

THE FUTURE OF MOLECULAR PROFICIENCY TESTING: STANDARDISATION, BEST PRACTICE, AND NEW TECHNOLOGY SURVEILLANCE

Paul Wallace MSc, PhD, QCMD.

Molecular Diagnostics has become the bench mark technology for the detection and determination of an increasing number of infectious diseases, many of which have only come to light within our live time over the last fifty years.

The speed at which molecular diagnostics have been introduced into the routine diagnostic laboratory setting has only been surpassed by the rapidity at which new advances in molecular technologies are developed.

Molecular diagnostics redefines where classical microbiology fits into the modern clinical diagnostic algorithm. By decreasing the time required for results from days to minutes, quantitation, as well as offering more specific and more sensitive assays over an increasing range of pathogens. This effectively advances the clinical decision making process and promotes fast and effective medical outcome.

However, this can only be true if the molecular tests are able to provide accurate, reliable, and robust results. Under current clinical laboratory regulation and the quality of molecular tests are reviewed and monitored on a continuous basis through the laboratories internal quality procedures and through participation in annual EQA / PT schemes.

Hence there is increasing demand on the proficiency testing provider to ensure that the schemes they provide are up to date with the current innovations within the molecular field. In addition, the schemes also need to consider both the analytical aspects of the molecular assay and its utility in the clinical setting. As a result, the proficiency test provider must be able to provide a balanced scheme that meets both regulatory requirement and provides educational feedback. The educational aspects of the scheme must consider the current clinical range of pathogens, patient groups, clinical sample type, intended use of the assay, and the influence of infectious disease prevalence on the clinical predictive value of the assay.

With the increasing availability of novel antiviral / microbial therapies and the convergence of diagnostics and therapy into theranostic approaches there will be further reliance on molecular quantitation and rapid pathogen characterisation. Within the clinical laboratory this will result in an increased demand for standardisation and quality. The role of the proficiency testing provider will remain to support these requirements and help facilitate further standardisation. In addition, the future independent proficiency testing provider may be well placed to support and expedite clinical guideline development as well as play a pivotal role with regards to the surveillance monitoring of new molecular technologies,

It is therefore important to reflect on what we have learnt to date and where appropriate apply these to findings to the next generation of molecular proficiency testing schemes to ensure they are capable of matching the quality challenges of the future.

NEW DEVELOPMENTS IN DIRECT SAMPLE-TO-ANSWER PCR

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Introduction:

Typical molecular diagnostic methods include steps required to isolate and purify nucleic acids from a given specimen matrix. Published studies have demonstrated that detection of target nucleic acids can be achieved without using extraction methods, but these studies typically required a dilution of the original specimen that results in decreased assay sensitivity. We have recently shown that direct detection of nucleic acids from patient samples is possible without specific dilution steps.

Objectives

We sought to prove the efficacy of this technology in a clinical setting by performing studies in a number of different facilities, including large testing centres in Australia. Results from samples run with conventional methods using extraction were compared to direct detection chemistry to determine the sensitivity and specificity of the direct methodology.

Methods:

Specimens from patients with clinical signs of disease were assayed using Simplexa direct chemistry (without nucleic acid extraction) using the 3M integrated cycler, and results were compared to those obtained using conventional methods. Organisms and specimen types tested included the following: nasopharyngeal or throat swabs (in transport media) for the detection of *Bordetella pertussis*, *Bordetella parapertussis*, influenza A, influenza B, respiratory syncytial virus, and Group A *Streptococcus*, swabs of lesions in transport media for varicella-zoster virus and herpes simplex virus, stool specimens for *Clostridium difficile*, and blood samples for detecting human genomic DNA.

Results:

The direct testing method was shown to be efficacious for a number of different targets, with good positive and negative concordance with methods using extraction. The direct methodology was able to be used in both high throughput and near-patient formats. A potential drawback from high-throughput direct detection is that it utilizes a relatively small sample volume, and it does not allow for concentration of purified nucleic acids, which could potentially limit assay sensitivity. A newly designed consumable with greater sample volume capacity should provide further improvements to assay sensitivity.

Conclusions:

Direct detection chemistry provides the opportunity to simplify molecular testing and reduce cost and time to result. Systems using direct methods will facilitate near patient testing, thereby leading to improved patient management, and improved patient outcomes.

ROLE OF THE WHO COLLABORATING CENTRE FOR REFERENCE AND RESEARCH ON INFLUENZA IN SURVEILLANCE AND CONTROL OF INFLUENZA IN AUSTRALIA

Kelso A

WHO Collaborating Centre for Reference and Research on Influenza, Australia

Since its inception in the late 1940s, the World Health Organisation (WHO) has paid special attention to the surveillance of influenza viruses infecting humans because of their potential to cause epidemics and occasional pandemics of devastating scale and severity. Today this task is undertaken by the WHO Global Influenza Surveillance and Response System, a growing network that now comprises 138 WHO National Influenza Centres, 5 WHO Collaborating Centres and several regulatory agencies. The WHO Collaborating Centre for Reference and Research on Influenza in Melbourne receives clinical specimens and virus isolates from diagnosed influenza cases submitted by laboratories around the Asia-Pacific region. At the Collaborating Centre, viruses undergo subtyping, antigenic analysis, gene sequencing and antiviral drug susceptibility testing. Unusual viruses are taken through more detailed investigation, for example to determine the role of specific mutations in antiviral drug resistance. Clinical specimens are also used for the direct isolation of candidate vaccine viruses in embryonated hen's eggs. Twice each year, representatives of the Collaborating Centres, regulatory agencies and other expert groups draw on the previous 6 months of data to develop the WHO's recommendations on suitable viruses for inclusion in influenza vaccines for the next southern or northern hemisphere winter.

Through its role in this global network and its close links with laboratories in all the Australian states and territories, the Melbourne WHO Collaborating Centre contributes to the control of influenza in Australia in several ways – in particular by monitoring the characteristics of influenza viruses circulating in the community and hospitalised patients, by providing recent viruses for the updating of seasonal influenza vaccines, by undertaking serological analyses of population immunity, and by contributing to government policy on seasonal and pandemic influenza. As shown in 2009, the Centre also maintains technical readiness to detect and characterise novel influenza viruses and to assist other laboratories in the early stages of an influenza pandemic.

RTIplex: A MULTIPLEXED AND AUTOMATED PCR BASED TEST FOR 16 UPPER RESPIRATORY TRACT ANALYTES

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Genera Biosystems Ltd., Australia

Introduction:

Genera Biosystems is an Australian biotechnology company that develops multiplex assays using AmpaSand® microsphere technology. The RTIplex assay has been designed to qualitatively detect the presence of Influenza A, Influenza B, Influenza A H1N1(2009), Influenza A H5N1(Avian), Respiratory Syncytial Virus types A and B, Human Parainfluenza Virus type 1, Human Parainfluenza Virus type 2, Human Parainfluenza Virus type 3, Human Parainfluenza Virus type 4, Human Metapneumovirus, Adenovirus (types B,C and E), Human Rhinovirus *spp.*, *Bordetella pertussis*, *Chlamydomphila pneumoniae* and *Mycoplasma pneumoniae* within purified total nucleic acid preparations from throat/nasopharyngeal swab specimens.

Methods:

Up to 88 unknown test samples of purified nucleic acid per 96 well reaction tray were subjected to a Solid-Phase PCR in an Eppendorf MasterCycler in which amplified product is simultaneously labeled with a fluorophore and loaded via hybridization onto distinct populations of silica microspheres. PCR plates were then loaded onto Genera's fully automated 'Sirocco' processing station which serves to wash beads and then analyze bead populations for the presence of fluorophore. The resulting data was analysed using Genera's proprietary QPlots software.

Results:

Using known copy numbers of either plasmid DNA or in vitro purified RNA corresponding to defined regions of the viral or bacterial genome of interest, the limit of detection (LOD) for the majority of analytes on the RTIplex panel was shown to be ≤ 125 copies. Furthermore, correct positive calls were made for each analyte in samples containing a pool of all 15 respiratory targets at, or near their LOD levels, as well as an internal RNA control, demonstrating the true multiplex nature of this assay. The total assay time from PCR set up to results was approximately 4h.

Conclusions:

RTIplex is a novel assay for rapidly assessing clinical samples for a wide range of known respiratory pathogens. RTIplex is currently undergoing prospective and retrospective clinical trials at both Healthscope Pathology and the Royal Women's Hospital to assess the usefulness of this assay in the Pathology setting. Genera will continue to work with Pathology providers (both National and International) to further clinically validate RTIplex.

DEVELOPMENT OF A NEW MULTIPLEX ASSAY FOR THE SUBTYPING OF INFLUENZA A VIRUS; A(H1N1), A(H1N1)pdm09 AND A(H3N2)

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Introduction:

The Virology, Molecular Diagnostics Laboratory uses high throughput real time PCR based methods for the detection of respiratory viral pathogens. As part of the respiratory screening protocol, all samples are tested in real-time for the Influenza A Matrix gene (present in all Influenza A strains) and the Haemagglutinin gene specific for A(H1N1)pdm09. Influenza A(H1N1)pdm09 had been the dominant subtype since the 2009 pandemic, almost completely replacing the A(H1N1) seasonal strain. However, late in the Influenza season of 2011 A(H3N2) emerged and is now the dominant subtype circulating in the population. This change in the dominant circulating subtype forced the laboratory to reassess its real-time typing strategies.

Objectives:

To develop a new TAQMAN based multiplex assay, to replace our current Influenza A(H1N1)pdm09 typing assay with one which detects Influenza A(H1N1)pdm09, A(H1N1)seasonal, and A(H3N2) in a single reaction vessel using the laboratories standard amplification parameters.

