WEDNESDAY 26\textsuperscript{TH} AUGUST 2009

THE IMPACT OF THE AUSTRALIAN NRL ON THE CANADIAN NATIONAL LABORATORY FOR HIV REFERENCE SERVICE – A QUALITY ACT

Kim J
National Laboratory for HIV Reference Services – Public Health Agency of Canada

The road to hell is paved with good intentions and so, as it is with implementation of quality systems, laboratories may want to do the right thing but still never reach that objective. This presentation describes the course of events and the trials and tribulations that the National Laboratory for HIV Reference Services (NLHRS) experienced in its path towards attaining official ISO 15189 accreditation. Currently the NLHRS remains the only lab in Canada to have this designation. One of the initial challenges in the early days was to overcome reluctance from many (including myself) as to why we really needed this in the first place. Many of the challenges and obstacles described will be familiar to those who have implemented quality management systems in their own facility and one purpose here will be to share in their experiences as well. This presentation and the discussion generated may be especially informative to those who are currently in the midst or considering implementation of quality systems in their own areas. The influence of the Australian NRL will be highlighted in this regard and additional examples will be shown where they continue to have had an influence on other quality activities, within the Canadian-NLHRS. One example shown will be in the area of external quality control monitoring in which a Canadian program, based on expertise from the Australian NRL led to major manufacturing changes and implementation of additional quality control measures in a major manufacturer of HIV EIA kits.
MEDICAL LABORATORY SCIENCE IN PACIFIC ISLAND COUNTRIES

Elliot J
Pacific Paramedical Training Centre, Wellington, New Zealand

This presentation will give an overview of the practice of medical laboratory sciences in Pacific Island countries. It will include a review of the training available for laboratory staff, the range of laboratory services provided and work that various organisations are doing and planning, to assist these laboratories improve the quality of the services that they provide."
STRENGTHENING THE QUALITY OF LABORATORY TESTING THROUGH PARTNERSHIP

Chalermchan W
National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Thailand

Objectives: To support the National HIV Prevention and Care Programs and to respond to quality policies in health laboratories of Ministry of Public Health Thailand, many quality assurance (QA) programs for HIV testing have been developed.

Method: The Thai National Institute of Health, Department of Medical Sciences, Ministry of Public Health Thailand has performed HIV test kit evaluations since 1994 to ensure the performance of test kits distributed within country are of a high standard. After many years of experience in pre-marketing control of test kits, the batch verification testing program is planning to implement the control at the post licensure stage. In 1999, the anti-HIV External Quality Assessment Scheme (EQAS) was established through the partnership with four Regional Medical Sciences Centers located in different regions all over the country. The EQAS is now expanded to cover HIV viral load, anti-HCV and recently HIV drug resistance. Since 2006, quality control (QC) samples have been prepared for more than 30 anti-HIV assays and supplied to more than 800 laboratories within the country. The QC program provides the basis for laboratories to fulfill their quality management requirements. All QA programs were implemented through the network within and between organizations. The international organizations such as World Health Organization, National Reference Laboratory, Australia and Central Diseases Control-Global AIDS Program also provided strong support with technical and financial assistance for the implementation of these programs.

Results: The three HIV QA programs are currently sustainable by continual supportive funding from the National Health Security Office. The implementation process through networking has facilitated the establishment, improvement and sustainability of the programs.

Discussion: The large national QA program is usually resource intensive and hard to sustain at a high level of performance. Working in partnership has made the establishment of the QA program faster, easier and with a high level of performance quality.
WEDNESDAY 26TH AUGUST 2009

ELISA SEROLOGY

Hueston L
Arbovirus and Emerging Diseases Laboratory
NEW IVD POLICY

Tang S
Therapeutic Goods Administration
Analysis of PRISM HCV Chemiluminescent Immunoassay Sample to Cutoff Ratios in a Blood Donor Population Highlights the Importance of Anti-HCV Confirmatory Testing

Walker K, Kiely P, Parker S and Cheng A
Australian Red Cross Blood Service, Melbourne, Brisbane and Perth, Australia

Introduction: The Australian Red Cross Blood Service (ARCBS) screens all donations for hepatitis C virus (HCV) both serologically for anti-HCV, and by nucleic acid testing (NAT) for HCV RNA. A final HCV status is assigned on the basis both anti-HCV serology (screening and confirmatory testing) and NAT results. However, a number of reports have suggested that, for samples with serological reactivity but non-reactive (NR) by NAT, an anti-HCV status can be assigned solely on the basis of screening immunoassay (IA) sample to cutoff (s/co) ratios without the need for serological confirmatory testing.

Objective: The primary aim of this study was to analyse the s/co ratio distributions for anti-HCV biological false reactive (BFR), indeterminate and confirmed positive results to determine whether screening IA s/co ratios are a reliable indicator for assigning an anti-HCV status in our donor population.

Methods: According the ARCBS Viral Testing Algorithms, the HCV/anti-HCV status of samples that test repeatedly reactive (RR) on the primary screening IA, Abbott PRISM HCV chemiluminescent immunoassay (ChLIA), is defined as follows. Anti-HCV BFR: NR on the secondary anti-HCV IA (Abbott-Murex anti-HCV EIA v4.0), not tested by immunoblot (Chiron RIBA-3) and NR on the NAT assay (Chiron Procleix); anti-HCV indeterminate: RR on the anti-HCV secondary IA, NR on the NAT assay and indeterminate on RIBA-3; anti-HCV confirmed positive: RR on the secondary IA, NR on the NAT assay, positive on RIBA-3; HCV confirmed positive: RR on the secondary IA and reactive on the NAT assay (RIBA-3 not required). For the period 07/06/00 to 30/06/08, all donor samples from 3 Australian Blood Services (Melbourne, Brisbane and Perth) that tested anti-HCV BFR, anti-HCV indeterminate, anti-HCV confirmed positive or HCV confirmed positive were included in the study. The s/co ratio distributions of all 4 result categories were analysed and compared.

Results:

<table>
<thead>
<tr>
<th>S/CO</th>
<th>Anti-HCV BFR</th>
<th>Anti-HCV indeterminate</th>
<th>Anti-HCV confirmed positive</th>
<th>HCV confirmed positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
<td>%</td>
</tr>
<tr>
<td>1.00-2.00</td>
<td>2098</td>
<td>84.09</td>
<td>154</td>
<td>66.09</td>
</tr>
<tr>
<td>2.01-3.00</td>
<td>340</td>
<td>13.63</td>
<td>55</td>
<td>23.61</td>
</tr>
<tr>
<td>3.01-4.00</td>
<td>47</td>
<td>1.88</td>
<td>19</td>
<td>8.15</td>
</tr>
<tr>
<td>4.01-5.00</td>
<td>8</td>
<td>0.32</td>
<td>2</td>
<td>0.86</td>
</tr>
<tr>
<td>5.01-6.00</td>
<td>2</td>
<td>0.08</td>
<td>1</td>
<td>0.43</td>
</tr>
<tr>
<td>6.01-8.00</td>
<td>0</td>
<td>0.00</td>
<td>1</td>
<td>0.43</td>
</tr>
<tr>
<td>8.01-10.00</td>
<td>0</td>
<td>0.00</td>
<td>1</td>
<td>0.43</td>
</tr>
<tr>
<td>10.01-14.01</td>
<td>0</td>
<td>0.00</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Total</td>
<td>2495</td>
<td>100.0</td>
<td>233</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Discussion and conclusion: The results showed that high s/co ratios (>5.00) were predictive of anti-HCV or HCV confirmed positive results and values less than 3.00 were predictive of anti-HCV BFR and anti-HCV indeterminate results. However, there is overlap between the s/co ratio distributions of all four result categories so that high s/co ratios were not always indicative of confirmed positive results and low values did not always indicate BFR or indeterminate results. These results highlight the importance of anti-HCV confirmatory testing before assigning a final anti-HCV status for donations that test RR on the primary anti-HCV screening IA but without concomitant HCV NAT reactivity.
DONOR DISCLOSURE — AN INDIAN EXPERIENCE

Dontula S., Mathur A., Kamaladoss T., Siddappa, Jagannathan L.
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HAL 3rd stage, Thippasandra Main Road, Bangalore 560067, India

Introduction: Rotary TTK Blood Bank, Bangalore Medical Services Trust, collects on average 22,000–25,000 blood units per annum from voluntary donors. Each blood unit is screened for HIV 1 and 2, anti HCV, HBs antigen using the fourth generation EIA, and for malarial parasite & syphilis. After the National Blood Transfusion Council of India permitted the disclosure of viral Transfusion Transmissible Reactivity (TTI) to the blood donor in 2004, we have set up a stringent donor disclosure programme. This study describes our pioneering experiences in donor disclosure and counselling during the period 2007–2008 and the particular challenges associated with them in a resource-limited environment.

Objective: To assess the success rate of donor disclosure. To study benefits of counselling to the donor and society.

Methods: All of Rotary TTK Blood Bank’s donors are voluntary; 40% of them are repeat donors. Blood units are screened as per our testing algorithm. The units that test repeat reactive for the viral TTI are discarded and the donor is informed of his/her reactive status. These donors are requested to attend confidential counselling session(s) at our blood bank.

At the time of donation, donors fill out a detailed questionnaire that contains specific questions on sexual risk behaviour. In the counselling session, the donor is informed about his/her TTI test result and its significance. The counsellor also tries to elicit more information on the donor’s sexual risk behaviour. The donor is advised on lifestyle changes and impact on immediate family. Finally, the donor is encouraged to get confirmatory tests done and are recommended to consult a physician or gastroenterologist.

Results: The number of units collected and tested at our blood bank in 2007 and 2008 were 21,700 and 22,479 respectively. Viral TTI reactivity at Rotary TTK blood bank in 2007 was 0.12%, 0.78%, and 0.18% for anti HIV, HBs Ag and anti HCV. In 2008, it was 0.16%, 0.73%, and 0.18% for anti HIV, HBs Ag and anti HCV.

Amongst donors who tested reactive for HBs Antigen in 2007, 58.33% attended counselling. Amongst donors who tested reactive for anti HIV and anti HCV, 42.87% and 46.15% attended counselling respectively. In 2008, 63.70%, 37.50% and 30.00% attended counselling for HBs Antigen, anti HIV and anti HCV.

