared with ICPMR. Technical issues encountered include low reagent volume, a non-human positive control containing antibodies at saturated levels and a set absorbance cut-off that does not take into account varying assay conditions. If required the assay could be performed using minimal equipment (dropper bottles, room temperature incubation). The kit insert indicates results can also be interpreted visually however this would be inaccurate as there is no cut-off control for colour comparison.

Conclusion: The IVD kit compared favourably with the ICPMR assay. Only one IVD result appeared to be inconsistent with the clinical background. The discordant analysis suggests the assay is more sensitive and is a suitable alternative to the ICPMR assay. The specificity of the assay must be taken into consideration when interpreting the results.
MALARIA ANTIBODY TESTING – A SAFE AND EFFICIENT STRATEGY TO MINIMISE THE RISK OF TRANSFUSION TRANSMITTED MALARIA

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Introduction: Malaria is a Protozoan infection that is almost always transmitted by the bite of the female *Anopheles* mosquito however can be acquired by direct inoculation with infected blood. Transfusion Transmitted Malaria (TTM) remains a rare but potentially fatal complication of blood transfusion with the last case in Australia recorded in 1991. Until recently and owing to the lack of a high-throughput laboratory test, screening for malaria in blood donors invariably involved collecting a comprehensive medical and travel history and excluding the potentially infectious ‘cellular’ blood components (red blood cells and platelets) from donors with a potential malaria exposure. Although this strategy minimised the residual risk of TTM in Australia to less than 1 in 10 million, it came at a substantial cost resulting in the unavailability for transfusion of approximately 35,000 red cell units per annum. In July 2005 the Australian Red Cross Blood Service implemented an alternative strategy based on screening donors with potential malaria exposure for malaria antibodies. In order to minimise the potential for ‘false negatives’ as a result of donors in the antibody ‘window period’, the protocol requires that testing to be conducted a minimum of 4 months after the last potential risk exposure.

Methods: Since July 2005, donations from blood donors identified as having had malaria and recovered, as well as those having travelled to or resided in a malarial country within the last 3 years were eligible for testing. Red blood cell units from these donors were collected and quarantined and samples tested using the Newmarket malaria antibody EIA (Newmarket Laboratories, Newmarket, UK). All donations returning a non reactive EIA result were defined as ‘malaria free’ and the quarantined components considered for transfusion. The associated donors were also re-instated for cellular component manufacture at their next donation. Reactive donations were re-tested in duplicate on the Newmarket EIA and where repeatedly reactive (RR) the donations were not issued for transfusion. RR donors were subject to supplementary testing by a malarial antigen immuno-chromatographic test (NOW ICT Pf/Pv. antigen assay, Binax Inc, Portland, USA) and a real time plasmodial PCR assay (*Realart* malaria PCR, Artus GmbH, Hamburg, Germany). Donors without reactivity on either test were considered ‘antibody positive’ without evidence of current infection. Donors reactive on either or both supplemental tests were considered potentially infected and referred immediately for clinical assessment.

Results: A total of 35,365 samples were tested using the Newmarket EIA between July 18, 2005 and 30 June 2006 of which 34,493 (97.5%) were non reactive and the remaining 872 (2.5%) RR. Of the 872 RR samples, one was weakly positive for *P. falciparum* on the NOW ICT Pf/Pv. antigen assay but plasmodial DNA was undetectable by PCR. The donor, who migrated from India in November 2005 had a history of malaria during childhood but was completely asymptomatic at the time of follow up. The donor was referred to an infectious disease specialist and a fresh blood sample was taken which showed no evidence of parasitemia on thick or thin blood film and was negative using the NOW ICT Pf/Pv. antigen assay.