Methods:

Sequence data spanning 11 years of 852 haemagglutinin genes was obtained from the NCBI Influenza Virus Resource database. Oligos were designed to amplify regions homologous to each genotype using conserved polymorphisms within the primers and probes to confer specificity.

432 total nucleic acid extracts from routine patient respiratory specimens and 30 Influenza A QAP samples were retrospectively tested using the new Influenza A Typing master-mix. In addition, 532 samples were tested in parallel with our current A(H1N1)pdm09 typing assay during routine testing.

Results:

Using the multiplex PCR, successful typing of Influenza A in the validation samples was increased from 21.4% using the single target A(H1N1)pdm09 PCR to 95.2% using the Multiplex PCR; 21.4% A(H1N1)pdm09, 0% A(H1N1) and 73.8% A(H3N2). All QAP results were concordant.

Conclusions:

The new multiplex typing assay provided a significantly improved real-time identification of circulating influenza strains. This assay has now replaced our Influenza A(H1N1)pdm09 typing assay in the laboratory.

THE LONG ROAD TO HIV POINT OF CARE TESTING (or don't throw the surveillance out with the syringe)

Levinia Crooks

Australasian Society for HIV Medicine, Australia

Introduction:

The Australasian Society for HIV Medicine formed in the mid 1980s in response to HIV and a growing need to better understand HIV within the medical and research community in Australia. It soon became involved in policy development and represented on policy committees. ASHM has applied its experience in the HIV field to the viral hepatitis and sexual health sectors. ASHM now hosts an HIV, hepatitis B and hepatitis C testing portal and chairs Expert Reference Panels in each of these areas which establish and regularly review testing policy.

Objectives:

This presentation reviews the development of the 2011 National HIV Testing Policy. It makes reference to the strategy undertaken in HCV and HBV areas to, where possible and feasible, have a consistent treatment across policies. The 2011 HIV Testing Policy broke with tradition and changed the hitherto embargo on point of care testing in Australia. The reasons for this are described.

Methods:

The presentation explores the caveats put on the introduction of point of care testing, including the training and regulation of operators and the steps being taken to get point of care HIV tests assessed for use in Australia. This work is currently underway and the presentation will, to the extent that this is possible in August, reveal the progress of Therapeutic Goods Administration (TGA) application. It also reviews policy and practice around PoCT in overseas settings.

Results:

The presentation will examine other strategies in place to maximise the uptake of HIV Testing using conventional laboratory based techniques and some of the procedures put in place to reduce the burden of HIV testing while maintaining high quality testing. Just what the trade-offs are with PoCT is still being debated and this presentation uses international comparisons to explore these.

Conclusion:

HIV Testing in Australia has always been high quality and we believe there has been considerable coverage of those people needing testing. Some recent research suggests that much transmission of HIV occurs from people who have themselves only recently become infected. Many approaches have been tried to get people at higher risk of HIV to test more frequently. PoCT will not pick up people at the earliest points of sero-conversion. But, if PoCT are more acceptable to those people unknowingly living with HIV who are reluctant to test using conventional tests, then this must be a good thing.

TRIALLING A POINT-OF-CARE (RAPID) HIV TEST IN A SEXUAL HEALTH CENTRE.

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2 Melbourne Sexual Health Centre, Alfred Health, Melbourne

Mathematical modelling suggests that increasing the frequency of HIV testing among homosexual men could reduce HIV transmission. Surveys and international experience indicate many individuals prefer rapid testing for HIV. This study aims to determine whether access to rapid HIV tests in a clinic increases the frequency of HIV testing among homosexual men.

We are conducting a randomised controlled trial where men reporting sex with men attending Melbourne Sexual Health Centre for HIV testing are offered access to point-of-care HIV testing using the Determine HIV1/2 Ag/Ab Combo test (Alere) over an 18 month period. The control arm consists of men with access to usual laboratory-based HIV serology over the same period. Participants complete a short questionnaire at baseline, 6, 12 and 18 months about HIV testing and their sexual behaviour. HIV tests reported at other clinics are confirmed by contacting those clinics.

Four hundred men were enrolled from September 2010 to March 2011 with 201 randomised to the rapid test intervention arm and 199 to the conventional serology control arm. In the intervention and control arms respectively, median ages were 30 and 29, and the median time since last HIV test was 6 months in both arms. The final patient will complete the study in early September 2012. Interim data on test results will be presented.

Some very faint reactive results, which were ultimately unconfirmed, presented challenges for testing staff who were uncertain how best to communicate these to patients. These were particularly stressful for patients, some of whom have declined further rapid tests. The public health benefits of rapid tests need to be weighed against their disadvantages, both of which vary according to the clinical environment and population prevalence of HIV.

Conflict of interest declaration: rapid tests were provided by the manufacturer Alere.

COMMUNITY HEALTH TO PHARMACY SHELF. THE DEVELOPMENT OF NON INVASIVE, RAPID HIV & HCV TESTS AND THEIR ROLE IN THE FIGHT AGAINST HIDDEN EPIDEMICS.

Reid.B

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“Silent” diseases such as HIV and HCV pose a significant global health problem. Whether these diseases benefit from stigmatization or lack of awareness, their spread continues, cloaked from accurate laboratory diagnosis by the fact that the infected individual is simply unaware of the ongoing infection.

The use of non invasive, accurate, rapid tests such as OraQuick HIV and HCV are helping to diagnose those undiagnosed in many areas around the world. Much discussion has taken place around utilisation of these and similar rapid tests, their accuracy - particularly for HIV (probably more than any other in vitro diagnostic test), algorithms for their use and the site at which the test should take place – in the laboratory or near patient. However, whatever the test, it is now widely accepted that testing is the foundation of disease diagnosis and subsequent linkage to treatment and care. Timely diagnosis can ensure that Antiviral therapy is initiated early, when its clinical benefits are greatest.³

In many countries with well developed laboratory infrastructure where detection of “silent “ diseases such as HIV and HCV have relied solely on laboratory analysis from an individual’s blood sample, it is now becoming recognised that significant value lies in the adoption of decentralised “point of care” testing (particularly in high prevalence community settings) utilising highly accurate rapid tests. Though laboratory methods of analysis may be highly accurate, the process relies on patient presentation and significant numbers of those being found as being HIV positive are presenting in late stages of infection² and significant numbers of patients may not receive their results at all⁴

In order to reach those individuals that have not attended a testing centre, workers in a number of countries are investigating and adopting novel means of testing; from provider based HIV Counseling and Testing (HCT) performed in the home and increasingly, supervised self testing¹; from Dental or Pharmacy testing to the introduction of “over the counter” models for providing test opportunities to those who would not attend a testing centre. In such circumstances a simple yet accurate, non invasive and non infectious test is highly desirable.

As comfort in the accuracy of the non-invasive OraQuick Rapid HIV test has grown, so the recognition of the simplicity of use and resulting suitability as a self test or home test has evolved. On 15th May 2012, the FDA’s Blood Products Advisory Committee gave its unanimous support to the commercialization of the first, oral fluid, In-Home self test for HIV.

Simple, Accurate and non-invasive, the OraQuick HIV & HCV rapid tests provide a convenient alternative to laboratory screening for detection of these “silent” diseases; ideal for near patient testing and community based screening and valuable tools in the fight against the spread of these global epidemics.

1. Feasibility, Accuracy, and Acceptability of Using Oral HIV Test Kits for Supervised Community-level Self-testing in a Resource-poor High-HIV Prevalence Setting: Blantyre, Malawi
Augustine Choko¹, N Desmond^{1,2}, E Webb³, K Chavula¹, S Mavedzenge³, S Makombe⁴, B Squire², N French³, V Mwapasa¹, and E Corbett^{1,3}
¹Malawi-Liverpool Wellcome Trust Clin Res Prgm, Blantyre; ²Liverpool Sch of Tropical Med, UK; ³London Sch of Hygiene and Tropical Med, UK; and ⁴Ministry of Hlth, Lilongwe, Malawi: Abstract presented at CROI 2011
2. dtb.bmj.com DTB CME/CPD accompanying Vol 49 | No 8 | August 2011
3. World Health Organization (2010) Towards universal access: scaling up priority HIV/AIDS interventions in the health sector, progress report 2010.
4. CDC MMWR June 25, 2004/53(24):523-526

ALGORITHM FOR THE SEROLOGICAL DIAGNOSIS OF HIV - A COMPARISON OF THREE HIV POINT OF CARE TESTING KITS FOR SECOND LINE TESTING

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Introduction:

The human immunodeficiency viruses (HIV) are enveloped single-stranded RNA viruses in the *Retroviridae* family. There are 2 major viral species, HIV-1 and HIV-2, with HIV-1 being the more virulent species with a worldwide distribution while HIV-2 is less pathogenic and has a more limited geographic distribution. Based on sequence diversity in the HIV-1 *gag* and *env* genes, HIV-1 can be further divided into groups M (major), O (outlier), and N (non-M, non-O). Screening tests are widely available to detect antibody to both HIV-1 and HIV-2. Such tests can be extremely sensitive but have a potential for being less specific, leading to false positive interpretations. Independent supplemental tests of high specificity are therefore necessary to further confirm the presence of antibodies to HIV-1 and/or HIV-2. At present the Virology laboratory, Pathology North Hunter which is a HIV reference laboratory undertakes:-

1. Screening HIV 1/2 antigen/antibody test on the ARCHITECT i2000. This is a random access assay using CMA technology which detects P24 Antigen and antibody to HIV simultaneously
2. Supplemental HIV BioRad GenScreen antibody test. This is a manual ELISA taking about 3 hours to complete. This assay detects HIV-1/2 antibody
3. Confirmatory BioRad GenScreen HIV-1antigen testing, using neutralisation assay. This is manual ELISA assay which takes 3 hours. This assay detects HIV-1 core antigen(P24)
4. Confirmatory HIV western blot MP Diagnostics HIV BLOT 2.2, detects antibodies to HIV-1 and HIV-2

Objectives:

To assess the validity of using a point of care test (PoCT) which detects only antibodies in established HIV infections, and replace the BioRad Genscreen™ HIV-1/2 Version 2 ELISA HIV antibody supplementary test. It is possible that a three hour labour intensive ELISA assay could be replaced with a point of care test which is cost effective and give a result in a few minutes. The study will assess the sensitivity, specificity and ease of use of three third generation PoCT kits using stored sera at the testing laboratory.