Conclusion: Our first experiences with the donor disclosure programme have been rewarding and positive in that it directly benefited the donor and their families. Most of the hurdles faced thus far in this programme were related to logistics of donor follow-up. There is also need for more awareness in India on the correlation between risk behaviour and TTI. Our results encourage us to continue our donor disclosure programme, and we hope it will eventually decrease the incidence of viral TTI in the community.
HIV VIRAL FITNESS

McPhee, D.1,2,3,4, Arnott, A.1,2,4, Jakobsen, M.4,5, Gorry, P.R.3,4,6, Wilson, K.1, Jardine, D.1, Merlin, K.7, Grey, P.8, Dax, E.1,3, Kelleher, A.7, Smith, D.7,8, and the Pulse Study Team.
1National Serology Reference Laboratory, Australia; St Vincent's Institute, Fitzroy, Australia
2Department of Microbiology, Monash University, Clayton, Victoria, Australia
3Dept of Microbiology and Immunology, University of Melbourne, Parkville, Victoria, Australia
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5Department of Infectious Diseases, Aarhus University Hospital Skejby, Aarhus 8200, Denmark
6Department of Medicine, Monash University, Clayton, Victoria, Australia
7National Centre for HIV Epidemiology and Clinical Research, Sydney, Australia
8Albion Street Centre, Sydney, Australia

Introduction: The HIV envelope glycoprotein mediates binding and entry into the host cell. While Clade B represented the predominant infections early in the epidemic HIV-1 Clade C now represents close to 60% of infections worldwide. Determination of the viral fitness for Clade C viruses that establish primary infection is a priority. The aims of the present study were i) to investigate the relationship between viral fitness and control of viral load (VL) in acute and early HIV-1 infection and ii) to assess the relative importance of the envelope gene in viral fitness. Relative to disease progression, it has been observed in several clinical studies that isolates which are less fit are also less pathogenic. Samples were obtained from subjects participating in two clinical studies in Australia where Clade B infections predominate. In the PULSE study, HAART was initiated before, or no later than six months following, seroconversion. Subjects then underwent a maximum of three structured treatment interruptions (STIs). Participants in the PHAEDRA study were identified after seroconversion, and became part of an observational cohort that did not receive HAART.

Objectives: To develop an assay to assess relative viral fitness of circulating patient HIV strains and determine the significance of the envelope gene in contribution to viral fitness.

Methods: A combined total of 51 isolates were obtained from cohort members. Isolates from more than one time point were obtained from 20 subjects (8 PULSE and 12 PHAEDRA subjects). Viral fitness was examined ex vivo using a standardised input of 600 pg of p24 in parallel with a highly pathogenic Clade B CCR5 using primary reference strain obtained from an individual with AIDS, HIV1MBC925. Cells were cultured for 96 to 158 hours, prior to quantification and analysis of total viral DNA production using a real time PCR assay developed in our laboratory. Viral fitness was determined relative to the reference strain.

Results: The relative fitness of isolates obtained from 6 PULSE and 9 PHAEDRA participants was investigated in this study. The fitness of isolates obtained from 5 of 6 PULSE subjects decreased over time, following intermittent HAART. Although decreasing VL correlated with decreasing viral fitness for 4 of 6 PULSE subjects, overall, viral fitness did not correlate with plasma VL. The fitness of paired isolates obtained from 7 of 9 PHAEDRA subjects increased significantly over time (p=0.03). Viral fitness did not correlate with VL for the PHAEDRA subjects investigated. The relative fitness of isolates obtained at baseline from PULSE subjects was equal to, or greater than that of isolates obtained 36 or 52 weeks subsequent to baseline from PHAEDRA subjects. At baseline, the majority of PULSE subjects were at an earlier stage of infection than PHAEDRA subjects, confirmed by Western blot. Furthermore, the relative fitness of several isolates obtained from plasma collected at baseline from PULSE subjects, before initiating HAART, was greater than that of the reference strain. The envelope genes for 3 of these early isolates, derived from individuals prior to seroconversion, have been cloned and expressed to determine function and contribution to viral fitness.

Discussion: Changes in relative viral fitness over time were observed for six PULSE and nine PHAEDRA participants. However, viral fitness did not correlate with plasma VL. Most unexpected was the high relative fitness of isolates obtained at baseline from PULSE subjects, before initiating HAART. It is widely thought that the fitness of strains present during the acute phase is low relative to strains present during chronic HIV-1 infection, due to the bottleneck imposed upon transmission. The results of this study provide evidence that the relative fitness of strains present during acute HIV-1 infection may be higher than previously thought. Furthermore, these findings may add considerable weight to the debate over when, during the course of HIV-1 infection, HAART should be initiated. Having established viral fitness for very early Clade B virus infections we now plan comparative testing with early Clade C virus infections.
WEDNESDAY 26\textsuperscript{TH} AUGUST 2009

ENHANCED SURVEILLANCE OF ACUTE HIV INFECTION IN SYDNEY

Cunningham P  
St Vincent’s Centre for Applied Medical Research
LACK OF CORRELATION AT THE LOWER LIMIT OF DETECTION OF THREE COMMERCIALLY AVAILABLE VIRAL LOAD ASSAYS FOR THE QUANTITATION OF HIV-1 RNA

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1NSW State Reference Laboratory for HIV & Molecular Diagnostic Medicine Laboratory, St Vincent’s Hospital, Sydney, Australia
2St Vincent’s Centre for Applied Medical Research, Sydney, Australia

Introduction: The diagnostic monitoring of plasma HIV-1 RNA levels by laboratory viral load testing is fundamental to the management of antiretroviral therapy for infected individuals. We conducted a comparative study of three viral load assays currently available in the market, with a focus on the clinically critical lower limit of quantitation.

Objective: To assess the performance characteristics of the Abbott RealTime HIV-1 assay (ABB) in comparison to the Roche COBAS Amplicor MONITOR HIV-1 v1.5 test (AMP) and the Roche COBAS AmpliPrep/COBAS Taqman HIV-1 test (CTM). Viral load correlation at the lower limit of quantitation was specifically analysed as all current treatment guidelines stress the importance of achieving a viral load less than the limit of quantitation.

Methods: We evaluated the sensitivity, precision, and the utility of the Abbott RealTime HIV-1 assay. We also assessed the correlation and agreement of viral load quantitation among ABB, AMP and CTM by testing HIV-1 RNA-positive plasma samples in parallel, with a particular focus at the lower limit of detection, i.e. ≤200 copies/mL.

All EDTA plasma samples had been previously submitted to the NSW State Reference Laboratory for routine HIV-1 viral load testing and held frozen at ≤-70˚C prior to this evaluation study. The majority of the patients were infected by HIV-1 subtype B and undergoing HAART.

Results were log10-transformed before statistical analyses, which involved scatter plots, linear regression, and Bland-Altman curves. Pearson’s coefficient was used to measure the strength of linear dependence between the assays. Values <40 and <50 copies/mL were assigned 39 and 49 copies/mL, respectively, and Cohen’s Kappa coefficient was used to inspect the agreement of the assays at the lower limit of quantitation.

Results: During the period of June 2008 to March 2009, we processed a total of 416 plasma samples from HIV-1 seropositive patients for viral load testing. Of these, 367 specimens generated paired results for ABB and CTM, 246 for ABB and AMP, and 202 for CTM and AMP. While good correlation was observed for ABB versus CTM (r = 0.94), ABB versus AMP (r = 0.93), and CTM versus AMP (r = 0.94), P = 0.0001, viral load differences exceeded 0.5 log10 copies/mL among paired results in 22.9%, 28.5%, and 22.8% of specimens, respectively.

For the low-end viral load correlation study, 160 specimens yielded paired results for ABB and CTM, 174 for ABB and AMP, and 135 for CTM and AMP; and the kappa statistics were 0.42 at 40 copies/mL (moderate agreement; 95% CI 0.28–0.56), 0.14 at 50 copies/mL (slight agreement; 95% CI 0.01–0.26), and 0.19 at 50 copies/mL (slight agreement; 95% CI 0.04–0.33), respectively. The coefficient of variation measured at approximately 50 copies/mL was calculated from the intra-assay precision data, and was found to be 8.37% for ABB, 8.52% for CTM, and 15.12% for AMP.

Conclusion: All three viral load assays exhibited substantial discrepancies and only slight to moderate agreement at the lower limit of quantitation but good overall correlation. Our data also demonstrated the increased precision of the real time PCR-based assays as compared to end-point detection PCR assay. While there might be inevitable debate regarding the true value of a viral load, the accurate quantitation of low-level viremia remains elusive. Furthermore, the relevance of low level viral load detected on the real time assays to clinical outcomes is still unknown and the interpretation of these results presents a challenge to the current paradigms driving clinical management.
WEDNESDAY 26TH AUGUST 2009

AN INTRODUCTION TO THE NEW SIEMENS VERSANT KPCR MOLECULAR SYSTEM

Walker E
Siemens Healthcare
WEDNESDAY 26TH AUGUST 2009

“SUCK IT AND SEE. . .” HIV SALIVA ASSAY DEVELOPMENT, VALIDATION AND SAMPLE TESTING.

Wilson K\(^3\), Buxton P\(^3\), McDonald T\(^3\), Panagiotopoulos L\(^3\), Pedrana A\(^1\)-\(^2\), Guy R\(^4\), Prestage G\(^4\), Best S\(^3\), Heiardi H\(^1\), Cunningham P\(^4\), Stoovè M\(^1\), Dax EM\(^3\)

\(^1\)Centre for Population Health, Burnet Institute, Melbourne, Vic, Australia; \(^2\)Department of Epidemiology and Preventive Medicine, Melbourne, Vic, Australia; \(^3\)National Serology Reference Laboratory, Melbourne, Vic, Australia; \(^4\)National Centre for Epidemiology and Clinical Research, Sydney, NSW, Australia;

In Victoria the numbers of HIV diagnoses have more than doubled between 1999 and 2006, with the majority of cases in MSM (men who have sex with men). From 2008 to 2009, a study was undertaken to estimate the prevalence of HIV infection and the proportion of these infections that were actually undiagnosed in the MSM community. Appropriately named “Suck it and See...” the study recruited volunteers from gay social venues and requested them to complete a questionnaire and provide a saliva sample for subsequent testing. Oral fluid specimens were tested at the National Serology Reference Laboratory, Australia using in-house assays to quantify total IgG and detect the presence of antibodies to HIV-1 by a capture EIA (GACELISA) and by Western blot.

The concentration of IgG in oral fluid is 1000 fold lower than that in plasma samples. As a result commercially available immunoassays for the detection of antibodies to HIV demonstrate low sensitivity when performed on oral fluid specimens. To ensure that the oral fluid specimens collected were of adequate quality to enable the detection of anti-HIV antibodies, the total IgG concentration in each saliva sample was quantified using an ELISA for total human IgG. For surveillance purposes 0.1µg/ml IgG was considered adequate.

An in-house anti-HIV IgG antibody Capture ELISA (based on the method developed by Parry et al.*) was validated using samples collected from 100 individuals known to be negative for antibodies to HIV-1 and 100 samples collected from individuals known to be positive for antibodies to HIV-1. This assay was then used to determine the HIV-1 antibody status of the 746 saliva samples collected during the study.

Samples that were repeatedly positive on the GACELISA were subjected to confirmatory Western blot. Western blots were considered positive according to The Centers for Disease Control and ASTPHLD criteria, i.e. at least one envelope band (gp41 and gp120/gp160) and a p24 band.

Of the 746 saliva samples collected, 101 were repeatedly reactive in the GACELISA and confirmed positive by Western blot, giving an estimated prevalence of 13.5% within the study population. The GACELISA performed exceptionally well providing a sensitivity of 100% and specificity of 98%. Oral fluid collection was well received within the MSM community, making this assay an excellent tool for future HIV surveillance studies.