Conclusions:
1. The RR rate for the Newmarket malaria EIA of 2.5% compares well with that predicted from an ARCBS pilot study of 2.3% (17/751).
2. One of the 35,365 samples tested showed evidence of circulating malaria parasites however the result was not reproducible on follow up indicating the likelihood of a ‘false’ positive original test. Assuming the alternative i.e the donor was parasitaemic, then the incidence of asymptomatic parasitaemia in blood donors is at most 1/35,364.
3. The testing protocol resulted in an additional 34,493 red cell donations with negative EIA test results being issued for transfusion. Donors re-instated by the negative EIA made a further 7,281 donations during the period of their original restriction which would also have been ineligible for transfusion.
4. The outcome is a marked efficiency dividend with a net gain of almost 42,000 red cell units available for transfusion from the same number of blood donors. Although it is too early to be definitive the lack of a reported TTM case since implementing the new strategy indicates that the improvement in efficiency has been achieved without impacting recipient safety.
PERFORMANCE OF THE ABBOTT ARCHITECT HIV Ag/Ab ASSAY IN A LOW RISK POPULATION WITH COMPARISONS TO THE ABBOTT AXSYM COMBO ASSAY

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Introduction: SNP laboratory services a population at low risk for Human Immune Deficiency Virus (HIV) infection. Combined HIV Antigen / Antibody testing has been used over the last 2 years for routine HIV screening in our laboratory, initially using the Abbott Axsym Combo assay, and since July 2005 the Abbott Architect Combo assay.

Objectives: To review the performance of the HIV Architect assay over the last 12 months, and to compare its performance with the previously used Axsym Combo Assay, and the Axsym antibody only assay.

Methods: Routine samples submitted for HIV testing were analysed using the Abbott Axsym Combo assay from January 2005 to July 2005, and then on the Abbott Architect to the present time. Prior to 2005 the Axsym HIV1/2gO assay was used. All initially reactive samples were repeated in duplicate on a previously stored sample, and repeat ably reactive samples were forwarded to QHPS laboratories for follow up by Western Blot and p24 antigen testing.

Results: The repeatable reactive rate was very similar between the three assays. For Architect Combo it was 0.28% (225 samples), for Axsym Combo 0.29% (93 samples), and Axsym HIV 1/2 0.28% (175) in 12 months preceding introduction of Combo assays. Some of these samples were repeat specimens on the same patients. All reactive samples were forwarded to QHPS for confirmatory testing. For the Architect Combo assay, 61 samples were confirmed positive by Western blot. A further 6 samples were p24 antigen only positive, and 6 were Indeterminate group 4 and p24 positive, giving a positive rate of 0.092% in the samples tested. This represented 32% of our repeat reactive samples. Of the 152 false positive samples, 116 were Western blot non-reactive, p24 antigen non-reactive. These included 82 with S/CO < 2.0, 26 with S/CO between 2.0 and 10.0, and 8 samples with S/CO > 10.0. Of this last group, 4 samples had S/CO > 300.0. A further 40 samples were returned as indeterminate (6 group 1, 7 group 2 and 27 group 3). These results give a specificity of 99.81%. For the Axsym Combo assay, 39 were confirmed positive by Western blot, a further sample was p24 antigen only positive, and 3 samples indeterminate group 4 and p24 positive, giving a positive rate of 0.136% during this period. Of the 50 false positive results, 38 were Western Blot negative, p24 negative. Thirty one (31) of these had S/CO < 2.0 and 7 had S/CO between 2.0 and 4.6. During the first year of use, the Architect Combo assay identified 6 patients as reactive who were only p24 antigen positive, and would have been negative in the HIV 1/2 antibody assay. In the previous 6 months the Axsym Combo assay identified 3 similar patients who were only p24 antigen positive. A number of the repeatable reactive samples were run on both the Axsym and Architect Combo assay, all of the confirmed reactive samples were reactive in both assays. Of the reactive samples on the Architect Combo Assay that were not confirmed by Western Blot or p24, as true HIV positive; 40 % of these initial repeatable reactive were non-reactive on the Axsym Combo assay and negative by Western Blot. A further 20% of repeat reactive Architect samples, were negative by Axsym and returned as indeterminate groups 1,2 or 3 on Western Blot.