Method:

Three point of care test kits were tested; BioRad Multispot HIV-1/HIV-2 Rapid Test, Alere Determine™ HIV-1/2, OraQuick ADVANCE® HIV 1/2 in this study. 90 sera were utilised consisting of 36 sera which were confirmed positive HIV by Western Blot or HIV p24 antigen neutralisation assay, 3 had indeterminate HIV status after supplementary/confirmatory testing and were excluded from final analysis and 51 were classed as negatives. 19 of the 51 negatives were false reactive by the Abbott ARCHITECT HIV Ag/Ab Combo assay.

Results:

Assay	Sensitivity	Specificity
BioRad Multispot HIV-1/HIV-2 Rapid Test	97.2%	100%
Alere Determine™ HIV-1/2	97.2%	92.2%
OraQuick ADVANCE® HIV 1/2	94.4%	98.0%
BioRad Genscreen™ ELISA	97.2%	89.2%

Conclusion:

The BioRad Multispot HIV-1/HIV-2 Rapid Test and OraQuick ADVANCE® HIV 1/2 PoCT are a suitable second line test to replace the current BioRad Genscreen™ HIV-1/2 Version 2 ELISA HIV antibody confirmatory test which has a sensitivity of 97.2% and specificity of 89.2%. The OraQuick false reactive specimen was ARCHITECT HIV Ag/Ab Combo screening negative hence would not be progressed to 2nd tier HIV confirmatory testing using the this testing laboratory's algorithm.

None of these 3 PoCT assays is recommended for use as a frontline assay in the laboratory as early seroconverting patients who are HIV P24 antigen positive only would be missed by these kits. The HIV Western Blot is still the "gold standard" assay for determining HIV status. For HIV PoCT to be a frontline test an antigen component needs to be added to the test kit.

Comparing the BioRad and the OraQuick for technical use, places the OraQuick as the preferred PoCT due to ease of use for an operator and favourable storage due to non refrigeration requirements.

EVALUATION OF THE IMMY CRAG LATERAL FLOW ASSAY FOR DETECTION OF CRYPTOCOCCAL ANTIGEN

Robert G Gibb, Susan P Clarke

Objective

The new IMMY CrAg Lateral Flow Assay for Detection of Cryptococcal Antigen (IMMY) was compared against the Meridian Cryptococcal Antigen Latex Agglutination System (CALAS) assay.

Methods

Sera and CSF that had previously been tested on the CALAS assay were tested using the IMMY assay. The IMMY assay required no sample pre-treatment making it a very quick assay to perform. Samples negative for cryptococcus antigen could be resulted in 10 minutes.

Results

Sixty-one samples from patients without cryptococcosis were tested and all were negative by both assays. Of the 15 samples from patients with cryptococcosis, all were detected by the IMMY assay but two were not detected by the CALAS assay. Galactomannan positive samples and samples from patients with candidiasis were also tested using the IMMY Assay to ensure its specificity. The IMMY assay demonstrated equal specificity to the CALAS assay but had greater sensitivity. The titres of positive samples were from 2 to 64 times higher with the IMMY assay than the CALAS assay. The IMMY assay was also shown to be superior for testing CSF samples with high concentrations of protein.

Conclusions

Overall, it was determined that the IMMY CrAg Lateral Flow Assay had greater sensitivity and its simple and rapid procedure made it the preferred assay.

NEW DIRECTIONS IN HIV MONITORING

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Alere Inc.

Introduction:

Access to high quality diagnostic services is one of the key barriers to improved HIV care in resource constrained settings. Despite significant efforts by ministries of health and the global health community early infant diagnosis, viral load and CD4 testing have remained persistently challenging to implement. This is partly due to the fact that the majority of tests on the market remain laboratory based, requiring skilled staff, technically complex instrumentation and reliable utilities infrastructure, some or all of which can be lacking in many settings. Where these needs are met and laboratories are functioning well they often cater for a relatively local population meaning that those outside urban centres may still not have access. While there remains a need to continue to improve laboratory services in resource constrained settings it is also generally accepted that high quality and robust testing options are needed at the point of care if HIV treatment access goals are to be achieved.

Discussion:

The Alere Pima™ Analyser and CD4 Cartridge were developed specifically to address the need of decentralised CD4 testing while maintaining laboratory-standard quality of results. The portable battery powered analyser and self contained cartridge have been evaluated and deployed in dozens of countries. With point of care testing and improved result turn around times of approximately 20 minutes, a number of studies have demonstrated improvements in rates of enrolment into care, retention in care and earlier treatment initiation than would have been the case with conventional testing, phenomena which are expected to continue to improve patient outcomes as uptake increases.

A similarly targeted point of care molecular testing platform is also under very late stage development by Alere Technologies. The first assay to be available when the platform is released is a fully quantitative HIV viral load assay. Early data indicate that the HIV viral load assay performs extremely well and may offer advantages over conventional assays for the purpose of treatment monitoring. The assay has also been assessed for the early infant diagnosis and has so far performed as well as conventional assays. Being a molecular platform capable of performing both PCR and isothermal amplification and with an amplicon detection system based a microarray and a single fluorophore, the system is extremely versatile and assays for many other infectious and non-infectious diseases are in active development.

TRAINING AND TEACHING HEALTH PROFESSIONALS IN POINT-OF-CARE TESTING: NATIONAL AND INTERNATIONAL EXPERIENCES

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A point-of-care test is a pathology test performed in a clinical setting at the time of patient consultation, generating a test result that is used to make an immediate informed clinical decision regarding patient care. The scope and application of point-of-care testing has expanded rapidly both within Australia and at a global level, as the array of test profiles, advances in technology and clinical demands for improved turnaround of pathology results continue to evolve.

Education and training of medical scientists and practising health professionals is a crucial component of building a workforce capable of conducting quality-assured point-of-care testing, particularly in non-laboratory, community-based clinical settings.

Innovative methods for delivery of training and competency assessment in point-of-care testing will be discussed using working examples from community-based point-of-care programs managed by the author's unit both nationally and internationally. Across these models, different health professional groups including medical, nursing and Indigenous health practitioners have been systematically trained in point-of-care testing using flexible training modes (including state-of-the art web-based technology), with training resources that are specifically tailored and/or culturally safe for the target audience. The development and delivery of point-of-care training programs provides innovative and challenging opportunities for today's medical scientists.

Integration of teaching point-of-care testing into the university sector will be explored, as a critical means of equipping the 'next generation' of medical scientists and health professionals with the knowledge and skill set required to become competent point-of-care field operators and network co-ordinators.

MULTISURE HCV ANTIBODY ASSAY: A NOVEL RAPID HCV TEST TO DETECTS AND DIFFERENTIATE HCV ANTIBODIES

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Background:

There is a necessity to develop a new HCV screening diagnostic assay which can detect and differentiate HCV antibodies and applicable at all level of health care system, to overcome the prolong test time and cost of current HCV diagnostics (screening and confirmatory assays).

The MPD MULTISURE HCV Antibody Assay is a novel reverse flow based immunochromatographic assay intended for the rapid detection of HCV antibodies and differentiate into four antibody profiles against Core, NS3, NS4 and NS5 HCV antigens in human serum, plasma or whole blood.

Objectives: To develop and validate one stop serology-based HCV diagnostic- MULTISURE HCV Antibody Assay using well-characterized HCV reactive positive and non-reactive human samples.

Methods: HCV structural (core protein) and non-structural proteins (NS3, NS4 & NS5) were used to develop MULTISURE HCV Antibody Assay using reverse flow technique. The performance of MULTISURE HCV Antibody Assay was evaluated using well-characterized archive and clinical specimens collected from various regions according to the common technical specifications (CTS) of The Commission of European Communities (2009). The sensitivity of MULTISURE HCV Antibody Assay was evaluated using 2618 specimens, 20 HCV sero-conversion and 223 Genotype specific (1-6) specimens. Specificity of MULTISURE HCV Antibody Assay was evaluated using >4000 HCV non-reactive specimens consist of healthy donors (n=1056), clinical specimens (n=2461), pregnant women samples (n=206) cross reactive panels (n=171) and samples with interference substances (n=114).

Results: MPD MULTISURE HCV Antibody Assay showed **99.39%** sensitivity (2602/2618, 95% CI: 99.01-99.65 %) against 2618 HCV reactive samples (positive with EIA and/or nucleic acid test) while **99.03%** specificity (3987/4026, 95% CI: 98.68% to 99.31%) against 4026 HCV non-reactive samples. MPD HCV MULTISURE Antibody Assay showed 100% positivity with 20 sero-conversions panels with average detected days of 24.95 days since first bleed compared to 26.8 days and 30.9 days by HCV EIA and HCV RIBA respectively. The MPD MULTISURE HCV Antibody Assay also detected all six genotypes of HCV (n=223) collected world-wide.

Conclusions: The newly developed MULTISURE HCV Antibody Assay is served as rapid screening with additional advantage of differentiating of HCV antibody profiles specific to four major HCV antigens and suitable for the diagnosis of HCV infection at all level of health care system.