PROSPECTIVE ESTIMATION OF INCIDENCE IN NEWLY DIAGNOSED CASES OF HIV INFECTION AT ST VINCENT’S HOSPITAL, SYDNEY, 2005 – 2009

Jane Cornwall¹, Philip Cunningham¹ ², Sara Evans¹, Leon McNally¹, AD Kelleher ¹ ² and DA Cooper¹ ²

¹NSW State Reference Laboratory for HIV/AIDS, St Vincent’s Hospital, Sydney, Australia
²National Centre in HIV Epidemiology and Clinical Research, University of New South Wales, Sydney, Australia

The objective of many HIV prevention strategies is to reduce the extent of HIV transmission in populations at risk of infection. The rate of HIV incidence is a key indicator for monitoring the rates of newly acquired HIV infection to assist characterizing the epidemic and to monitor the effectiveness of prevention programmes.

A number of immunoassays have been developed which distinguish between individuals with established HIV infection and those which have been infected within the previous six months. There has been growing interest in the application of laboratory methods to measure HIV-1 incidence to monitor the effectiveness of prevention programs, targeting resources and second generation surveillance. In 1997, Janssen et al (CDC USA) developed the ‘detuned’ or ‘Less sensitive’ ELISA by modifying a first generation lysate EIA for HIV-1 infection (infection within <176 days). More recently, the BED HIV-1 IgG incidence EIA (Calypte Biomedical, USA) estimates incidence by measuring HIV-1 specific IgG with total IgG (infection within <153 days).

These testing strategies are known as STARHS (serological testing algorithms for recent HIV seroconversion). It is well known that these assays have limitations including overestimation of HIV incidence in individuals with advanced HIV infection and individuals who have received long term antiretroviral therapy (ARV) and being virologically suppressed.

The NSW State Reference Laboratory (SRL) for HIV at St Vincent’s Hospital identifies more than 60% of newly identified cases of HIV infection for NSW. In 1995, the SRL commenced incidence testing of all newly identified cases of HIV infection with the OTC ‘detuned’ LS ELISA and since 2005, has been performing parallel testing with the BED HIV incidence EIA.

<table>
<thead>
<tr>
<th>Year of Diagnosis</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>(n=286)</td>
<td>(n=230)</td>
<td>(n=255)</td>
<td>(n=220)</td>
</tr>
<tr>
<td>Incident HIV infections</td>
<td>37.9%</td>
<td>38.6%</td>
<td>37.5%</td>
<td>44.5%</td>
</tr>
<tr>
<td>Established HIV infections</td>
<td>62.1%</td>
<td>61.4%</td>
<td>62.5%</td>
<td>55.5%</td>
</tr>
</tbody>
</table>

Over the four years, the ‘detuned’ OTC assay (44.9%) estimated the number of individuals with incident HIV infection on average 5.7% higher than the BED assay (39.6%). Subsequent analysis of discordant results revealed ‘detuned’ OTC assay misclassified established cases as incident. In 2008, the detuned assay was discontinued commercially.

This study shows that approximately 40% of newly identified cases of HIV are presenting with incident HIV infection on average 5.7% higher than the BED assay (39.6%). Subsequent analysis of discordant results revealed ‘detuned’ OTC assay misclassified established cases as incident. In 2008, the detuned assay was discontinued commercially.

Although the number of cases of incident infection using the BED assay is increasing, it does not reach statistical significance (p=0.157; Wilcoxon rank sum – test for trend).

STARHS for newly identified cases of HIV-1 infection and provides an important means of enhanced surveillance of HIV incident infection and may represent opportunities for prevention.
THURSDAY 27TH AUGUST 2009

ARCHITECT HCV AG: CHANGING HCV DIAGNOSTICS?

Pereira LF
Abbott Diagnostics
EVALUATION OF THE NEW ROCHE ELECSYS® ANTI-HCV ASSAY IN COMPARISON TO THE ABBOTT ARCHITECT® ANTI-HCV

Bell CV¹, Robson, J¹, Cherian, S¹, Fredline DR¹, Melchiior W², Längin T²
¹Biochemistry Department, Sullivan Nicolaides Pathology, Taringa, QLD 4068
²Clinical Trials, Roche Diagnostics GmbH, Penzberg, Germany

Introduction: Sullivan Nicolaides Pathology (SNP) performs routine hepatitis C testing using the Abbott ARCHITECT Anti-HCV assay and confirms any reactive results with the Abbott Anti-HCV Murex test. We have recently evaluated a new Hepatitis C assay produced by Roche.

Objective: To evaluate the performance of the Roche ELECSYS Anti-HCV assay in comparison to the Abbott ARCHITECT Anti-HCV.

Methods: Routine patient samples submitted for Hepatitis C antibody testing were analysed using both the Abbott ARCHITECT and the Roche MODULAR ANALYTICS E170 Anti-HCV assays. All reactive and discordant results were run in duplicate as well as tested using the Abbott Murex Anti-HCV assay.

Inter-assay precision for the Roche assay using the provided kit controls was evaluated over 18 runs and 11 days. Assay performance was further assessed using 3 commercial seroconversion panels (BBI Diagnostics, West Bridgewater, Massachusetts).

Results: 497 (96.5%) of 515 patient samples tested negative by both assays, 9 (1.7%) were positive by both assays as well as by the Murex assay. Nine samples (1.7%) had discordant results. These included 4 samples that were ELECSYS positive and ARCHITECT negative. These were all negative when tested on the Murex assay.

Five samples were grey zone or positive on the ARCHITECT assay and ELECSYS negative. Four of the 5 of these were Murex negative and one was weak positive (Murex definition of grey zone). Only one of the 8 discordant samples had hepatitis C qualitative PCR performed and this was negative. All discordant patients had no known risk factors for HCV infection, the majority being insurance testing.

Inter-assay precision was 33.4% and 6.7% coefficient variation at Anti-HCV cut off index (COI) of 0.17 and 14.1 respectively.

An earlier detection of Anti-HCV of 3 and 7 days respectively was seen in the Roche ELECSYS assay in 2 of the 3 seroconversion panels.

Conclusion: These results suggest that the Roche assay may serve as an alternative to the Murex assay as a supplementary assay although this algorithm could result in more specimens that are positive on a supplementary assay rather than discordant. Resolution of the patient status is likely to require not only the tests results but also consideration of risk factors, liver function results and qualitative HCV PCR results. The advantage of the Roche assay is that it can be automated and tested from the source tube whereas currently the Murex assay is performed manually and results are delayed. To ensure carry-over is not present, an automatic re-run of the second sample is performed if 2 consecutive reactive results occur. Laboratories that process high volume infectious serology may find kit preparation time consuming as they are supplied not ready to use and need to be reconstituted the day before.
THURSDAY 27TH AUGUST 2009

EVALUATION OF THE ROCHE ELECSYS® HBsAg II ASSAY IN COMPARISON TO THE ABBOTT ARCHITECT® HBsAg ASSAY

Bell CV¹, Robson, J¹, Cherian, S¹, Fredline DR¹, Melchoir W², Längin T²
¹Biochemistry Department, Sullivan Nicolaides Pathology, Taringa, QLD 4068
²Clinical Trials, Roche Diagnostics GmbH, Penzberg, Germany

Introduction: Sullivan Nicolaides Pathology (SNP) performs routine testing for Hepatitis B surface antigen (HBsAg) using the Abbott ARCHITECT HBsAg assay.

Objective: To evaluate the performance of the Roche ELECSYS HBsAg II assay in unselected samples received in the diagnostic laboratory compared to the Abbott ARCHITECT HBsAg assay.

Methods: Routine diagnostic serum samples submitted for hepatitis B testing were analysed both on the Abbott ARCHITECT and then the Roche MODULAR ANALYTICS E170. All reactive results are run in duplicate. Neutralisation was performed on any ARCHITECT results <250.00 s/co using the Abbott ARCHITECT Hepatitis B confirmation assay. Results >250.00 were confirmed using the Abbott AxSym HBsAg assay. The Hepatitis B e antigen (HBeAg), Hepatitis B e antibody (HBeAb) and Hepatitis B core antibody (HepBcAb) results were reviewed to further confirm a positive HepBsAg result.

Precision for the Roche assay was evaluated using the kit controls. Intra-assay precision was evaluated by testing the one negative sample 50 times on the same run. Inter-assay precision was evaluated using the negative and positive samples over 41 runs and 11 days.

13 recombinant mutant specimens (Roche Diagnostics, Penzberg, Germany) were run on the Roche ELECSYS, Abbott ARCHITECT and AxSym. Sensitivity was further assessed through the use of 2 seroconversion panels (Zeptometrix Corporation, Buffalo, New York).

Results: 2108 (99.4%) of 2120 samples tested negative by both assays, 11 (0.5%) were positive for both assays and 1 ((0.1%) was discordant. The discordant sample was ARCHITECT positive and ELECSYS negative. Upon neutralisation, the sample was repeat reactive non-confirming. Roche ELECSYS and Abbott ARCHITECT achieved specificity of 100% and 99.95% respectively.

Intra-assay coefficient variation (CV) for the negative kit control with a stated cut off index of 0.39 was 18.7%. Inter-assay precision for the negative (0.46) and positive (2.6) kit control respectively was 17.75% and 3.17% CV.

All recombinant mutant samples were reactive when performed on the Roche ELECSYS and Abbott ARCHITECT, although 12 of the 13 ARCHITECT results were in the grey zone (0.05 – 1.0). Three of the 13 samples were non-reactive when performed on the Abbott AxSym.

HBsAg was detected 3 days earlier in both seroconversion panels by the Roche ELECSYS HBsAg II assay.

Conclusion: Our results demonstrate that the Roche ELECSYS HBsAg II assay is very sensitive and specific method for the detection of Hepatitis B surface antigen and shows a better inter-assay precision at higher levels.
THURSDAY 27TH AUGUST 2009

EVALUATION OF THE ABBOTT ARCHITECT ANTI-HBC II ASSAY FOR ANTIBODY TO HEPATITIS B CORE ANTIGEN

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Introduction
The Abbott Architect anti-HBc assay was originally evaluated in 2004 and found to have a high false positive rate of 6%. The Architect anti-HBc II assay was released in 2008 and was evaluated on the Architect i2000SR at Pathology Queensland Central laboratory.

Method
Samples were chosen for evaluation based on the original results obtained by the Abbott AxSYM CORE assay. These were then tested on both the original Architect anti-HBc assay and the new Architect anti-HBc II assay. Discordant were assayed using the bioMerieux VIDAS anti-HBc total II assay and the Architect anti-HBe assay. Measurable performance indicators were determined, including precision (using NCCLS guidelines), sensitivity, specificity, analytical limit of detection and suitability for use on cadaveric serum.