Conclusion: The performance of the Architect HIV Ag/Ab Combo assay has more than met our expectations. The specificity seen has been as expected. The percentage of repeatable reactive specimens by the three screening assay formats was the same in the population tested over time, but it appears that repeat reactive results on the Architect Combo assay comes from a different group of specimens to that seen on the Axsym Combo assay. This difference is probably due to the different antibody formats used in the two assays. The majority of the false positive results had initial S/CO of <10.0 on the Architect, although two samples had levels > 300.0 on repeat collections.
INTRODUCING THE COBAS® AmpliPrep AND COBAS® TaqMan® 48 SYSTEM – AN AUTOMATED SOLUTION FOR THE MOLECULAR LABORATORY

Burnside J. M.
Roche Diagnostics Australia

The COBAS® AmpliPrep together with the COBAS® TaqMan® 48 provides real-time PCR technology coupled with automation of the PCR process, from sample preparation through amplification and detection, to calculation of results. The COBAS® AmpliPrep/COBAS® TaqMan® 48 System delivers high throughput capability, minimum hands-on-time and reduced contamination risk\(^1\). Broad dynamic ranges offer potential improvements in clinical utility for HIV-1\(^2\), HCV\(^2\), and HBV viral load testing\(^1\). Genotype and subtype inclusivity allows for worldwide utility of the assay\(^1\).

The COBAS® AmpliPrep Instrument automates the sample preparation process, replacing time-consuming manual preparation. Once the samples and reagents are loaded onto the system, the operator is free to perform other tasks. The workflow is greatly simplified with minimum instrument setup time and ready-to-use reagents. COBAS® AmpliPrep allows multiple tests and continuous loading with up to 72 samples onboard at one time. Additional features of the COBAS® AmpliPrep include a closed sample tube format, incorporation of quantitation standards throughout the entire assay process combined with clot detection and volume input surveillance. The COBAS® AmpliPrep platform provides a flexible, reliable and contamination-free method of automated sample preparation.

AMPLILINK software manages the overall operation and maintenance of the COBAS® AmpliPrep/COBAS® TaqMan® 48 System. Configuration management tools allow the laboratory to customise the functionality of the System. Full sample tracking by automated barcode reading ensures no transcription errors. The AMPLILINK software delivers an easy to read graphic display of samples and instrument status, with the capability for bidirectional interfacing to laboratory information systems.

Performance data on the sensitivity, linear range, specificity, genotype and subtype inclusivity of the HIV-1\(^2\), HCV\(^2\) and HBV tests will be presented, along with correlation data with other platforms and technologies.

1. COBAS AmpliPrep/COBAS TaqMan 48 package inserts
2. Pending TGA approval

Roche, COBAS, COBAS AmpliPrep, AmpliPrep, AMPLILINK, COBAS TaqMan and TaqMan are trademarks or registered trademarks of a member of the Roche Group.
USE OF REAL TIME DNA PCR FOR THE DETECTION OF HTLV-I AND -II INFECTION.

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Introduction: The National Serology Reference Laboratory (NRL) provides screening and confirmatory testing for blood-borne pathogens, such as HTLV-I and HTLV-II. There are times when it is not possible to interpret an individual’s infection status from serological testing alone.

The NRL has developed a multiplex real time PCR assay for the detection of both HTLV-I and HTLV-II proviral DNA, in conjunction with an automated nucleic acid extraction method, for routine diagnostic use.

Objectives: To develop a real time PCR multiplex to simultaneously detect HTLV-I and HTLV-II DNA, which maintains the sensitivities of the previous in-house PCR assay, which was based on conventional PCR.

Methods: Nucleic acid extraction was performed using the Roche MagNA Pure LC DNA Isolation Kit. Real time PCR was carried out using Stratagene’s MX3000P. Primers and Taqman probes were chosen for their ability to detect a wide range of subtypes. The assay includes an internal control using cellular primers and probes for the human albumin gene.