NEW DEVELOPMENTS IN INFECTIOUS DISEASE TESTING

Maine GT

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Introduction:

Infectious disease diagnostic algorithms have been devised to help guide physician interpretation of laboratory results, determine what additional follow up testing is required, and assist the physician to prescribe the best possible patient care.

Objectives:

To examine the different test algorithms used for EBV testing. To review the recent data that supports the diagnostic algorithms for CMV and Toxoplasma testing in pregnant women. To review the outcome of a Rubella meeting held in Paris in June where the problem with standardization of Rubella IgG assays was discussed.

Methods:

Test results from the ARCHITECT EBV assays under development were used to compare four different EBV test algorithms. The ARCHITECT CMV and Toxo assays were used to test patient samples from pregnant women undergoing documented seroconversion. Test results from the different commercial Rubella IgG tests were reviewed and discussed at the Paris meeting.

Results:

Application of the EBV EBNA screening algorithm minimized the number of follow up tests, when the grayzone resolved approach was used. The combination of CMV and Toxo IgG, IgM and IgG avidity test results, when used in a diagnostic algorithm, provides accurate staging of the CMV or Toxoplasma infection. Large differences in Rubella IgG IU/mL titers were reported between commercial Rubella IgG assays standardized to the current WHO 1st International Standard RUBI-1-94 for Anti-Rubella Immunoglobulin

Conclusions:

The most effective way for the correct diagnosis and staging of EBV infection was obtained when initial testing was done by the ARCHITECT EBV EBNA-1 IgG assay combined with a simple approach to evaluate grayzone results. Current test algorithms for CMV and Toxo were shown to be valid and fit for use with the ARCHITECT CMV and Toxo assays. The problem of the lack of standardization of Rubella IgG assays needs to be addressed and the "true" meaning of the individual Rubella IgG assay cutoffs needs to be better defined.

HTLV, A HISTORY – FROM OBSCURITY TO AUTOMATED ASSAY

Birger Jansson

R&D, DiaSorin S.p.A, Saluggia, Italy

While still remaining in the shadows of a more infamous retrovirus, the importance of Human T lymphotropic virus 1 and 2 should not be underestimated. Asymptomatic carriers of HTLV-1 or HTLV-2 pose a large problem to the blood supply, particularly in endemic regions. An estimated 20 million people worldwide are infected with HTLV-1. It is essential that adequate assays for blood screening and diagnosis of HTLV infection are developed and distributed.

This presentation will discuss aspects of HTLV biology and diagnosis and their impact on the design, development and performance of a novel, automated chemiluminescent assay, LIAISON XL murex rec HTLV I/II immunoassay, detecting antibodies against HTLV-1 and HTLV-2.

CDC'S STRATEGY FOR BUILDING SUSTAINABLE LABORATORY CAPACITY IN RESOURCE POOR COUNTRIES: PEPFAR APPROACH

Moore, C - CDC

The President's Emergency Plan for AIDS Relief (PEPFAR) is the U.S. Government's program to assist partner countries in the battle against HIV/AIDS. PEPFAR's early efforts focused on prevention, care and treatment strategies. As national programs have grown and matured, PEPFAR's strategy is shifting from intervention to sustainability. Strong laboratory systems are essential for the accurate detection of HIV and other diseases. With PEPFAR support, The International Laboratory Branch (ILB) within the CDC's Center for Global Health develops laboratory capacity in resource poor countries through service delivery, laboratory system strengthening, and organizational development. ILB works with Ministries of Health to write National Laboratory Strategic Plans that will guide development of a hierarchy of quality laboratories. Training and technical support are core services provided by the branch. Emphasis is placed on comprehensive quality management leading to sustainable country ownership. ILB programs provide a stepwise approach to laboratory capacity building that encourages and supports the pursuit of internationally recognized accreditation standards.

ONE HEALTH – CROSSING MEDICAL AND VETERINARY BORDERS

Professor Martyn Jeggo

Director, Australian Animal Health Laboratory, CSIRO, Geelong Victoria

The One Health initiative has been in existence for a number of years. However as the threat from new and emerging infectious diseases grows, and it becomes recognized that many of these diseases emerge in wildlife, cause significant losses to our livestock and can seriously affect human health, so we increasingly begin to approach the problem differently. Fundamental to this approach, is the recognition that bringing together wildlife ecologists, animal health experts and the medical profession can offer additional and crucial insights both into the disease emergence process as well alternative ways of control and prevention. But the approach has proved difficult to implement for a number of crucial reasons. Whilst the logic of working together is clear, issues of resource sharing, of complexities of missions and mandates and of language and understanding have limited what might have been achieved.

At the level of animal health and medical laboratories, there are few examples of a One Health approach, yet the technologies used in laboratories are fundamentally the same, the reagents often identical and the needs for quality assurance, biosafety and biosecurity paramount. An examination of these areas would argue strongly that we could do a great deal more through a shared approach both in terms of resources and facilities. Whilst a pooling of expertise and resources is unlikely much can be done now in terms of harmonization, allocation of roles and responsibilities and sharing of resources, particularly for those agents where high containment is a prerequisite.

This presentation explores many of the problems of adopting a One Health laboratory approach to the diagnosis and monitoring of emerging infectious diseases and proposes a number of actions that could improve the way we currently operate.

NRL STEPS: SUSTAINABLE TRAINING, EDUCATION AND PARTNERSHIPS

Geraldine KONG

NRL, Melbourne Australia

Introduction:

NRL has played an active role in international laboratory capacity building in the Asia Pacific region since its inception in 1985. By working in collaboration with partners in-country and in the region, and aligning with national and regional health strategies, our aims in each country are to establish, implement and promote best practice testing for infectious diseases. This will consequently ensure reliable and reproducible results, minimise false test results, and improve blood safety. NRL has distilled the experience and expertise gained over the years into a model for laboratory capacity building known as NRL STEPs: Sustainable Training, Education and Partnerships.

Objectives:

To explain the philosophy and principles behind NRL STEPs and demonstrate how NRL has applied the model to its laboratory capacity building activities.

Discussion:

NRL STEPs is a structured, stepwise and culturally sensitive approach to strengthening laboratory systems. It builds on countries' existing capacity and provides advocacy, customised training and mentorship based on identified needs. NRL works in collaboration with in-country partners, especially Ministries of Health, to implement NRL STEPs in harmony with existing national strategies for laboratory development. The four key stages of NRL STEPs will be explained, and relevant case studies presented to demonstrate its application in some of the countries NRL has worked with.

Conclusion:

While the same philosophy and principles drive the delivery of NRL STEPs, its implementation is customised to each country. Nevertheless, the ultimate aim of NRL STEPs is to support laboratories in delivering accurate test results and better patient outcomes.

ACCREDITATION REQUIREMENTS FOR ASSAY VALIDATION AND VERIFICATION

Griffin A

National Association of Testing Authorities, Australia

The guiding principles for the validation and/or verification of testing is governed by the overarching requirements of ISO/IEC 17025 and ISO 15189. These are generic standards which outline the requirements that laboratories “shall use only validated procedures” and that the validation be as extensive as is necessary to meet its intended needs in the given application or field of application and that methods selected for use shall be evaluated and found to give satisfactory results before being used for medical examinations (ISO 15189 5.5.2).

Essentially, the intent is that the validation and verification needs to be as extensive as is necessary bearing in mind the application of the test. Who determines what one needs to do and how much is enough is left to each facility to determine. Some form of validation and/or verification however is required for any assay brought into a medical testing environment, no matter how routine.

NATA is consistently asked by facilities how much is enough and whether there are approved publications which can be used as procedural documents for the validation and verification of new assays. There are many publications relating to validation and verification of assays which may be useful for facilities to follow, however, they are often discipline specific and there is no one publication or validation method universally agreed upon.

NATA Technical note 17 - *Guidelines for the validation and verification of quantitative test methods* which was re-published in June 2012 is intended as a guidance document to cover those areas which should be considered when undertaking method validation and/or verification. It is by definition a generic document covering a broad range of scenarios. It is not intended to be used as a procedure for method validation or verification.

Laboratories performing In-house In vitro diagnostic (IVD) testing (Classes 1 – 3) are required to achieve accreditation to ISO 15189 and NPAAC *Requirements for the Development and Use of In Vitro In house Diagnostic Devices* (IVDs) and to register any In-house IVD with the TGA by 1 July 2014. Class 4 IVDs will require TGA conformity assessment but will also be subject to accreditation requirements should the facility require recognition of these assays on the NATA Scope of Accreditation.

There may be facilities performing in-house IVDs which have a diagnostic or clinical use which have not previously considered the requirement for accreditation, especially facilities who do not claim Medicare reimbursement. As the TGA IVD framework may now require accreditation for these assays, facilities may need to formalise their validation and verification reports to meet accreditation expectations.

REGULATION OF IN-HOUSE IVDs IN AUSTRALIA

Kaylock P

Therapeutic Goods Administration (TGA), Australia

Introduction:

On 1 July 2010 a new regulatory framework for in vitro diagnostic (IVD) medical devices commenced in Australia. In-house IVDs that are developed by laboratories for their own use are regulated under the new framework.

The regulatory requirements for in-house IVDs will be presented, along with an overview of the roles played by laboratories, the TGA and the National Association of Testing Laboratories, Australasia (NATA).