Results
511 anti-HBc negative samples were tested. The number of false positive samples on the original Architect anti-HBc assay reflected the data of the 2004 evaluation (6.0%). Of the 30 samples detected by the anti-HBc assay, none were detected by the anti-HBc II assay. These were also negative by the VIDAS anti-HBcore II assay and the anti-HBe assay. This indicated that these samples were falsely positive on the Architect anti-HBc assay.

All 45 HBsAg Positive samples were positive on both the anti-HBc and anti-HBc II assays. Three of 53 patients (6.5%) with evidence of past hepatitis B infection on the AxSYM assays (CORE Positive) were negative on the Architect anti-HBc and anti-HBc II assay. They were also negative on the VIDAS anti-HBc II assay and the Architect anti-HBe assay, suggesting that these are true negative samples and these were falsely reactive on the AxSYM assay. The analytical limit of detection of the Architect anti-HBc II assay and the AxSYM Core assay were shown to have identical sensitivities.

Anti-HBc cadaveric samples tested on the AxSYM CORE and the Architect anti-HBc II assays showed no difference in specificity. Twenty spiked cadaveric samples (100%) were detected by the Architect anti-HBc II assay and the limit of detection was the same for both cadaveric and control samples.

Conclusion
The evaluation showed the Architect anti-HBc II assay to be of superior sensitivity to the AxSYM CORE assay and the previous version of the Architect anti-HBc assay. The Architect anti-HBc II assay also appears to be more specific than the AxSYM CORE assay. This is of particular importance for its use in organ and tissue donor screening. Based in the resolved data, the Architect anti-HBc II assay was determined to be 100% sensitive and specific. The Abbott Architect anti-HBc II assay has been shown to be fit for use in Pathology Queensland laboratories.
THURSDAY 27TH AUGUST 2009

SERO-NEGATIVE HBsAg RESULT ON A PATIENT WITH HEPATITIS B INFECTION AND DETECTABLE HBV DNA.

Bautista CJ, Gibb R, Bletchly C, Schomberg C, Bowden S.
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2Pathology Queensland, Princess Alexandra Hospital, Brisbane, Queensland.
3Victorian Infectious Diseases Reference Laboratory, North Melbourne, and Department of Microbiology, Monash University, Clayton, Victoria, Australia.

Introduction: A 66 year old male with diffuse large B-cell lymphoma presented for investigation mildly abnormal liver function tests (ALP: 312 U/L, GGT: 285 U/L, ALT: 54 U/L, AST 100U/L). The patient had no detectable HBsAg, but had detectable levels of anti-HBs (94 mIU/mL), anti-HBc and HBeAg. HBV DNA was detected by an in-house qualitative PCR procedure and a level of $2.8 \times 10^3$ IU/mL was detected by the Roche TaqMan quantitative method.

Results: The sample was also found to be negative for HBsAg on the following assays: bioMerieux VIDAS, Abbott PRISM, Abbott AxSYM, Abbott Murex, ADVIA Centaur, Roche Elecys, Vitros ECI and Immunolite 2000. Sequencing of the POL/Env region demonstrated a number of substitutions. A HBsAg vaccine escape mutant is suspected, although this may also be due to an occult HBV infection where the levels of HBsAg are undetectable.

Conclusion: This demonstrates the importance of performing nucleic acid testing on patients where the clinical symptoms and history of hepatitis are not supported by serology. There are also public health concerns where the available HBsAg screening assays may not detect all HBV infected individuals.
THURSDAY 27TH AUGUST 2009

EMERGING RESPIRATORY VIRUSES

Jennings L
Virology Section – Canterbury Health Laboratories
THURSDAY 27TH AUGUST 2009

CLOSTRIDIUM DIFFICILE: CULTURE VERSUS ELISA

Nguyen T
Inverness Medical

abstracts
Abstracts

THURSDAY 27TH AUGUST 2009

BRUCELLA SEROLOGY – COMPARISON OF SIX COMMERCIAL IGG AND IGM ENZYME IMMUNOASSAYS (EIA’S)

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Introduction: Brucellosis is a classic bacterial zoonosis. Common Brucella species that are pathogenic in humans and their usual animal reservoirs include B. melitensis in sheep and goats, B. abortus in cattle, and B. suis in swine. In Australia less than 50 locally acquired cases are reported annually. Most cases occur in those involved in the recreational pursuit or processing of feral pigs. Human brucellosis may be acute, relapsing or chronic in presentation and also can be associated at any stage with focal complications. Serology is the mainstay of diagnosis for acute disease and is cross reactive across the species for all except the rare B. canis. Sullivan Nicolaides Pathology (SNP), a private pathology company servicing the population throughout country Queensland, the main focus of locally acquired infection, screens patients with a commercial IgG and IgM EIA (Panbio, Brisbane). If either is reactive, serum agglutination (SAT) and complement fixation (CFT) are performed.

Objective: To evaluate six commercial Brucella IgG and IgM EIA’s (DRG (DRG Diagnostics, Germany), Novagnost (NovaTec Immunodiagnostica, Germany), NovaLisa (NovaTec Immunodiagnostica, Germany), Vircell (Vircell Microbiologists, Spain), Virion/Serion (Insitut Virion/Serion, Germany) and Virotech (Genzyme Virotech, Germany) against the current Panbio assays (Panbio, Australia) for which production is ceasing.

Methods: Fifty-six archived serum samples from 52 patients were tested by each of the commercial assays. Samples were categorized into 5 groups (early infection, recent infection, past infection, false positives and no infection) based on clinical history and the serological profile. The negative panel included 8 patients undergoing insurance testing, 3 with acute Q fever and 3 with acute leptospirosis and 6 patients being investigated for zoonoses but with no serological evidence of brucellosis. The performance characteristics of the 6 assays was assessed on the specificity of the IgM response as it is well recognised that false positive isolated IgM responses without confirmation by a reference method such as SAT and demonstration of seroconversion can be misleading. Persistence of IgG even in chronic focal disease was also considered important in continuing to rule in brucellosis in the differential diagnosis.

Results: The ViroTech, Vircell and Virion/Serion assays produced fewer non-specific IgM results on the panel tested however the sensitivity of their IgG assays was lower than the Panbio assay. The DRG IgG assay appeared to be oversensitive with high levels detected in the early infection and negative group. The DRG, NovaLisa and Novagnost IgM assays had similar disappointingly poor levels of specificity comparable to the Panbio assay. The NovaLisa and Novagnost IgG assays had comparable sensitivity to the Panbio IgG assay. At SNP the Brucella IgG and IgM assays are used as screening assays, with positives being further tested by SAT and CFT. Therefore assays demonstrating high sensitivity are of paramount importance and specificity of secondary importance.

Conclusion: Either the NovaLisa or Novagnost assays would be suitable alternative assays to the current Panbio assays. It will be important to emphasize that the predictive value of an isolated IgM requires confirmation with supplementary (SAT and CFT) and convalescent testing.
EVALUATION OF THE VITAL DIAGNOSTICS EPSTEIN-BARR VIRUS ENZYME IMMUNOASSAYS

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Introduction: Epstein-Barr virus, frequently referred to as EBV, is a member of the herpesvirus family and the cause of one of the most common human diseases, infectious mononucleosis (IM). The virus occurs worldwide, and most humans are infected with EBV during their lives. Serological testing for antibodies to several viral-specific antigens is common in the diagnosis of EBV. These antigens are the viral capsid antigen (VCA), the early antigen (EA), and the EBV nuclear antigen (EBNA). At St Vincent’s Pathology, Melbourne the validated testing algorithm for EBV serological testing consists of testing for VCA IgM and IgG, and EBNA IgG antibodies.

Due to ongoing issues with the EBV assays routinely used in the laboratory, the VITAL Diagnostics EBV assays was evaluated as potential replacements for the kits currently in use.

Methods: All testing was performed at St Vincent’s Pathology. The testing was performed on the Triturus. A subset of the validation samples were also tested using the manual method. A range of well-characterised samples, supplied by the NRL were used in the evaluation. The Euroimmun EBV IgG Western Blot was used to determine the EBNA (n=117) and VCA IgG (n=125) status of the samples prior to testing. Samples from individuals with a clinical history of IM which had a reactive Monospot test and reactive lymphocytes in the blood film were selected (acute samples). As no IgM Western blot was available, the acute samples (n=52) were tested in three other EBV VCA IgM EIAs. Only samples reactive in all three VCA IgM EIAs were included in the acute sample panel.

Results: Of the 185 samples that had been previously assigned a VCA IgM status (Positive status n=52, Negative status n=133), all Vital Diagnostics VCA IgM assay results were concordant with the reference laboratory results. Of the 125 samples assigned a VCA IgG status (Positive status n=100, Negative status n=25) testing using the Vital Diagnostics VCA IgG assay, 122, were concordant using VCA IgG assay. Of the 117 samples assigned an EBNA IgG status (Positive status n=87, Negative status n=30) testing using the Vital diagnostics VCA IgG assay 116 were concordant. These discordant samples results were examined considering the St Vincent’s Melbourne reporting algorithm and all reports would have been clinically appropriate.

Conclusions: St Vincent’s Pathology have implemented the Vital Diagnostics EBV assays to replace previous assay for the diagnosis of EBV infection and for screening for past EBV exposure. The validation testing presented in this report supports the use of these assays. The introduction of the Vital Diagnostics EBV assays has lowered the number of repeat testing due to invalid runs, thereby offering cost savings benefits.
VICTORIAN MEASLES OUTBREAK 2009

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\(^1\)Victorian Infectious Diseases Reference Laboratory
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**Introduction:** From January to the end of March this year, twenty-eight laboratory-confirmed cases of measles have been identified in Victoria, a sharp increase in the previous four years’ annual average of just 4.5 cases\(^1\). An investigation was undertaken to identify the index case and the source of infection.

**Methods:** Measles cases were laboratory confirmed by detection of measles-specific immunoglobulin M (IgM) in serum by EIA and detection of measles RNA by RT-PCR from serum, throat and nose swabs. Genotyping was performed on the PCR positive samples.

**Results:** There were 28 laboratory confirmed cases of measles during this period, 25 of which were genotyped. Three remaining cases (negative by PCR) were confirmed by serology. Molecular genotyping identified 4 distinct circulating clusters with each cluster characterised by a different genotype. The index case in each cluster was determined to be a returned traveller. Of the four clusters, the largest two were found to be the H1 genotype and the D8 genotype, the index cases for each having returned from Iran and India respectively. Where known, none of the cases in either cluster were fully vaccinated, and transmission in both clusters occurred locally in emergency departments, GP clinics, between family members, in schools, hotels and shopping centres. The remaining two clusters consisted of genotypes D4 in a case with epidemiological linkage to a Tasmanian case who had travelled to India and D9 in a child who had travelled from the Philippines but no further cases of transmission were identified.

**Conclusions:** The genotyping of measles has enabled us to identify multiple clusters of measles, consistent with measles elimination in Victoria and identify each index case as an importation. Transmission has occurred in families and individuals who were not vaccinated, highlighting the importance of vaccination and herd immunity.
VALIDATION OF HIV, HCV AND HBsAg IMMUNOASSAYS FOR USE WITH CADAVER SERUM.