Separate real time PCR assays were developed for both HTLV-I and HTLV-II. Primers and probes, as well as PCR conditions were optimised to provide maximum sensitivity, specificity and reproducibility in each assay. Following the optimisation of each single-analyte assay, primers and probes for HTLV-I and HTLV-II and the human albumin gene were combined in a multiplex assay. Adjustments to the reagents and PCR conditions were made to maintain the optimum levels of specificity and sensitivity.

Evaluation of the sensitivity of the NRL HTLV multiplex real-time DNA PCR assay was conducted by testing DNA extracted from 29 clinical samples from HTLV infected individuals. Specificity of the multiplex assay was estimated by testing DNA extracted from 208 specimens from uninfected individuals.

The reproducibility of the multiplex assay was assessed by determining the coefficient of variation (CV%) between the threshold thermal cycle (Ct) values for seven five-fold dilutions of the HTLV-I and HTLV-II proviral standards and for six five-fold dilutions of the cellular (albumin) standards.

Results: Twelve specimens from individuals infected with HTLV-I and 17 specimens from individuals infected with HTLV-II were assayed using the NRL’s HTLV multiplex real time DNA PCR assay. All of the HTLV positive specimens were reactive by the HTLV multiplex assay. No viral DNA was detected in any of the 208 sero-negative samples; the presence of extracted DNA was verified by the detection of the internal control gene in all samples.

The HTLV Real Time PCR multiplex successfully amplified and detected the HTLV-I and HTLV-II standards. The coefficient of variation (%CV) ranged between 2.08 and 9.03% for the Ct values determined for HTLV-I, HTLV-II and human albumin amplifications.

Discussion: Development of the HTLV multiplex real-time DNA PCR at the NRL maintains the NRL’s ability to detect HTLV DNA with comparable sensitivity and specificity to that previously available using the original in-house method. Not only did the Real Time PCR multiplex successfully amplify and detect the viruses of interest, it also did so in a fraction of the time required by conventional PCR.
PROCLEIX<sup>®</sup> TIGRIS<sup>®</sup>/ULTRIO<sup>®</sup> EVALUATION: THE ARCBS EXPERIENCE WITH FULLY AUTOMATED NUCLEIC ACID TESTING.

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<sup>1</sup>Australian Red Cross Blood Service, Australia

The Australian Red Cross Blood Service (ARCBS) commenced human immunodeficiency virus type 1 (HIV-1) and hepatitis C virus (HCV) nucleic acid testing (NAT) in June 2000 using the Chiron PROCLEIX<sup>®</sup> HIV-1/HCV (Multiplex) assay. This duplex assay, run on the Chiron semi-automated (SAS) testing platform, was implemented using a combined testing strategy of individual donor testing (IDT) and pooled donor testing (PDT) across five testing sites.

Following 6 years of routine screening at the ARCBS, the PROCLEIX<sup>®</sup> HIV-1/HCV assay and its semi-automated testing platform have proven to be reliable with excellent operational performance. However, the technology has remained restricted to the detection of HIV-1 and HCV RNA and remains very labour intensive despite upgrades to its equipment and software. More recently, advances in NAT technology have seen the development of ‘second generation’ NAT assays on fully automated testing platforms that now include the simultaneous detection of HIV-1 and HCV RNA along with HBV DNA. One such candidate system is the Chiron PROCLEIX<sup>®</sup> ULTRIO<sup>®</sup> assay (HIV-1, HCV and HBV) using the PROCLEIX<sup>®</sup> TIGRIS<sup>®</sup> platform.