INCLUDING IN-HOUSE IVDs ON THE ARTG: SUGGESTIONS FOR LABORATORIES

Susie Braniff, Wayne Dimech and Mark Lanigan
NRL, Melbourne, Australia

The implementation of the IVD legislation in 2010 means that laboratories that use in-house tests are required to include them on the Australian Register of Therapeutic Goods (ARTG). The legislation defines an in-house IVD as

- a. within the confines or scope of an Australian medical laboratory or Australian medical laboratory network:
 - i. developed from first principles; or
 - ii. developed or modified from a published source; or
 - iii. used for a purpose, other than the intended purpose assigned by the manufacturer; and
- b. not supplied outside that medical laboratory or medical laboratory network.

The legislation classifies IVDs according to the public health and personal risk associated with their use. The requirements that must be met to include IVDs on the ARTG become more comprehensive with increasing risk class. Laboratories that wish to include in-house IVDs on the ARTG will be required to meet the requirements specified for the risk class into which the IVD falls.

NRL has been contracted by TGA for more than 20 years to conduct performance evaluation of IVDs. These evaluations include examination of manufacturer's dossiers of evidence that support the application for inclusion of an IVD on the ARTG. In examining these dossiers we have identified key areas where information is often lacking or unclear *viz* scientific protocols, identification of appropriate acceptance criteria, performance testing of the device and communication of the findings. This presentation will focus on the aspects of the dossier relating to clinical evidence and product validation and verification and discuss pitfalls that laboratories can avoid when preparing documents for ARTG application.

EVALUATION OF AN IN-HOUSE RUBELLA IgG IMMUNOASSAY

J Cai, W Dimech and K Wilson.
NRL, Melbourne Australia

Introduction:

We have developed an anti-rubella immunoglobulin G (IgG) in-house enzyme-linked immunosorbent assay (IH-EIA), designed specifically to quantify low levels of human anti-rubella IgG. This assay was calibrated with the WHO International Standard (RUBI-1-94) and results were reported in international unit per millilitre (IU/mL). The IH-EIA was evaluated to determine its suitability to screen NRL's sample bank for rubella IgG-negative samples for use as diluent in NRL's anti-rubella IgG quality control (QC) sample. The assay's sensitivity, specificity, variability and precision were assessed. Well-characterised samples, a secondary standard dilution series (2°STD, AcroMetrix, Ca.) and an anti-rubella IgG external QC (AcroMetrix, Ca.) were used to evaluate the performance of IH-EIA.

Objectives:

The aim of the study was to evaluate IH-EIA for screening sample bank specimens for rubella IgG-negative samples.

Methods:

IH-EIA was optimised by varying the concentrations of the rubella viral lysate (HPV-77 stain) use to coat the solid phase and several horseradish peroxidase-coupled (HRP)-conjugated anti-human IgG conjugates were compared. The optimal concentration of rubella viral lysate was 10µg/mL and a mouse monoclonal HRP-conjugated anti-human IgG was selected and used at a diluted of 1 in 1000. The IH-EIA was calibrated using RUBI-1-94.

In this evaluation, a positive anti-rubella IgG status was assigned to samples reactive in at least seven out of the eight of commercial immunoassays (CIA) and reactive in a haemagglutination inhibition (HAI) assay. A negative anti-rubella IgG status was assigned to samples non-reactive in a CIA, HAI and commercial western blot.

A total of 189 blood donor plasma specimens were assigned a positive status and 57 samples were assigned a negative status. The quantitative IH-EIA results were compared with the assigned anti-rubella IgG status and analysed using a receiver operating characteristic (ROC) analysis.

The 2 °STD, calibrated against RUBI-1-94 and consisting of seven doubling dilutions, was tested three times in two test runs to verify the accuracy of IH-EIA. The QC was tested at least once in each test run to determine the inter-assay precision and tested a further 39 times in the same test run to determine the intra-assay precision.

Results:

Of the 246 samples tested the sensitivity and specificity with 95% confidence interval (CI) estimated by ROC analysis, using 10 IU/mL as the cut-off, were 99.5% (CI: 96.6 - 99.9%) and 64.9% (CI: 51.1 - 76.8%) respectively.

The results of 2 °STD tested in IH-EIA were plotted against the expected values. The coefficient of determination (R^2) was estimated as 0.95 and the equation describing the correlation was $y=1.1x$.

A total of 23 QC test results obtained from 10 test runs had a mean of 25.1 IU/mL and coefficient of variation (CV) expressed as a percentage of 19.6%. A further 39 QC test results, obtained from a single test run had a mean of 26.5 IU/mL and a CV of 9.4%.

Conclusions:

An IH-EIA, calibrated with the RUB-1-94, was evaluated to determine its suitability for screening NRL's sample bank for rubella IgG-negative samples. The IH-EIA demonstrated good accuracy when compared with an independent secondary standard; with R^2 of 0.95. The level of precision was comparable to commercial immunoassays. A sensitivity of 99.5% indicated confidence in IH-EIA's ability to detect the presence of anti-rubella virus IgG. However, poor specificity would result in up to 35.1% false-positive test result. Therefore, some rubella IgG negative samples would be mis-classified and therefore not be available for use as diluent. The results of the evaluation indicated the IH-EIA would be suitable for the screening negative anti-rubella IgG samples in NRL's sample bank; however the poor specificity limits its usefulness. Further optimisation of the IH-EIA and a repeat evaluation would be required to improve the specificity of the IH-EIA. A secondary standard would be required to replace RUB-1-94 as the calibrator if IH-EIA was used routinely.

MONITORING TREATMENT RESPONSES IN CHRONIC HEPATITIS C: TRANSITIONING FROM THE STANDARD OF CARE INTO THE NEW ERA OF DAAS

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Director, WHO Regional Reference Laboratory for Hepatitis B

Hepatitis C virus (HCV) remains a major public health problem worldwide. The WHO has estimated a global prevalence of about 3% with the virus affecting 170 million of the world's population in 1999, and the global annual incidence of HCV infections was estimated to be 683,000 in 2002. Updated figures from the 2011 survey will be released by the WHO on World Hepatitis Day, July 28 this year and are expected to exceed 200 million.

Ultimately, the current treatment for chronic HCV infections has been based on a combination of pegylated interferon (P) and ribavirin (R), but its efficacy varied substantially and was HCV genotype dependent. The HCV is classified into six major genotypes, and genotype has been identified as a major predictor of response to PR containing antiviral therapies. PR antiviral regimens have been optimized for infections with HCV genotypes 1-4, although treatment strategies for genotypes 5 and 6 have yet to be properly evaluated. For genotype 2 and 3, 24 weeks PR therapy is usually sufficient, achieving an 80% sustained virological response (SVR) or cure. In contrast, 48 weeks is typically required for non-genotype 2/3 infections, and the SVR is usually less than 50%. Rules based response guided therapy (RGT) involving quantitative HCV RNA measurement (RVR4, EVR12, etc) combined with host IL-28 SNP (CC vs no-CC) testing has resulted in the emergence of rather complex treatment regimens during PR therapy. The molecular basis for these differences in response of the different HCV genotypes has yet to be determined.

Recently, there has been significant advances in developing direct acting antiviral (DAA) therapies for chronic hepatitis C. There are many DAA agents in various stages of clinical development, and a substantial number of them are in Phase 2 or 3 of clinical trials and at least 2 are now approved, Telaprevir and Boceprevir, in combination with PR. These DAAs work by directly targeting essential HCV proteins such as NS3/4A, NS5A and NS5B to inhibit their function during active viral replication. While DAAs are effective, the ability of HCV to replicate to high levels with substantial genetic heterogeneity means that drug-associated resistance mutations can arise rapidly. Data from the first round of clinical trials of these DAAs and subsequent clinical experience has reported emergence of resistance within days of therapy initiation. This clearly has immediate clinical implications and substantial challenges in the management of patients with DAA resistance. Already, particular signature substitutions have been identified with DAA resistance associated changes in the HCV genome: codons R155, A156, D168, V36 and T54 of the HCV NS3/4A protease; codons P495L and S282T for the HCV NS-5B RdRp polymerase, and codon Y93H for the NS5A.

In the short term, antiviral resistance to the DAAs will emerge as the single most important factor in treatment failure after patient compliance, but the future looks very promising for PR free regimens of much shorter duration and higher cure rates. The new horizon for hepatitis C monitoring will be simpler and cheaper than current protocols, probably only requiring a qualitative PCR at the beginning and the end of treatment, with no need for genotype testing, but rather ensuring the patient takes ALL the oral medication for the prescribed period, but this will probably be only for 12 weeks. The new horizon does indeed look very promising for patients with chronic hepatitis C.

EVALUATION AND UTILITY OF THE ABBOTT ARCHITECT HCV ANTIGEN ASSAY

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Objective

The Abbott Architect HCV Antigen assay (HCV Ag) is used where an assessment of HCV viraemia is required. An evaluation was performed to determine its sensitivity compared to PCR, specificity and utility in resolving indeterminate HCV profiles.

Method and Results

Sixty-one samples with detectable HCV RNA ranging from 85 IU/mL to 7.7×10^5 IU/mL were tested for HCV antigen. The HCV antigen assay showed a sensitivity of around 3000 IU/mL. All 69 HCV PCR negative samples were also negative on the HCV Ag assay. The assay was determined to be 100% specific.

HCV Ag was introduced to routine use in 2011 as a supplementary assay for samples with discordant anti-HCV serology. Samples positive for anti-HCV are confirmed with a second EIA. Previously, discordant samples were resolved with the Recombinant Immunoblot Assay (RIBA) or recollection for HCV PCR. It has since been shown that the RIBA is ineffective in resolving discordant HCV samples. However, the RIBA is still of value in weakly reactive anti-HCV samples. The HCV Ag assay can now be performed on the same collection tube as the anti-HCV.