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Introduction: The Australian Code of Good Manufacturing Practice – Human Blood and Tissues: 2000, (clause 836) specifies that for donor testing 'where a kit is not registered on the Australian Register of Therapeutic Goods, has been modified for use, or there is an "in-house" protocol used, there must be validation data to verify acceptability of the test system'. Using sample types aside from those specified in the manufacturer’s test kit instructions, such as cadaver samples, is considered a modification that requires validation.

Objective: To investigate the performance of the Abbott Murex HIV-1.2.0 EIA, the Abbott Murex anti-HCV version 4.0 EIA and the bioMerieux Vidas HBsAg Ultra ELFA with serum samples collected from cadavers.

Methods: In 2005, the Victorian Institute of Forensic Medicine (VIFM) donated to NRL a repository of cadaver serum samples. The samples were transferred with appropriate ethics approval allowing NRL to use the samples for assay validation purposes. VIFM also provided test results for anti-HIV, anti-HCV and HBsAg obtained with the test kits that were in use at the time of collection.

To assess the sensitivity of the serology test kits used at the NRL when testing cadaver serum, a panel of samples was constructed from the VIFM sample repository selecting samples that had been reactive at VIFM. Sixty anti-HCV, 45 HBsAg and 17 anti-HIV reactive samples having adequate sample volume were available. To confirm this reactivity, samples were tested in the Roche COBAS AmpliScreen HCV, HBV or HIV-1 assay. Those samples giving a positive nucleic acid test (NAT) result were deemed to have been collected from a cadaver infected with the relevant virus and would, therefore, be expected to have detectable antibody or antigen. To increase the number of samples in the HIV positive panel, samples reactive at VIFM and negative on AmpliScreen were tested using an HIV-1 Western blot. Samples positive on Western blot were also included in the HIV positive panel. An additional HIV positive sample was obtained from a commercial source. The final positive panel contained 38 HCV, 41 HBV and 18 HIV-1 positive cadaver samples.

To assess the specificity of the NRL’s serology test kits when testing cadaver serum, 37 presumed antibody negative cadaver samples were sourced from a commercial supplier. In addition, 100 cadaver serum samples from the VIFM sample repository that had screened negative for HBsAg by VIFM were tested in the HBsAg assay under validation.

Samples from each panel were tested once in the relevant test kit according to the manufacturers’ instructions. Samples from the negative panel that showed reactivity were further tested according to the NRL’s confirmatory testing algorithm.

Results: All 18 HIV, 38 HCV and 41 HBsAg positive samples were reactive in the Abbott Murex HIV-1.2.0 EIA, the Abbott Murex anti-HCV version 4.0 EIA or the bioMerieux Vidas HBsAg Ultra ELFA respectively. Of the 37 presumed antibody negative cadaver samples all were negative in the Abbott Murex HIV-1.2.0 EIA and the Abbott Murex anti-HCV version 4.0 EIA. Of the 137 presumed antigen negative cadaver samples, five were initially reactive on the VIDAS HBsAg Ultra ELFA, but none of these were confirmed positive by neutralisation.

Conclusion: Although the numbers of samples available for this study were limited, the results support the use of the Murex HIV-1.2.0 EIA, the Murex HCV version 4.0 EIA and the bioMerieux HBsAg ELFA for testing serum from cadavers.
HEPATITIS A VIRUS OUTBREAK: MOLECULAR EPIDEMIOLOGY INDICATES A COMMON SOURCE.

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Introduction: Hepatitis A is a widespread infectious disease affecting the liver caused by hepatitis A virus (HAV). Transmission occurs predominantly through the faecal-oral route via contaminated food and water. The incidence of hepatitis A in Australia over recent decades has fallen with improvements in sanitation and the availability of a vaccine for groups at risk, however, sporadic outbreaks do occur. Recently, a substantial increase in the number of cases of hepatitis A was reported in Victoria and correlated with increased reporting of hepatitis A cases in other Australian states. Sequence analysis of HAV isolates has enabled the virus to be divided into six genotypes, designated I-VI of which I and III are the most prevalent. Using a molecular approach, we carried out genotyping and phylogenetic analysis to try and determine any relationship between the samples.

Methods: RNA was extracted from samples and initially tested in the RealArt HAV LightCycler RT PCR system (QIAGEN). Samples shown to be HAV RNA positive were re-assayed using primers to the HAV VP1/P2a junction and the resulting PCR product was directly sequenced with internal primers. Sequences were aligned using Clustal X and unrooted neighbour joining trees were generated using the Seqboot, DNAdist, neighbour and consense programs available at the Phylip website. Trees were resampled by bootstrapping (1000 replicates) and visualised using Treeview.

Results: Of eighty samples shown to have detectable HAV IgM, 75 (94%) were positive by the real-time PCR assay and of these, sequence information was obtained from 71 (96%). Sequence alignment showed that 68/71 samples had identical sequences for the region encoding the HAV VP1/P2a junction. Phylogenetic analysis showed this large group of identical sequences were classified as HAV genotype IB. Of the remaining three samples, genotyping showed them to be classified as HAV IA, HAV IIIB and the other to be another HAV IB but in a distinct clade to the other IB samples.

Discussion: The high percentage of HAV RNA positive samples suggests recent acute hepatitis A infection because HAV RNA usually becomes undetectable after 7-8 weeks from the initial exposure. The phylogenetic analysis gives a strong indication of a likely epidemiological link between most cases and implies the outbreak originated from a common source. When combined with a traditional epidemiological investigation, the putative source of the outbreak may be identified and steps taken to further reduce the chances of future hepatitis A outbreaks.
THURSDAY 27\textsuperscript{TH} AUGUST 2009

INNO-LIA SYPHILIS SCORE TEST FOR THE RESOLUTION OF EIA/TPPA DISCORDANT SERA.

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Introduction: Current syphilis testing guidelines recommend a reactive EIA screen be confirmed by TPPA. A second treponemal test is required to resolve the status of EIA/TPPA discordant sera. FTA-ABS currently fulfills this role in our laboratory. However, issues with test subjectivity are well documented. FTA-ABS sensitivity and specificity are also lower than the TPPA. The INNO-LIA Syphilis Score\textsuperscript{®} recombinant line blot immunoassay has been recommended as a suitable alternative confirmatory test to FTA-ABS.

Objective: Part 1: Assess INNO-LIA Syphilis Score\textsuperscript{®} diagnostic accuracy when testing clearly defined sera. Part 2: Assess INNO-LIA ability to further characterise EIA/TPPA discordant sera and its suitability as a replacement for the FTA-ABS.

Methods: Part 1: Diagnostic accuracy was assessed against; 1) A consecutive sample of Abbott Syphilis TP\textsuperscript{®} EIA AND Serodia TPPA\textsuperscript{®} positive sera classified as “syphilis infected” (n=72) and 2) Randomly selected EIA/TPPA negative sera classified as “not infected with syphilis” (n=86). Part 2: INNO-LIA and FTA-ABS (Biomerieux) were compared for measures of agreement with a composite reference standard (CRS) defined as a 2/3 consensus result by EIA, TPPA and a whole cell lysate western blot (T.pallidum IgG Marblot\textsuperscript{®}), using discordant sera from study 1 (n=13) and a stored panel of EIA/TPPA discordant sera (n=34).

Results: Part 1: INNO-LIA sensitivity = 94.4\% (86.5\% - 97.8\%), specificity = 97.7\% (91.9\% - 99.4\%), likelihood ratio LR(+) = 56 (12 - 278), LR(-) = 0.007 (0.0005 - 0.12). FTA-ABS sensitivity = 88.9\% (79.6\% - 94.3\%), specificity = 94.2\% (87.1\% - 97.5\%), LR(+) = 15 (6.5 - 35), LR(-) = 0.118 (0.06 - 0.23). Part 2: Almost half (21/47) of the EIA/TPPA discordant sera could not be resolved by the CRS due to high numbers of indeterminate results. The CRS re-classified 8 sera positive and 18 negative. The INNO-LIA re-classified 5 sera as positive and 20 negative. Agreement with the CRS for both the INNO-LIA and FTA-ABS tests was poor, although the INNO-LIA had higher agreement overall (49\% vs 40\%). Agreement for the FTA-ABS suffered due to the lack of an indeterminate category for this test.

Discussion and conclusions:
- The INNO-LIA was at least as sensitive and specific as the FTA-ABS test when testing clearly defined positive and negative sera but was less sensitive than has been reported previously (94.4\% (86.5\% - 97.8\%) vs. 100\% (98.3\% - 100\%)).
- The ability of the INNO-LIA to resolve EIA/TPPA discordant sera was not significantly better than the FTA-ABS. However the INNO-LIA may be more appropriate in this setting as the binary FTA-ABS test is unable to deliver an indeterminate result.
- From our study there appears to be only marginal benefit in further testing specimens that are EIA/TPPA discordant. Rather, it may be more useful to follow immune response over time by serial testing patients with discordant EIA/TPPA results. Further studies are required.
EVALUATION OF THREE COMMERCIALLY AVAILABLE FTA-ABS KITS FOR THE DIAGNOSIS OF SYPHILIS

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Introduction: Syphilis is a sexually transmitted disease caused by the spirochete Treponema pallidum (T. pallidum). The organism is difficult to culture so the diagnosis of syphilis is largely serologically based.

The Fluorescent Antibody Absorption (FTA-ABS) is a treponemal test that uses T. pallidum as its antigen. It is considered to be the most sensitive test in all stages of syphilis and is therefore used as a confirmatory test.

Objectives: To find a suitable commercially available FTA assay to replace the current in-house method.

Method: All test systems were performed according to the procedure listed below One hundred routine syphilis serum samples were tested in parallel by all four methods. The breakdown of samples tested were: 44 reactive, 46 non-reactive and 10 equivocal.

Test sera were diluted 1:5 in sorbent (a Reiter's treponeme) to remove group treponemal antibodies that may have been produced by non-pathogenic treponemes. The absorbed serum was placed on the appropriately labelled well on the slide. Slides were incubated at 37° C in a moist chamber for 30 minutes and then washed (x3) gently in PBS and once in water. The Fluorescein isothiocynate (FITC) labelled anti-human immunoglobulin was added (at a dilution determined by the manufacturer) and the slide was incubated at 37° C in a moist chamber for a further 30 minutes. Slides were washed as previously described, air dried and mounted with coverslips and examined by fluorescent microscopy.

Results:

<table>
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<tr>
<th>Method</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive Predictive Value</th>
<th>Negative Predictive Value</th>
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<tr>
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<td>Mardx</td>
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<tr>
<td>Trepo-Spot</td>
<td>78</td>
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Discussion: All results were compared to the in house method which was considered the gold standard. This method required the production of treponemes in rabbits and subsequent preparation and quality control of slides. We could no longer justify the production of treponemes in animals if a commercial source was available.