During evaluation of this assay/platform combination, the 95% detection limits were determined using serial dilutions of WHO standards. These were found to be 42.2 IU/mL for HIV-1, 2.0 IU/mL for HCV and 12.2 IU/mL for HBV. Testing of 10397 Hong Kong donor samples by IDT was performed to assess the operational performance of the TIGRIS. In addition to a daily test throughput and workflow analysis, the calculated test performance characteristics were compared to those produced by the PROCLEIX<sup>®</sup> HIV-1/HCV assay on the enhanced semi-automated platform (eSAS) currently in routine use at the ARCBS.

The test performance comparison showed:

<table>
<thead>
<tr>
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<th>ULTRIO &amp; TIGRIS</th>
<th>HIV-1/HCV &amp; eSAS (June – August 06)</th>
</tr>
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<tbody>
<tr>
<td>False Reactive Test Rate</td>
<td>0.10%</td>
<td>0.15%</td>
</tr>
<tr>
<td>Invalid Test Rate</td>
<td>0.05%</td>
<td>0.14%</td>
</tr>
<tr>
<td>Failed Run Rate</td>
<td>2.92%</td>
<td>1.98%</td>
</tr>
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The TIGRIS<sup>®</sup> demonstrated a high degree of system automation and enables high throughput IDT blood donor screening and a greater flexibility in results availability. The TIGRIS<sup>®</sup>/ULTRIO<sup>®</sup> assay combination was found to be reliable with an operational performance that was comparable to that of the current PROCLEIX<sup>®</sup> HIV-1/HCV assay on the eSAS system but with the addition of simultaneous HBV DNA detection.
EVALUATION OF THE bioMerieux NucliSENS easyMAG NA PLATFORM

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Introduction: The bioMerieux NucliSENS easyMag NA platform (eM) is an automated system for extraction of nucleic acids from clinical samples based on silica extraction technology.

Aim: To evaluate the bioMerieux NucliSENS easyMAG NA platform and another automated extraction system (Roche MagNA Pure LC (MPLC)) focusing on: nucleic acid recovery, preparation and extraction times for various run sizes, consumables and reagents required, software features, ease of use and environmental considerations.

Methods: A total of 97 samples comprising routine clinical samples and EQAP samples representing various specimen types were extracted by both platforms. Samples were extracted on the eM using the generic protocol with 200 µL sample and a final elution volume of 55 µL. Samples were extracted on the MPLC using the Total NA – Variable Elution Volume protocol with 200 µL sample and a final elution volume of 50 µL. Extraction runs on both platforms were in run sizes of 8, 16 or 24 samples and controls. Extracts were then analyzed using LightCycler real-time PCR assays for either HSV 1 and 2 DNA, VZV DNA or porA DNA (N. gonorrhoeae confirmatory assay).

Results: HSV DNA was detected in 29 clinical samples in extracts from both platforms. For HSV EQAP samples, 10 samples extracted on the eM were detected, compared to 9 extracted on the MPLC. VZV DNA was detected in 1 clinical sample from extracts from both platforms, and 5 EQAP sample extracts from both platforms were detected. N. gonorrhoeae DNA from routine samples was detected in 3 MPLC extracts, whereas only 2 eM extracts were detected. 5 EQAP samples had N. gonorrhoeae DNA detected from extracts from both platforms. The crossing points (Ct) for eM extracts were less than MPLC extracts in >90% of samples (range 4.46 cycles earlier to 0.67 cycles later), indicating an increased NA recovery for the eM in most instances. Extraction times were less for the eM, with the run time for 24 extractions being 47 minutes compared to 83 on the MPLC.

The eM required 9 plastic consumables/24 samples compared to 87 on the MPLC. eM required fewer reagents which remained on board between runs. The eM software was touch screen, with reagents and consumables bar coded and scanned into position. Reagent use is monitored and each sample in a run can be of a user defined volume, with different (software defined) elution volume. The width and depth of both platforms are identical, with the weight of the eM being 125 kg (MPLC = 151 kg). The height of the eM is 530 mm (830 mm with reagents compartment lid open) while the height of the MPLC is 890 mm (1500 mm with lid open).