Since introduction of the Abbott Architect HCV Antigen assay, 12 patients with discordant or indeterminate anti-HCV serology have had HCV antigen detected. These patients were shown to have recently acquired HCV infection. Under previous reporting protocols, these would have been reported as indeterminate anti-HCV serology, possibly due to false reactivity of the EIA assay, and a repeat collection requested. Detection of HCV antigen in such cases is essential to exclude recent infection. The following example demonstrates this.

A male patient was involved in needle-stick incident on 6 June 2011. The anti-HCV result was discordant and may previously have been considered to be a non-specific reaction. The sample showed high HCV Ag levels ($> 20,000$ fmol/L) indicating that the patient had a current HCV infection. A sample collected one month prior had negative anti-HCV, but high reactive HCV Ag indicating recent HCV infection. Testing 12 days later showed full anti-HCV reactivity.

Date	anti-HCV Screen		anti-HCV (Murex)		HCV Ag fmol/L		HCV RNA
03.05.11	NR	0.08	NR	0.20	R	17,485	
06.06.11	R	3.38	NR	0.64	R	$>20,000$	
18.06.11	R	4.24	R	4.56	R	17,993	1.5×10^5

Conclusion

The HCV antigen assay has proven effective in determining viraemia in patients with HCV infection. The assay is useful for confirmation of discordant anti-HCV samples and adds more information than provided by the HCV RIBA assay.

PERFORMANCE OF THE ABBOTT ARCHITECT HEPATITIS C ANTIGEN TEST IN A COMMUNITY LABORATORY

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Sullivan Nicolaidis Pathology, Taringa, Queensland, Australia

Introduction:

Assessment and treatment of confirmed cases of HCV infection (HCV antibody reactive using two assays) requires qualitative or quantitative HCV RNA estimations usually on a subsequent dedicated sample. Recently a quantitative Abbott ARCHITECT HCV Antigen (HCV Ag) assay which is a two-step chemiluminescent microparticle immunoassay (CLMIA) dedicated to the Abbott ARCHITECT *i*System (*i*2000 and *i*2000SR) has become available.

Methods:

To determine the performance of the HCV Ag assay on 256 samples that were HCV antibody (HCV Ab) reactive by two different assays that also had corresponding HCV viral load (HCV VL) estimations performed using the Roche Ampliprep/Taqman assay. HCV Ab non reactive samples (40) were also compared to the HCV Ag assay.

Results:

HCV Ag versus HCV VL (n=256)

HCV Ag	PCR		
	Detected	Not Detected	Grand Total
Non Reactive	92	49	141
Reactive	115	0	115
Grand Total	207	49	256

There was good correlation with the quantitative HCV Ag and HCV VL. The majority of the 92 HCV Ag non-reactive and HCV VL detected samples had viral loads less than log 3.6 suggesting the lower limit of detection is approximately 5000 IU/mL. The differences could not be explained by genotype. More than 60% of these patients were known to be on treatment at the time. All HCV Ab non reactive samples were also HCV Ag non reactive yielding 100% specificity.

Conclusions:

Automation and testing of the same sample for quantitative HCV Ag assay can provide a more rapid and cost effective confirmation of active infection in anti-HCV positive patients. These patients usually have high viral loads. As this is the more common scenario following acute infection, a significant number of samples will not require follow up HCV RNA testing thus representing a significant cost saving. The reduced sensitivity at lower viral loads is likely to be most significant in patients on treatment.

INVESTIGATION OF LOW POSITIVE/HIGH NEGATIVE RESULTS ON THE HIV AMPLISCREEN ASSAY

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NRL tests samples from tissue donors for HIV-1 nucleic acid using the Roche AmpliScreen test. This test is included on the Australian Register of Therapeutic Goods; approved sample types are serum or plasma from living or cadaver donors. NRL's testing strategy dictates that when a positive result is obtained with a donor's sample, the testing is repeated in duplicate. If either or both of the repeat tests is negative, the nucleic acid test is reported as "Inconclusive"; if both the repeat duplicate tests are positive the result is reported as "Nucleic Acid Detected".

Since we commenced using this test in February 2008 we have intermittently encountered low positive results that are just above the assay cut-off (optical density of > 0.200). In the past the majority of these samples returned negative results on retesting. From May to September 2011 we observed these low positive results occurring with increased frequency using kit lot number N09375. Quite often these samples produced low positive results on retesting. We also observed a significant increase in the optical densities obtained in negative samples ($P < 0.05$). We were of the opinion that the low positive results we were observing were not true, but were a result of the overall increase in the optical densities in negative samples, resulting in some negative samples producing optical densities just above the assay cut-off. Analysis of evaluation data also indicated that these low positive results were not consistent with those we would expect to obtain with a true positive sample, irrespective of its viral load.

Discussions and data sharing with Roche and the other Australian laboratories using the AmpliScreen HIV-1 test, revealed that the problem was not confined to NRL. All laboratories were using lot number N09375 and all reported a significant increase in optical densities obtained with negative samples. Similar results were obtained with a second lot number (N17497).

This situation took several months to resolve during which HIV-1 AmpliScreen testing was halted at NRL and donated tissue quarantined pending a resolution, except in urgent cases. This action was preferable to the possibility of obtaining false positive results which would lead to the unnecessary discard of donated tissue. Despite this measure, some tissues were discarded. Although scientific and manufacturer's evidence indicated that the low positive results were false, the affected tissues could only be released exceptionally. This imposed requirements that made the processing of affected tissue untenable.

THE EXPERIENCES OF THE CHINA INTERNATIONAL TRANSFUSION INFECTION CONTROL (CITIC) EXTERNAL QUALITY ASSESSMENT SCHEMES (EQAS)

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2. NRL, Melbourne, Australia

Introduction:

In China, there are over 430 independent Blood Transfusion Services (BTSs) with broad diversity in operational scale. Since the majority of BTSs conduct blood screening for transfusion transmissible infections within their own laboratories, many of which differ in management and professional experience, EQAS is one of the key mechanisms to measure laboratories' performance and improve the reliability and standardization of the blood screening results. In order to further improve the standard and quality of blood screening in China, a CITIC EQAS was established in June 2011 through collaboration between Shanghai Blood Center (SBC) and NRL, Australia.

Objectives:

To review the experiences of the first three test events (TEs) of CITIC EQAS and assess the level of quality of blood screening in China by comparing the CITIC EQAS data with those of other laboratories in the world.

Methods:

CITIC acts as a marketing and customs support department for NRL EQAS in China, but works beyond these fields, including EQAS orders, importation, transportation, data collection, report translation and troubleshooting, all with a focus on establishing a quality assurance system in China based on the CITIC EQAS platform. This system is guided by two professional committees, CITIC Management Committee and the Quality Assurance Experts Committee. A series of training workshops, intercommunication activities and collaborative scientific research works are also integral to the system.

Results:

Over 60 registered CITIC participants have participated in the past three EQAS TEs. The Quality Assurance Experts Committee (10 members) was established in April 2011, working towards guidance for quality control of blood screening either on technology or on management initiatives. The CITIC Management Committee (composed of 17 of the largest BTSs in China) was established in April 2012, focusing on blood screening technical services and CITIC marketing services for the local blood banks. Three training and intercommunication workshops were carried out during the past one and half a years, and more than 100 technicians have achieved the certificate issued by the Quality Assurance Experts Committee. From the NRL EQAS report, the rate of laboratories with unacceptable results was 1.61%, 1.61%, 2.50% (except for anti-HIV in TE of November 2011), 0.00% and 22.58% for HBsAg, anti-HCV, anti-HIV, Syphilis serologic testing and nucleic acid testing (NAT), respectively. The rate of serologic testing was significant lower than that of participants outside mainland China (6.45%, 4.74%, 6.96% and 17.28%, respectively), yet the rate for NAT was higher than other participants (21.48%) but not statistically significance ($\chi^2=0.01$, $P=0.91$).

Conclusions:

CITIC EQAS was established in China successfully. It is considered valuable to further extend the experiences of the collaboration between SBC and NRL for CITIC EQAS to developing countries to ensure blood safety. The quality of NAT blood screening of CITIC participants should be improved in the future under the guidance of the professional committees and the practices of CITIC EQAS.

EXTENDED GREY ZONE PHENOMENON

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Introduction:

Rotary Bangalore TTK Blood Bank collects on average 25,000–30,000 blood units per annum from only voluntary donors. Each blood unit is screened for HIV, HCV, HBs antigen using the fourth generation EIA, and for malarial parasite & syphilis.

For ELISA based assays, kit criteria prescribe that all samples which have an OD (Optical Density) below cutoff (CO) is defined Non-Reactive (NR). Samples with an OD higher than or equal to CO are defined Initially Reactive (IR) and retested in duplicate before final interpretation. Samples that fall within a 10% Grey Zone (GZ) are also retested and interpreted with caution. After retesting, the sample is considered reactive if any of the duplicates are higher or equal to cutoff value. The sample is considered nonreactive if both values are less than cutoff value.

We observed that some samples fell in a different population as compared to other nonreactive samples. These samples had OD below cut off (still non-reactive as per kit criteria). These on retesting in duplicate tested reactive as per kit criteria. Such samples were classified as Extended Grey Zone (EGZ) samples, which had an OD 40% below cutoff.

Objective:

To quantify percentage of EGZ samples that re-test reactive with a view to improve screening strategies in developing countries.

Methods:

Total of 88,291 donor samples were screened between 2008 to 2011 using Biorad kits: anti HIV 1 & 2 (Genscreen™ Plus HIV Ag- Ab), HBs Ag (Monolisa™ HBs Ag Ultra) and anti HCV (Monolisa™ Ag Ab Ultra). Before availability of HCV Monolisa™ Ag Ab Ultra, we used Ortho^R HCV 3.0 and Abbott Murex anti-HCV version 4.0. We added an additional step in our testing algorithm, i.e. all samples, which test in the EGZ (40% below cut off), were considered as IR and retested in duplicate. If one or both duplicate samples tested reactive or in EGZ, the unit was defined as not fit for issue and discarded. If both duplicate samples tested non-reactive, unit was defined as non-reactive and taken into stock for transfusion purposes.