• Trepo-Spot had numerous thin filamentous treponemes on the slide making it difficult to read. This resulted in greater numbers of false positives and false negatives being reported compared to the other methods. Several batches displayed beaded treponemes which resulted in retesting and difficulty in reading.

• Mardx slides were difficult to differentiate between reactive minimum and < reactive minimum This resulted in an increase in costs and turn around time.

• Zeus slides had sufficient numbers of treponemes on the slide which did not display beading and so were easier to read. There was a good distinction between the reactive minimum and < reactive minimum. This method was the easiest to use and correlated closely with the in-house method.

Conclusion: The current study suggests that the Zeus FTA-ABS kit is a reliable and acceptable alternative to the in-house method.
THE AUSTRALIAN NATIONAL SEROSURVEILLANCE PROGRAM

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Introduction: Serosurveillance is an important component of any comprehensive surveillance system for vaccine preventable diseases. It is the gold standard for measuring immunity in a population, thereby complementing traditional disease surveillance methods. National coverage data are limited for immunisation of adolescents and adults and population immunity is also influenced by naturally occurring infection. Serological surveys can be used to evaluate the relative contributions of immunisation coverage versus the age specific incidence of disease.

Objective: To establish a regular national serosurveillance program that can be used to measure immunity to antigens relevant to vaccine preventable diseases.

Methods: At appropriate time intervals (1996-1999, 2002 and 2007), a bank of sera were collected opportunistically from a geographically representative group of diagnostic laboratories receiving samples from hospitalised and ambulant persons throughout Australia. The sera were residual from specimens submitted for diagnostic testing and would otherwise have been discarded. For each antigen to be tested, sample sizes were calculated for each age group of interest. In each age group for both males and females, states and territories were sampled proportionally to their population size. All testing was performed at the Centre for Infectious Diseases and Microbiology (CIDM), except for pertussis in the 2002 serosurvey which was tested in Italy. Approval for each serosurvey was obtained from the Western Sydney Area Health Service Human Research Ethics Committee.

Results: The first national serosurveys were conducted using sera collected in 1996-1998 and 1999, i.e. either side of the Measles Control Campaign. Immunity to the following antigens was tested; measles, mumps, rubella, varicella, hepatitis A, hepatitis B, hepatitis C, diphtheria, tetanus, polio and pertussis. Nineteen publications resulted from this serosurvey. Results from the tetanus serosurvey showed that over 90% of children had tetanus antitoxin levels \( \geq 0.01 \) IU/mL (immune), but levels decreased significantly with age. The elderly, especially females, had the lowest antitoxin levels. These results provide a graphic picture of the effect of past and current vaccination policies on immunity in each age/sex cohort, and suggest that the current recommendation for a booster at age 50 years is necessary.

The second serosurvey was conducted using sera collected in 2002 and immunity to the following antigens was tested; measles, rubella, varicella, hepatitis B, pertussis, meningococcal C, cytomegalovirus and helicobacter pylori. Eight publications resulted from this serosurvey. The results of the Hepatitis B serosurvey from 2002 were compared to those from 1996-99 to evaluate the impact of universal infant vaccination and school-based catch-up programs for adolescents. Immunity improved significantly overall, but especially in 1-2 year olds (40% versus 67%) and 12-17 year olds (29% versus 46%). These serosurveys demonstrated successful implementation of universal infant hepatitis B vaccination in Australia and that school-based immunisation programs for adolescents are effective.

The third serosurvey is currently being conducted using sera collected in 2007. Antigens being tested include measles, mumps, rubella, varicella, pertussis, meningococcal C, diphtheria and tetanus.

Conclusion: The national serosurveillance program conducted by NCIRS is a valuable resource for estimating vaccine coverage by population immunity, for immunisation program evaluation and to contribute to disease modelling.
EVALUATION OF THE VIDAS EBV ASSAYS FOR VCA IGG, VCA IGM AND EBNA IGG.

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Introduction: Our laboratory screens for EBV infection by testing for EBV VCA-p18 IgG and EBV VCA IgM using Panbio reagents on the Bio-Rad EVOLIS immunoassay analyser. If the sample is positive for both VCA IgG and IgM, EBNA IgG is also tested to discriminate acute infection from convalescent or past infection. This requires a manual EIA procedure to be performed the next day.

Objective: The bioMerieux VIDAS has released a panel of assays for EBV serology. These include EBV VCA IgG, EBV VCA IgM and EBV EBNA IgG. These were evaluated against the Panbio assays to:

a. Determine the utility of the VCA IgG and IgM assays for urgent or after-hours testing.
b. Decrease the turn-around time to provide EBNA IgG results on patients with current EBV infection.

Method: Samples were chosen for evaluation based on the original results obtained by the Panbio assays. These were then tested on the bioMerieux VIDAS assays: EBV VCA IgG, EBV VCA IgM and EBV EBNA IgG. Discordant samples were assayed using IFA.

Results:

<table>
<thead>
<tr>
<th></th>
<th>VCA IgG</th>
<th>VCA IgM</th>
<th>EBNA IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Panbio</td>
<td>VIDAS</td>
<td>Panbio</td>
</tr>
<tr>
<td>EBV Negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 14</td>
<td>Pos</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Neg</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Past EBV Infection</td>
<td>Pos</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>n = 21</td>
<td>Neg</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Current EBV Infection</td>
<td>Pos</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>n = 14</td>
<td>Neg</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Past / Convalescent EBV – persistent IgM</td>
<td>Pos</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>n = 6</td>
<td>Neg</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

All 14 patients with no evidence of current or previous EBV infection were concordant by all three VIDAS assays.

There was 100% concordance between all assays in patients with past EBV infection. One sample was positive for EBNA IgG but negative for VCA IgG in both assays whilst two samples gave the reverse results with EBNA IgG negative and VCA IgG positive in both assays.

Patients with current EBV infection showed some variability. 13/14 samples were positive in the VIDAS VCA IgM assay. All samples were negative for EBNA IgG by both assays. There was some variability with the VCA IgG assays. It has been previously shown that antibodies against the VCA p18 antigen are not always produced early in the infection. This variability was demonstrated with 4 samples negative on the Panbio assay and only 2 negative on the VIDAS assay.

There were 6 samples that were positive for all 3 EBV markers when tested on the Panbio assays. All 6 samples were positive for VCA IgG and EBNA IgG by the VIDAS assay. However, 4 of the 6 samples were negative for VCA IgM on the VIDAS assay.

Conclusion: The results indicate that for determining immune status (evidence of past infection), the Panbio and VIDAS assays for VCA IgG and EBNA IgG are equivalent. There were some differences noted in patients with recent infection where the VCA IgG seems to appear earlier in the VIDAS assay. The VIDAS VCA IgM did not detect 1 out of 14 acute EBV samples and was negative in 4 out of 6 samples positive for all markers. This may indicate that either these were falsely reactive by the Panbio assay where a non-specific IgM response can be found, or that the VIDAS was falsely negative.

This trial indicates that the VIDAS VCA IgG and EBNA IgG assays meet our objectives and are suitable for use in our laboratory. Further investigation is required to determine the performance of the VCA IgM assay.
THURSDAY 27TH AUGUST 2009

RCPA SEROLOGY QAP – MOLECULAR DIAGNOSTICS CASE STUDIES

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Introduction/Objectives: The RCPA Serology Quality Assurance Program (SQAP) introduced a Molecular Diagnostics Module in 2005. Ongoing development has enabled module expansion to currently include quality assurance for the detection and/or quantitation of Hepatitis B DNA, Hepatitis C RNA, Hepatitis C genotype, HIV RNA, Alphavirus/Flavivirus, HPV DNA and Avian Influenza. The results are peer reviewed and participating laboratories can use these reports to make comparisons and discuss procedures, algorithms or other issues. A number of issues have been identified and addressed utilising the Molecular Diagnostics Module.

Methods: The SQAP aims to issue a range of specimens in order to allow the assessment of intra-run and inter-run reproducibility, detection of significant log differences, detection of similar levels of different subtypes and the use of negatives to assess contamination. On review of reports from the Molecular Diagnostics Module, several issues were identified. Discussion with participants regarding potential problems associated with each issue and resolutions were addressed.

Results/ Discussion: A number of areas were identified as potentially problematic by the return of false positive/false negative results, results that differed between surveys and to the other participants and the resulting of negative samples. Cases highlighted factors including specimen storage and handling procedures, assay sensitivity, testing strategies and post analytical errors. Generally the resolution of the identified issues was appropriate.

Conclusion: There are a number of potential areas from which erroneous results can originate. Quality assurance programs are designed as ‘snapshots’ of a laboratory’s performance and if used correctly can help indicate potential problems before they become major issues. It is encouraging that participants who have identified issues using the SQAP Molecular Diagnostics Module are proactive in addressing them. These issues also highlight the need for continuous education and training in the laboratory.
LESSONS LEARNED FROM THE RESULTS OF PROFICIENCY TESTING FOR THE LEPTOSPIROSIS MICROSCOPIC AGGLUTINATION TEST

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²Royal Tropical Institute, Amsterdam, The Netherlands
³County Hospital, Hereford, UK

The microscopic agglutination test (MAT) for detecting antibodies against leptospiral bacteria remains in wide use throughout the world. The ability of a laboratory to deliver accurate and meaningful test results depends on the application of quality assurance measures within the context of a quality management system. The MAT has a particular need for quality assurance, because it uses, as a test reagent, cultures of live leptospiral bacteria, which are difficult to standardise. There are also differences between laboratories in how the test is performed, and a subjective element in reading the test.

Proficiency testing is an essential element of laboratory quality assurance. However, it may sometimes not be readily available, or may be prohibitively expensive for some laboratories. For this reason, the International Leptospirosis Society (ILS) offers a low cost proficiency testing scheme for the MAT. Seven rounds have been distributed, and there are currently 92 participants in 48 countries.

The use and interpretation of the MAT are greatly complicated by the antigenic complexity of leptospiral bacteria. The genus *Leptospira* includes more than 200 serovars within more than 25 serogroups. The MAT is broadly serogroup-specific, so that cross-reactions between serovars are usually observed within but not between serogroups. However, proficiency testing, which generates many individual results, shows that serogroups – which have no taxonomic status - are arbitrary. Unexpected cross-reactions may occur, and may differ depending on the reference laboratory from which a test culture was sourced.

The antigenic complexity of leptospires, combined with the inherent difficulties in standardising the MAT, makes participation in proficiency testing imperative. Proficiency testing is designed to help individual laboratories to improve performance. However, it also helps us to understand the complexity of cross-reactions, and factors that may influence MAT results.
DIGITAL POLYMERASE CHAIN REACTION: A PRIMARY REFERENCE METHOD FOR ACCURATE QUANTIFICATION OF NUCLEIC ACIDS?

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Objectives: Accurate quantification of DNA or RNA is vital for many diagnostic applications and routine analysis. However, the lack of nucleic acid certified reference materials dictates an urgent requirement for DNA and RNA reference standards traceable to the International System of Units (SI). At NMI we have been investigating a new polymerase chain reaction (PCR) technology, called digital PCR, which provides an increased scope for accurate measurement of gene copy numbers independent of a calibrant.