Conclusions: The NucliSENS easyMAG consistently extracted higher yield NA than MPLC in more than 90% of clinical and EQAP samples representing a variety of specimen types. The eM has user-friendly and intuitive software with sample tracking and reagent ID via bar code scanner. There is a single generic extraction protocol requiring a single set of reagents that remain on-board. The eM was found to have a high throughput with fast turn-around times for extraction runs of all sizes. Limited data was generated with regard to the effectiveness of eM for removal of inhibitors and possible interfering factors and more extensive studies would be required.
2nd INTERNATIONAL STANDARD FOR HIV-1 RNA

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Introduction. Studies at the NIBSC in the early 1990s to examine PCR sensitivity in a range of assays highlighted the incidence of false reporting of positive and negatives; in one study only 46% of laboratories returned the expected results. This, combined with the rapid development of commercial assays for HIV RNA nucleic acid-based amplification techniques (NAT) and the appreciation of the scope of this new technology by many diagnostic and research laboratories, led to a decision that there was a need for HIV-1 reference materials. As a WHO collaborating centre, NIBSC developed the 1st International Standard for HIV-1 RNA, as well as three HIV-1 RNA working reagents and an HIV-1 RNA Genotype Reference Panel.

The 1st International Standard for HIV-1 RNA NAT (NIBSC code 97/656), was established by the Expert Committee on Biological Standardisation (ECBS) in 1999 and has since been extensively used for the calibration and validation of assays and the calibration of secondary standards such as working reagents. However, due to low stock levels and the discovery of the presence of HBV DNA within the lyophilised material, the 1st International Standard was replaced by the 2nd International Standard for HIV-1 RNA in 2005.

Methods. Eight laboratories from 6 countries participated in an International collaborative study to evaluate the 2nd International Standard for HIV-1 RNA. Participants were sent three vials of the candidate replacement standard (one of the candidate standards evaluated in the original study to establish an International Standard for HIV-1 RNA) along with three vials of the 1st International Standard; vials were labelled sample 1 and sample 2 respectively. Both samples were lyophilised preparations and participants were asked to reconstitute one vial of each reagent just prior to use. Participants assayed one vial of each reagent in three independent assays. In the first assay, each sample was tested neat and at 10 fold dilutions up to $10^{-4}$ to determine an end point. In the two subsequent assays, participants performed half log dilutions around the end point ascertained in assay one. The relative potency of the candidate standard relative to the 1st International Standard was calculated as the difference in estimated log ‘detection units per ml’ ($XX – YY$).

Results. Overall eight laboratories reported results from eight different commercial and in-house NAT systems, 5 (63\%) of which were from quantitative assays and 3 (37\%) from qualitative assays. Of the qualitative assays, different extraction volumes and methods were used and this was taken into account during data analysis and calculation of potency in log$_{10}$ IU/ml. Laboratory mean estimates (log$_{10}$ copies/ml) were calculated for both data formats, the mean estimates for the quantitative assays were based on the geometric mean estimates of copies/ml across dilutions and across assays. In the case of qualitative assays, mean estimates were calculated from the series of number positive out of number tested. For the purpose of assigning an IU to the proposed candidate standard, values obtained in this study were compared and combined with those seen in the original study to establish the 1st International Standard. The overall potency of the proposed 2nd International Standard for HIV-1 RNA was 5.56 Log$_{10}$/ml.