Results:

The TTI reactivity during this period was 0.13%, 0.70% and 0.19% for anti HIV 1 & 2, HBs Ag, and anti HCV respectively. The samples which tested in EGZ were 0.15% (n=132) for anti HIV 1 & 2, 0.18% (n=157) for HBs Ag and 0.23% (n=201) for anti HCV. The number of EGZ samples which tested repeat reactive were 6.82% (n=9) for HIV, 10.19% (n=16) for HBs Ag and 7.46% (n=15) for anti HCV. These 40 samples were defined as not fit for transfusion. We were unable to subject these samples to further confirmatory tests.

Conclusions:

If such EGZ samples are indeed confirmed positive, it would mean that 10% Grey Zone criteria alone is not sufficient. Our concern was these 40 samples, which may have been reactive. Confirmatory tests would lend concrete basis to confirm a final status of such samples.

In India where Hepatitis B is highly prevalent and NAT testing is not mandatory, this could be an additional layer of safety in transfusion centres. It leaves us with questions such as can EGZ predict a positive result, are these cases of low antigen/ antibody titre or seroconverting samples, and is similar EGZ phenomenon observed with other EIA kits. Another possibility is these kits may not detect viral variants prevalent in India. In view of all this, do we eventually need to increase the 10% Grey Zone criteria?

A PILOT STUDY FOR SCREENING BLOOD DONORS IN HANGZHOU CHINA BY NUCLEIC ACID AMPLIFICATION TECHNOLOGY

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Introduction:

Nucleic acid amplification test (NAT) has not yet been mandated in China. The risk of enzyme immunoassay (EIA)-negative, NAT-reactive donations was not well understood. This study aimed to screen for blood donors in China by triplex individual-donation (ID)-NAT.

Objectives:

To evaluate the effects of NAT on blood donations screening test in blood center of Zhejiang province which locates in the southeast of mainland China and to increase the recognition of yield of NAT testing for blood donors in the mainland of China.

Methods:

HBV, HCV and HIV-1 testing was implemented for individual donation from August 1st 2010. All samples were also tested with serology method using two different EIA assays for each of these three viruses. Samples with discordant results between NAT and EIA were further tested with alternative NAT assays. Donor follow-up testing for those potential yield cases were further evaluated when possible.

Results:

A total of 178,447 donations were included. 169 HBV NAT yield cases (0.095%) were detected. No NAT yield cases were found for HIV-1 or HCV. Follow-up results showed that 11(6.51%) were probable or confirmed HBV window period infection, 5(2.99%) were chronic HBV carrier, and 153(90.53%) were probable or confirmed occult HBV infection. The rates of NAT-positive were 0.472% and 0.146% for first-time donors and repeat donors and the difference was significant ($\chi^2=142.526$, $p<0.05$).

Conclusions:

Our data demonstrate that the potential HBV yield rate was 1:1056 for blood donor populations in China. Implementation of NAT will provide a significant increment in safety relative to serologic screening.

PREVALENCE AND PATTERN OF HEPATITIS C VIRUS GENOTYPES IN BANGLADESH

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Introduction: Of hepatotropic viruses (A-E), hepatitis C virus (HCV) infection has residual effect on liver. In the long run, infection may progress to chronic liver diseases leading to cirrhosis, liver failure and hepatocellular carcinoma. Important associated factors for the disease progression include the viral genotypes, their subtypes and viral load. To our knowledge, no reports are available on the prevalence of HCV genotypes in Bangladesh. This study was thus conducted to see the prevalence pattern of HCV genotypes to facilitate the health care providers and clinicians in designing the therapeutic strategies for the sustained HCV clearance after therapy.

Methodology: The study was carried out on 370 HCV RNA positive samples submitted to clinical laboratory services of icddr,b to determine the genotypes during 2006-11. The samples were subjected to nucleotide sequence and the genotype was then determined using bioinformatics tool, the BLAST.

Results and discussion: Of 370 patients, 67% were males. Higher number of samples was received in 2010-11 (62-63%). In all years, genotypes 3b (47%) were more common followed by 3a (19%), 1b (15%), and 1a (13%) except 2008 when genotype 1 was the highest (61%). The least common genotypes were 4a, 4c, 2a, and 2c. Males and female ratio was almost 2:1. The age group 30-50 years were more infected (58%). HCV genotyping is important for epidemiological, clinical and therapeutic perspectives. It helps in selecting the type and duration of anti-viral therapy and predicting the likelihood of sustained viral response (SVR) which varies with genotype.

Conclusion: Information on the prevalence of HCV genotype has implication on the options in determining the therapeutic regime for the management of HCV infected patients and epidemiologic importance for research. However, study should be carried on a broader scale of samples to compare the efficacy of different options.

EVALUATION OF A FASTER, EXTRACTION-FREE MOLECULAR ASSAY FOR HSV 1&2, VZV, AND CMV

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Human herpes viruses are endemic and it is highly desirable to provide robust, efficient and timely diagnostics to identify and treat affected individuals. Conventional virus isolation and identification techniques are time-consuming, expensive, insensitive, and fail to accurately differentiate the different herpes virus types, and are highly susceptible to the quality and adequacy of specimen collection. In the context of possible herpes related acute encephalopathy investigations and there is also a need for urgent identification and treatment. Therapeutic treatment monitoring of patients with active human cytomegalovirus infection is also highly desirable in the context of immunodeficiency in solid organ and bone marrow transplant recipients and advanced HIV infection. Over the last decade there has been a dramatic increase in the application of nucleic acid techniques for the identification and differentiation of human herpes virus infection. In particular, vesicle diagnostics for the alpha-herpes viruses, herpes simplex (HSV-1, HSV-2) and varicella zoster (VZV), and also in the central nervous system compartment the involvement with alpha and beta-herpesvirus infections such as human cytomegalovirus CMV. As with most nucleic acid detection methods nucleic acids are required to be extracted from clinical samples using chaotropic agents in either manual or automated processes.

We undertook a study to evaluate the Simplexa™ HSV 1 & 2 and VZV Molecular Assay (Focus Diagnostics, CA, USA), in a routine molecular diagnostic laboratory which proposed a simplified sample preparation (extraction free) method. In our study, 103 specimens were tested on the Simplexa™ HSV 1 & 2 and VZV Molecular Assay, and a combination of the our routine benchmark nucleic acid methods of Roche LightCycler® HSV 1/2 Detection Kit (Roche Molecular Systems, NJ, USA), Qiagen RealArt® VZV PCR kit, and the Qiagen RealArt® HSV PCR kit (Qiagen GmbH, Germany). Simplexa™ results showed 94 specimens (91.3%) had 100% correlation with the results generated against routine benchmark tests, with an additional 4 specimens newly detected and confirmed, and 5 indeterminate. The Simplexa™ test saved 1-2 hours of processing time without the requirement of any nucleic acid extraction procedure. Essentially, dry swabs were eluted in a sample diluent of which 4 microlitres was transferred directly to the amplification mixture.

A second arm to the evaluation looked at samples collected from individuals undergoing either pre-emptive treatment for CMV reactivation or treatment monitoring of patients undergoing antiviral therapy for active CMV infection. 89 specimens consisting of samples with (60, 67%) and without (29, 33%) detectable CMV DNA on the Roche COBAS Amplicor CMV Monitor Assay were tested. The Simplexa™ CMV Molecular Assay results showed that 52 (87%) of specimens with detectable CMV DNA fell within $\pm 0.5 \log_{10}$ difference between both assays, while 27 (93%) of specimens without detectable CMV DNA were confirmed as negative. Using EasyMag automated extraction system (Biomerieux, France), the results of the CMV quantitation was available 3 hours from specimen reception in the laboratory compared with 6 hours.

CONCLUSION: The Simplexa™ system appears to be a more rapid detection, differentiation and quantification platform for nucleic acid investigation with comparable performance to established assays. Further evaluation will be required due to the limited number of samples evaluated to date.

DETERMINATION OF CMV VIRAL LOAD USING THE ABBOTT M2000 PLATFORM

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Introduction:

CMV is a ubiquitous virus which can lead to significant morbidity and mortality in immunocompromised patients. CMV disease is a major cause of organ-transplant complications. Monitoring of CMV disease in susceptible patients is of critical importance in these patient groups. CMV viral load testing has become a best practice method for the diagnosis of active CMV infection. The quantitation of CMV in blood allows clinicians to provide timely anti-viral therapy and importantly monitor disease progression. With increasing numbers of transplant and immunosuppressed patients in the health care system, the demand for this test is growing. To deal with this demand an automated testing platform is required.

Objectives:

(1) To evaluate the Artus CMV PCR Kit with the Abbott m2000 system (ART) for the detection and quantification of CMV in patient samples; (2) Compare the automated method with the Roche COBAS Amplicor CMV Monitor Assay (RCA).

Methods:

Patient samples referred for routine CMV testing were analysed using the RCA and tested retrospectively on the ART. Inter assay reproducibility of the ART assay was assessed using an external CMV DNA standard (Optiquel) over a range of dilutions and a standardised CMV DNA extraction control.