Methods: In digital PCR, the PCR solution is typically distributed across a panel comprising several hundred individual partitions. The concentration of target DNA in the PCR is carefully adjusted such that not all partitions will contain a target DNA sequence. Following amplification, the target DNA copy number is estimated using binomial approximation based on the proportion of partitions containing amplified DNA. Our study, using a plasmid certified reference material, was designed to identify factors contributing to the uncertainty associated with this method and develop approaches for minimising bias.

Results: Accurate digital PCR measurements rely on two key assumptions; firstly, that all target molecules are amplified and secondly, that the molecules are randomly distributed through the digital panel. Our study demonstrated that target molecules are partitioned with a high degree of randomness and independence and that single molecule amplification efficiency is significantly improved when DNA is digested into short fragments containing intact target DNA prior to amplification. The precision of the digital PCR measurements can be improved by ensuring that the number of target molecules per panel falls within an optimal range and by increasing the number of partitions analysed.

Conclusions: Digital PCR is an alternative technique for quantifying gene copy number which may provide more accurate measurements than other approaches currently available as it is not dependant on amplification efficiency. Of particular importance, digital PCR measurements are made independent of any calibrant and, therefore, this technique has the potential to be considered as a primary method for use in certification of nucleic acid reference materials. We believe this approach could also be adapted for certification of viral reference materials with a value that is traceable to the SI.
ONE PLATFORM – TWO AUTOMATED REAL TIME HCV ASSAYS; VIRAL LOAD AND HCV GENOTYPING

Van Asten M
Diagnostic Technology
LOW POSITIVE PREDICTIVE VALUE OF A NUCLEIC-ACID AMPLIFICATION TEST FOR NON-GENITAL *NEISSERIA GONORRHOEAE* INFECTIONS IN HOMOSEXUAL MEN

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**Objectives:** Nucleic acid amplification tests (NAAT) for the detection of *Neisseria gonorrhoeae* are only validated for use with genital samples. To determine the test performance of the BD ProbeTec assay for the detection of *N. gonorrhoeae* at non-genital sites, we performed supplemental testing using a previously validated assay targeting the gonococcal porA pseudogene on non-genital samples collected from participants in the community-based Health in Men (HIM) cohort of homosexual men in Sydney, Australia.

**Methods:** A total of 327 (289 oropharyngeal and 38 anorectal) samples initially reactive for *N. gonorrhoeae* DNA using the ProbeTec assay were tested on the porA supplemental assay. An additional 100 (50 oropharyngeal and 50 anorectal) samples negative on the ProbeTec assay for *N. gonorrhoeae* DNA also underwent supplementary porA testing.

**Results:** Of the 327 samples initially reactive for *N. gonorrhoeae* DNA on the BD ProbeTec assay, 88 of 289 oropharyngeal samples and 28 of 38 anorectal samples tested positive on the porA assay. The positive predictive value of the ProbeTec assay for the detection of *N. gonorrhoeae* DNA was 30.4% (95% CI 25.2-36.1%) in the oropharynx and 73.7% (95% CI 56.9-86.6%) in the anorectum. All oropharyngeal and anorectal extracts negative for *N. gonorrhoeae* DNA on the ProbeTec assay were also negative on the porA assay resulting in negative predictive values of 100% at each site.

**Conclusion:** Supplemental testing of reactive non-genital BD Probetec *N. gonorrhoeae* samples resulted in greatly improved accuracy of gonorrhoea diagnosis at these sites. More research involving a larger number of specimens is required to determine the performance characteristics of the BD assay on anorectal samples. However, our results indicate that a positive result of a single NAAT cannot be relied on for diagnosis of pharyngeal gonorrhoea in Australian homosexual men.
INCREASED FREQUENCY OF HIV-1 VIRAL LOAD BLIPS USING ROCHE COBAS AMPLICOR/TAQMAN HIV-1 ASSAY

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¹PaLMS, Royal North Shore Hospital, St Leonards
²Northern Clinical School, University of Sydney.

Introduction: The detection of transient low level HIV-1 viremia in patients with previously stable undetectable viral load (blips) is a cause of confusion & distress to patients & physicians. International reports suggest the introduction of the Roche COBAS Ampliprep/COBAS TaqMan HIV-1 real time PCR assay to be accompanied by an increase in frequency of viral load blips compared to the Roche Amplicor HIV-1 Monitor, version 1.5, ultrasensitive assay. We reviewed our patient data to determine the prevalence of HIV-1 blips in our testing population & to determine whether the introduction of the Ampliprep/Taqman assay had increased the rate of blips.

Methods: Patient records for the previous four years were reviewed. All assays for the previous two years were performed on the Ampliprep/Taqman assay & included 1669 samples from 355 patients. All assays for the two years prior were performed by Amplicor Monitor assay & included 1128 samples from 215 patients. Blips were defined as quantifiable viral load counts of < 1000 copies per ml which occurred after a year or more of undetectable (< 50 Amplicor, <40 Taqman)) viral load counts and followed by a return to undetectable levels.

Results: The prevalence of blips increased two fold from 6 % to 11% with the introduction of the Ampliprep/Taqman assay. The mean blip size was 107 copies per ml for the Ampliprep/Taqman assay. The frequency of blips was 6 fold higher by the Ampliprep/Taqman assay when a blip was defined as any low positive result (<1000 copies/ml) preceded & succeeded by an undetectable viral load count.

Discussion: Our study confirms published reports of increased blip rate associated with the introduction of the Ampliprep/Taqman assay. Published evidence suggests that blips are due to both assay error & true biological variations. The reasons for the increased blip rate associated with the Ampliprep/Taqman assay remain unresolved but it is likely that contributing factors include extended assay sensitivity combined with imprecision at low concentrations, biological variation & differing standardisation between the Ampliprep/Taqman & Amplicor Monitor 1.5 kits.
FRIDAY 28TH AUGUST 2009

DEVELOPMENT OF MULTI-TARGET REAL-TIME PCR ASSAY FOR HEPATITIS B VIRUS

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2 QPID Laboratory, RCH, Herston, Brisbane

Introduction: Detection of hepatitis B virus (HBV) DNA by nucleic acid tests remains an important part of diagnosing HBV infection and is the mainstay for monitoring response to anti-HBV therapy. Pathology Queensland (PQ) currently use the Roche CobasTaqman HBV assay to monitor HBV DNA levels; however, the assay is not intended as a diagnostic test to confirm the presence of HBV infection. It is also a time-consuming and expensive test. Requests for HBV PCR at PQ have increased significantly over the last three years.

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

Method: A multi-target HBV real-time PCR (mtHBV) assay was developed targeting highly conserved sequences on the HBV large S protein and X protein genes (using previously published primers and probes). The mtHBV assay was validated by testing 201 clinical specimens submitted to PQ for HBV testing, and the results were compared to those obtained using the quantitative COBAS Taqman HBV Test (Roche Diagnostics).

Results: In total, 129 specimens provided positive results and 52 provided negative results in both assays. A further 20 specimens provided discrepant results, all of which contained low viral loads evidenced by qualitative cycle threshold values (>37) or by quantitation (load <29 IU/mL, which is the lower lineal limit of that assay). Of the 20 specimens, 13 were positive in the qualitative assay only, while 7 specimens were positive in the quantitative assay only.

Conclusion: The results show the mtHBV real-time PCR assay is suitable for routine detection of HBV DNA in clinical samples, and represents a significant cost saving compared to the Roche method. In addition, the two-target system of the mtHBV assay decreases the potential for sequence-related HBV false-negative results. This approach may enhance the detection of a broad range of infectious agents, particularly those exhibiting extensive genetic variation.
VALIDATION OF ROCHE COBAS AMPLISCREEN HIV-1 AND HCV TESTING FOR USE WITH SAMPLES UP TO 10 YEARS OLD

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\textsuperscript{1}National Serology Reference Laboratory, Australia.

Introduction: Samples for HIV and HCV testing, including those collected from donors of tissue and cord blood, may be stored frozen for long periods before testing. The present protocol was developed to investigate the testing by Roche AmpliScreen of plasma samples that have been stored frozen for extended periods of time. Concerns over prolonged storage relate to the potential instability of RNA, and therefore the potential for a falsely negative result in a positive sample that has been stored.

Objective: To show if samples up to ten years old give the same qualitative HIV-1 and HCV RNA results as were obtained when originally tested.

Methods: Eighteen HIV-1 RNA positive and 18 HCV RNA positive samples were tested in the relevant Roche AmpliScreen tests. Nine RNA negative samples were tested in both AmpliScreen tests. The samples had been collected between 1994 and 1999 and had since been stored frozen at -20\degree C or -70\degree C. There were paired aliquots of some samples, one aliquot having been stored at -20\degree C, the other at -70\degree C. In addition 18 replicates each of the same HIV-1 RNA or HCV RNA positive samples were tested in the relevant Roche AmpliScreen tests. These plasma samples were members of a well-characterised commercial dilution series and had been stored at -70\degree C since 2000. All testing was performed according to the test kit manufacturer’s instructions. Testing on samples that returned invalid results was repeated. The negative samples were each expected to give a negative result and positive samples were each expected to give a positive result.

Results: Sixteen of eighteen HIV-1 RNA positive samples tested gave positive results in the Roche AmpliScreen HIV-1 test. Seventeen of eighteen HCV RNA positive samples gave positive results in the Roche AmpliScreen HCV test. All nine RNA negative samples gave negative results on both the HIV-1 and the HCV AmpliScreen tests. All 18 replicates of each of the HIV-1 RNA and HCV RNA samples were positive on the relevant Roche AmpliScreen tests.

Discussion: Thirty-six samples collected from different individuals and positive for HIV-1 or HCV RNA were tested in the relevant HIV-1 or HCV AmpliScreen tests. Testing results showed that 33 of the 36 were positive on initial testing. Of the three that were not positive, two were negative and one was repeatedly inhibited. All three samples had been stored at -20\degree C. Repeat testing on two of the samples using aliquots that had been stored at -70\degree C gave the expected positive results. An aliquot of the third sample stored at -70\degree C was not available. The viral loads of the three samples that gave aberrant results were all < 20,000 RNA copies per mL when tested approximately 10 years ago.

Conclusion: Although the numbers of samples available for this study were limited, the data shown suggest that the Roche AmpliScreen tests are appropriate to detect seroconversion in samples that have been stored at -70\degree C or less for up to 12 years.
FRIDAY 28TH AUGUST 2009

DEMAND AND SUPPLY FOR HIV THERAPIES: MAKING THE GLOBAL MARKET FOR GENERIC ANTIRETROVIRAL PHARMACEUTICALS

Funder J
GBS Venture Partners
THE ANALYSIS OF FACTORS AFFECTING ON KCDC HIV EQAS BY SOURCES OF EQAS PANELS IN 2008

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Introduction/ Objectives: KCDC has operated HIV EQAS program for HIV laboratories twice per year as a provider of proficiency testing programs. We analyzed the results of KCDC HIV EQAS under different sources of EQAS panels to apply following EQAS panel productions.