Conclusion. The results of the study show the proposed candidate material (sample 1) to be suitable for establishment as the 2nd International Standard for HIV-1 RNA. Sample 1 showed no evidence of drift in overall detectable titre between this and the original study, indicating that there were no problems with stability. Based on the results of the current and previous collaborative studies, NIBSC proposed that sample 1 (NIBSC code 97/650) should be established as the 2nd International Standard for HIV-1 RNA NAT assays and assigned a unitage of 5.56 log$_{10}$ (363,078) IU/vial. This proposal was endorsed by the ECBS in autumn 2005; the 2nd International Standard for HIV-1 RNA is now available from the NIBSC.
A MATRIX ORGANISATIONAL STRUCTURE TO ACHIEVE QUALITY MANAGEMENT IN A MULTIDISCIPLINARY MOLECULAR DIAGNOSTIC LABORATORY

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Introduction: In 2004, a plan was developed to rationalise the delivery of metropolitan serology, virology and molecular diagnostic (SVM) services in Queensland Health Pathology Service (QHPS) to the Royal Brisbane and Women’s Hospital (RBWH) site. In mid 2005 SVM staff and most of the work from the other two major metropolitan QHPS laboratories, Princess Alexandra Hospital and The Prince Charles Hospital, transferred to the RBWH. In February 2006 SVM services moved into the new QHPS-Central laboratories located at the RBWH campus and were split over three floors. The Molecular Diagnostic Unit (MDU) moved into purpose built laboratories, known as the Molecular Suite, sharing facilities with molecular units from other pathology disciplines (Microbiology, Immunology, Haematology and Chemical Pathology). A matrix organisational structure was employed to achieve quality management in this facility.

Methods: In 2004 SVM was a single operational unit with staff reporting to one supervisor. Following the centralisation of services in 2005, SVM was split into two work units, serology and MDU/virology, each with a separate supervisor. In 2006, following the move into QHPS-Central, a manager was appointed to manage the Molecular Suite. A matrix organisational structure has evolved to suit the diverse requirements of the work groups using this facility.

Results: The matrix structure created dual lines of authority. Staff in the Molecular Suite have two managers – their discipline supervisor and the suite manager. The discipline supervisor manages the human, financial and physical resources of the work unit and supervises its scientific and technical operations. The Molecular Suite manager, in consultation with the discipline supervisors, manages the core services of the facility including management of equipment and resources, maintenance of instruments and quality checks, booking schedules for instruments, rooms and core services, supply of common consumables, cleaning of the facility, contamination control policies and procedures, and maintenance of a program to meet with accreditation and certification requirements.

Discussion: A strength of the matrix structure is its ability to facilitate coordination of multiple complex interdependent activities. The direct and frequent contact between different specialities in the matrix can make for better communication and more flexibility. The dual lines of authority reduce the tendency of staff to protect their own work units at the expense of the organisation’s overall goals. The matrix facilitates the efficient allocation of specialists. It also achieves economies of scale by providing the organisation with both the best resources and an effective way of ensuring their efficient deployment. The major disadvantages of the matrix structure lie in the confusion it creates, its propensity to foster power struggles, and the stress it places on individuals. It can also lead to problems where rapid decision-making and conflict resolution are required. Despite a few disagreements between the diverse work units in the Molecular Suite, the matrix organisational structure appears to be a success six months after its inception.
Toxoplasma gondii infection is common in many species of livestock. Clinical Toxoplasmosis in cattle has been debated, whether T. gondii can cause clinical disease and abortions in cattle. In present study serum samples from 195 problem animals (86 deshi cows, 61 cross bred cows and 48 buffaloes) with reproductive disorders and 10 serum samples each from desi cows, crossbred cows and buffaloes having normal breeding history were screened for detection of toxoplasma antibodies using IHA Test. A titre of 1:18 or more was taken as positive for toxoplasma infection.

Only 11 serum sample out of 147 serum samples from desi cows and crossbred cows could be found positive for toxoplasama antibodies; whereas toxoplasama antibodies were detected in 30 out of 48 serum samples of buffaloes. Higher percentage of buffaloes carrying toxoplasama antibodies indicates the susceptibility of buffalo species for toxoplasma infection. Two serum samples from 30 apparently normal buffaloes also gave positive reaction. This study reveals the importance of toxoplasmosis in cattle and buffaloes, which as less known previously and further economic importance of this disease is being established in these animals.