Methods: HIV EQAS panel consisted of 2~3 undiluted positive and negative samples with HIV antibody. The diluted positive sample with target values ([(BioMerieux] Vironostika HIV-Uni-Form II plus O, ratio 3~5) was additionally supplied to HIV laboratories at university hospitals. The raw-material (ACD1 plasma) was treated with CaCl$_2$ to convert the plasma to serum for the first EQAS in 2008. However the second EQAS panels were produced without CaCl$_2$ treatment as plasma. University hospital laboratories (1$^{st}$; n=61, 2$^{nd}$; n=63) and hospital/clinic laboratories (1$^{st}$; n=149, 2$^{nd}$; n=155) participated in this program through on-line. HIV ELISA results were analyzed by sample sources (plasma/serum, undiluted/diluted) to assess testing accuracy using analytical sensitivity and specificity.

Results: The analytical sensitivity of undiluted positive sample was 99.6% (559/561) for the first EQAS, 100% (386/386) for the second one, while the analytical specificity of negative samples was 90.0% (550/561) for the first EQAS, 99.7% (385/386) for the second one respectively. The false-positive was higher in CaCl$_2$ treated samples than plasma. Especially the 4 cases of false positive results were shown in one testing kit. The analytical sensitivity of diluted positive sample was 94.7% (118/122) for the first EQAS, 92.1% (116/126)) for the second one; the 14 cases of false negative results were mainly shown in two testing kits.

Conclusions: The effect of defibrination on analytical sensitivity was not significant for undiluted strong positive samples. However, the false positive results were found in negative samples for some test kits. It may be due to micro-fibrin generated on defibrinated serum. The diluted samples can be used as replacement of positive sample, but it would be more appropriate to be applied for comparison of the HIV testing accuracy among laboratories using the same test kit.
USING PHOTOGRAPHED RAPID HIV TEST RESULTS IN A PILOT EQAS

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Introduction: Effective management of HIV testing requires accurate testing for accurate diagnosis. Because larger numbers of HIV tests need to be performed in varied situations, operators without laboratory experience and working in settings outside laboratories will increasingly be performing testing. HIV rapid diagnostic tests (RDTs) are well suited for maximising testing in various settings including health clinics and by outreach teams in the field. RDTs are immunoassays that are accurate, quick, and easy to use, but their interpretation may sometimes present difficulties. Therefore, it is crucial to maintain the quality of testing. External quality assessment schemes (EQAS) are designed to ensure the quality of the whole testing process from sample reception to reporting of the result. We conducted a pilot EQAS using photographed results of RDTs, to assess the accuracy of interpretation, which is the end-point of the testing process. Our aims were 1) to assess whether photographed results of RDTs could be used in EQAS to increase operators’ proficiency, and 2) to tailor and evaluate training in interpretation for the particular group involved in the assessment.

Methods: Four RDTs were selected: Advance Quality HIV Rapid Test (Intec Products), Determine HIV 1/2 (Inverness Medical), Insti HIV-1/HIV-2 (bioLytical Laboratories) and SD Bioline HIV 1/2 3.0 (Standard Diagnostics Inc). The interpretation of results of the chosen kits involved reading of dots or lines; SD Bioline was designed to differentiate HIV-1 from HIV-2, but the other three RDTs were not. Panels of 10 samples were designed to assess the ability of participants to recognise common patterns illustrated in kit inserts, and to read the presence of faint lines or dots. There were three types of participants: non-laboratory personnel (n=19), laboratory personnel (n=22) and pathologists (n=34). Each participant was identified by a confidential ID, and they were surveyed about their previous experience with RDTs generally and with the specific tests used. Participants were given, for each kit, 10 photographed RDT results to interpret, along with photographed positive and negative sample results, in two sessions. Answers were given to participants after each session and no corrections were allowed. In the first session, participants were required to complete the scoring sheet without discussion. In the second session, participants received a simplified kit insert for each kit, and a 5-min tutorial on interpreting 2-line and 3-line RDTs. The same photographed samples were provided in the second session, but in a different order, and discussion before reporting was allowed. All reported results were compared with the reference results to establish the accuracy of interpretation. Differences between the results reported by groups of participants, and differences between the types of RDT, were assessed statistically using the Chi square test.

Results: Before training, there were significant differences in the accuracy of interpretation by the three groups of participants with all RDTs except Determine; presumably participants were more familiar with Determine owing to its extensive use in the region. After training, the only remaining significant differences in interpretation among the three groups were with SD Bioline. This suggests that training standardised the quality of interpretation of results of Advance Quality, Determine and Insti by the three groups. Over all, training increased the correct interpretation for the four RDTs as follows: SD Bioline from 44% to 82%; Determine from 85% to 98%; Advance Quality from 86% to 99.5%; Insti from 87% to 99%; p<0.001. Non-laboratory personnel made the least accurate interpretations: 74% for the subtyping RDT SD Bioline, and 96% for the non-subtyping RDTs, after the training session. The relatively poorer accuracy of interpretation of SD Bioline highlighted a need for further training of the participants in this study in the interpretation of the results of this test.

Conclusion: This study demonstrates that using photographed RDT results can improve the accuracy of interpretation of results, and also provide information to allow tailoring of appropriate training for specific participants.
EXTERNAL QUALITY ASSESSMENT SCHEMES FOR HIV-1 VIRAL LOAD AND HCV VIRAL LOAD NUCLEIC ACID TESTING, 2008

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\(^1\)National Serology Reference Laboratory, Australia

Introduction: The National Serology Reference Laboratory, Australia (NRL) provides Quality Assurance (QA) programmes to laboratories that test for blood-borne infectious diseases by serology and/or nucleic acid testing (NAT). One of the components of QA is External Quality Assessment Schemes (EQAS). The aims of EQAS are to: monitor the performance of the laboratory testing process, assess the quality of assays used, assist in identification and resolution of testing problems, facilitate an information exchange network and educate participants in QA issues.

Objective: To examine the performance of the assays used by the participants of the NRL HIV-1 Viral Load (VL) and HCV VL EQAS in 2008.

Methods: Each HIV-1 VL and HCV VL EQAS consisted of three five-member panels. These panels were composed of well characterised plasma samples designed to mimic normal human samples. EQAS samples contained known viral loads designed to test the linearity, intra- and inter-run reproducibility and geno/subtype quantification of VL assays used. Participants were asked to test the panels in the same manner as routine samples, in order to reflect the day-to-day functions of the laboratory. The results of testing were submitted to NRL via an internet-based application called DigitalPT (www.digitalpt.com, HealthMetrix, Vancouver). Test results submitted by participants testing in the same test methods were grouped for analyses (designated as peer groups). The results were log\(_{10}\) transformed and peer group means were calculated. Outlying results were identified using Tukey’s Outlier Filter and removed prior to statistical analyses. Results that differed by more than ± 0.5 log\(_{10}\) from the peer group mean were identified as aberrant and also removed prior to statistical analyses.

Results: In 2008, 27 participants from nine countries testing using eight different test methods and 22 participants from four countries testing using six different test methods participated in the HIV-1 VL and HCV VL EQAS, respectively. Two HIV-1 VL and two HCV VL results were identified as outlying. One HCV VL result was aberrant (i.e. did not agree with the reference results). Outlying and aberrant results were removed from analyses.

The inter-run reproducibility of the HIV-1 VL assays used showed that the results differed by no more than 0.48 log\(_{10}\). HIV-1 VL intra-run reproducibility showed that the results differed by no more than 0.41 log\(_{10}\). The inter-run reproducibility results of HCV VL assays did not differ by more than 0.56 log\(_{10}\) while intra-run reproducibility showed that the results did not differ by more than 0.3 log\(_{10}\).

Lines of best fit for linearity suggest that the assays utilised in the HIV-1 VL and HCV VL EQAS reported viral loads that displayed a linear relationship with respect to the changing viral loads.

Different HIV subtypes and HCV genotypes included in each panel were detected in the various assays and instances of quantification bias were not observed.

Conclusion: EQAS provides participants with an independent mechanism for assessing both laboratory and assay performances. The data from the two EQAS analysed suggest that the assays utilised by the participants are behaving in the manner described by the assay manufacturers.
FRIDAY 28TH AUGUST 2009

A QUALITY ASSESSMENT SCHEME WITH A SCORING SYSTEM TO STANDARDISE THE OUTCOME OF HIV GENOTYPIC RESISTANCE TESTING IN A GROUP OF ASIAN LABORATORIES

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Objective The TREAT Asia Network is evaluating HIV drug resistance (HIVDR) in a comprehensive capacity building programme in the Asian region. The aim is to facilitate surveillance for transmitted HIVDR across subtypes and to monitor the development of acquired HIVDR in individuals receiving antiretroviral therapy. The TREAT Asia Quality Assessment Scheme (TAQAS) aims to standardise HIV genotypic testing (genotyping) outcomes and to permit meaningful and confident comparison of results from clinics and testing centres throughout the region.

Methods Since 2006 six TAQAS panels have been tested by up to 18 Asian laboratories and a reference laboratory. Panels of 5 samples were tested. Samples were either plasma from HIV-infected persons or amplified virus spiked in to negative plasma. Participating laboratories performed genotyping using their standard protocols. Quality assessment (QA) of all participants’ results, including the reference laboratory’s results, included comparison of nucleotide sequences, detection of drug resistance mutations (DRMs), and interpretation of HIVDR. A system was applied to the outcomes of the 6\textsuperscript{th} TAQAS panel whereby participant’s performance outcomes were scored.

Results Phylogenetic analysis confirmed accuracy and lack of contamination in all nucleotide sequences included in the QA analysis. High level nucleotide sequence agreement (>98%) and detection of DRMs (>95%) was achieved by most participants testing their second and, in some cases, first TAQAS panel. The scores given to 5 of 18 participants testing TAQAS 6 reflected their suboptimal detection of DRMs. A high level of agreement (up to 100%) in interpretation of HIVDR was achieved when all participants used the same system (www.HIVDB.stanford.edu) for interpretation, regardless of sequencing methodology.

Conclusion High quality genotyping results, comparable to the reference laboratory’s test outcomes, were achieved among 18 Asian laboratories. Some differences in the outcomes of sequencing, sequence editing, detection of DRMs, and interpretation of HIVDR were identified. Despite the use of different sequencing schemes and platforms, the use of a common interpretation system yielded agreement on interpretation of HIVDR across participating laboratories. A scoring system was found to be effective in summarising and quantifying participants’ outcomes on TAQAS panels. Consistent results with respect to the detection of mutations and interpretation of HIVDR allows meaningful comparisons across drug regimens, clinical sites, HIV-1 subtypes and sequencing methods to identify the causes and consequences of virologic failure.

Key words: HIV genotyping, resistance, quality assessment