Results:

A total of 195 patient samples were tested by both the RCA and the ART methods. All samples with a viral load of >600 copies/mL by RCA (n=28) were detected by ART. RCA reported 166 samples as <600 copies/mL. When tested by ART, seven of these samples were reported as >600 copies/mL, 12 were reported as between 30-600 copies/mL and five samples were reported as detected at <30 copies/mL. PCR was inhibited in one sample tested by RCA, however was reported as CMV DNA not detected by ART. Intra-laboratory reproducibility was assessed using CMV DNA standards over a range of viral load concentrations. Results for standards with viral loads >30 copies/mL were within $\pm 0.35_{\log}$ (1SD). Additionally, the ART was noted to report the viral load for an external CMV DNA processing control consistently as $2.61_{\log} \pm 0.26_{\log}$ (1SD) from 210 replicates tested over 13 months.

Discussion/Conclusion:

The CMV DNA values determined by the ART were higher than RCA for samples with a detectable viral load. This may be due to an increase in ART sensitivity or due to the additional freeze/thaw cycle these samples underwent before testing.

The ART assay includes quantitative standards that are amplified with each kit, however this does not control for extraction processes. An external DNA control is required to enable full assay performance monitoring. Therefore we implemented an external DNA control (Optiquel CMV DNA) as a process control to monitor test performance. We found the reproducibility of the external DNA control result produced by ART to be consistent.

In addition, the greater dynamic range of the ART (<30 copies/mL) compared to the RCA (<600 copies/mL) may provide earlier detection of CMV activation in patient samples.

The introduction of the ART enabled complete automation of the CMV assay, requiring less hands-on time compared with the RCA. Turn around time for the ART was also superior to the RCA. However, the ART assay requires a minimum of 600 μ L sample volume compared to 200 μ L for the RCA assay.

Since this evaluation, Abbott Diagnostics have released new CMV PCR Assay and is currently under evaluation in our laboratory.

INCREASED ROUTINE SCREENING FOR SYPHILIS AND FALLING SYPHILIS INCIDENCE IN HIV POSITIVE AND HIV NEGATIVE MEN WHO HAVE SEX WITH MEN: IMPLICATIONS FOR SYPHILIS AND HIV PREVENTION.

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Background:

Similar to many developed countries, syphilis has re-emerged in Australia. Dramatic increases in syphilis have overwhelmingly affected men who have sex with men (MSM) and appear likely to have contributed to increased HIV transmission. In response, high MSM caseload clinics in Melbourne, Australia, have maintained strategies to increase routine syphilis serology for MSM since 2005. We examined trends in syphilis testing and incidence among MSM attending high caseload clinics in Melbourne to investigate the effect of this intervention.

Methods:

Syphilis testing data among MSM attending high caseload clinics in Melbourne (January 2007 to December 2010) were analysed. Testing rates and infectious syphilis incidence were determined over time and disaggregated by HIV status and MSM reporting 'high risk' behaviours. Poisson regression was used to test the significance of trends.

Results:

Over four years 16,806 syphilis tests among 6,441 HIV positive MSM and 24,142 syphilis tests among 17,440 HIV negative MSM were conducted. Average annual increases in testing among HIV positive and negative MSM were 7% (95%CI=6%-8%) and 12% (95%CI=11%-13%), respectively. Between 2007 and 2010 infectious syphilis incidence declined from 6.07 to 2.58 per 100PY among HIV positive MSM (21% average annual decline), and from 3.27 to 0.96 per 100PY among HIV negative MSM (29% average annual decline). Incidence declined even more substantially in 'high risk' HIV negative MSM - 4.10 to 0.76 per 100PY among those reporting >10 partners in the previous 6 months and 4.29 to 0.82 per 100PY among those reporting inconsistent condom use. All testing and incidence trends were statistically significant ($p < .01$).

Conclusions:

Enhanced routine syphilis testing appears to have contributed to increased syphilis detection and subsequent reductions in syphilis incidence among MSM. These findings support recommendations that syphilis can be controlled among MSM by sustaining high frequency testing, with important implications for HIV control among MSM.

Table 1: VPCNSS; syphilis testing, proportion positive and incidence in MSM by HIV status, 2007-2010

		2007	2008	2009	2010	IRR*	95% CI
HIV positive MSM	Total tests	3616	4225	4496	4469	1.07	1.06-1.08
	Average number of tests per individual	2.50	2.63	2.65	2.64	1.02	1.00-1.03
	Proportion syphilis positive	2.43	1.73	1.60	1.03	0.77	0.69-0.86
	Infectious syphilis incidence (per 100 PY)	6.07	4.34	4.06	2.58	0.79	0.68-0.90
HIV negative MSM	Total tests	4932	5880	6256	7074	1.12	1.11-1.13
	Average number of tests per individual	1.31	1.37	1.39	1.45	1.03	1.02-1.04
	Proportion syphilis positive	1.89	1.73	1.85	0.99	0.84	0.77-0.92
	Infectious syphilis incidence (per 100 PY)	3.27	2.15	1.86	0.96	0.71	0.60-0.83
	>10 partners in 6 months (incidence) [†]	4.10	3.66	2.69	0.76	0.67	0.50-0.88
	Inconsistent condom use (incidence) [†]	4.29	2.84	1.81	0.82	0.60	0.47-0.76

*Poisson regression used to provide average annual change. [†]These data are not available for HIV positive MSM

VERIFICATION OF THE APTIMA TRICHOMONAS VAGINALIS ASSAY ON THE GEN-PROBE PANTHER AUTOMATED PLATFORM

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Introduction:

Trichomonas vaginalis (*T. vaginalis*) nucleic acid testing is currently performed by in-house real time PCR targeting the 18S ribosomal RNA gene but a commercial fully automated assay is available which may be a more cost effective option for the laboratory.

Objectives:

To compare the GenProbe Aptima *T. vaginalis* assay with the in-house PCR and to determine if the Aptima assay is a suitable replacement for the current test system.

Methods:

Six hundred and fifty nine genital samples were tested retrospectively. Nucleic acids for the in-house assay were extracted from samples using the Ambion total nucleic acid extraction kit on the automated MagMax extraction platform (Life technologies). LightCycler Version 2.0 (Roche) was used for amplification and detection.

The Aptima *T. vaginalis* assay (Gen-probe) using transcription mediated RNA amplification (TMA) was performed on the automated Panther testing platform as per the manufacturer's instructions and tested in parallel with the in-house assay. Endpoint analysis was performed from serial dilutions of positive control materials.

Results:

Six hundred and fifty nine samples comprising 255 urine specimens, 114 cervical/endocervical swabs, 49 genital swabs, 223 vaginal swabs, 1 thinprep, 1 urethral swab and 16 swabs where the site was not recorded were tested in parallel. Six hundred and forty eight of the 659 results were concordant, 113 positives and 535 negatives. Eleven results were discordant of which ten were negative by the in-house assay and positive by the Aptima assay. Six of the 11 discordant results were resolved by retesting, the remaining five had low Aptima RLU results suggesting a low number of *T. vaginalis* organisms present. The comparative endpoint testing showed the Aptima assay was approximately a ten fold dilution more sensitive than the in-house PCR.

Conclusions:

The Aptima *T. vaginalis* assay performed well when compared with the in-house assay. No significant discordance was noted. Improvements in sample traceability, workflow, turn around time and reagent control were all apparent. Based on this study, Panther automated system for detecting *T. vaginalis* is recommended for use in molecular diagnostics laboratory.

CTNG AND HSV NAT EQAS ANALYSIS 2011

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Introduction:

NRL conducts External Quality Assessment Schemes (EQAS) for laboratories that perform serological and/or nucleic acid testing (NAT) techniques for infectious diseases. Samples provided for EQAS are designed to be treated in the same manner as clinical samples. In 2010, NRL examined the stability and viability of using dry swab samples for the CTNG and HSV-1/2 NAT EQAS, the data from which were presented at the 28th NRL Workshop on Infectious Diseases. Based on these data, the 2011 CTNG and HSV-1/2 NAT EQAS were each provided to participants as two panels of swab samples in addition to the traditional frozen liquid format EQAS.

Objective:

To examine the performance of the swab samples in NAT assays used by participants of NRL's CTNG and HSV-1/2 NAT EQAS in 2011.

Methods:

Ten-fold serial dilutions of *C. trachomatis* elementary bodies (CT) and *N. gonorrhoeae* bacteria (NG) were suspended in separate solutions of sterile phosphate-buffered saline. Cell culture supernatants of herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) were suspended in separate solutions of Optimatrix, a matrix designed to mimic cerebrospinal fluid. For swab manufacture, 250µL of each solution was aliquotted directly onto sterile, polyester-tipped plastic swabs and allowed to air dry inside a Class II biological safety cabinet. In addition, 1.2mL suspensions of the same analytes were directly aliquotted into tubes and frozen at -20°C. The results from testing the liquid samples were compared with those from testing the swab samples to estimate rates of detection, cross-contamination and analyte cross-reactivity.

Results:

For participants enrolled in the CTNG NAT EQAS, the detection of CT DNA was similar in both liquid (100%, CI:93.4-99.9%) and swab (98.9%, CI:92.9-99.9%) samples, irrespective of the assay. The detection rates of NG DNA was analogous in both liquid (96.8%, CI:88-99.4%) and swab (97.3%, CI:89.7-99.5%) samples. For laboratories that used in-house NG confirmatory assays, NG DNA was detectable at the same rate as their screening assay, with 100% concordance between liquid and swab samples in both the screening and confirmatory assays.

For participants enrolled in the HSV-1/2 NAT EQAS, the detection rates of HSV-1 DNA were similar between the liquid (94.9%, CI:81.4-99.1%) and swab (92.3%, CI:78-98%) samples, irrespective of assay. The detection rates of HSV-2 DNA between liquid (92.3%, CI:78-98%) and swab (87.2%, CI:72-95.2%) samples were also similar.

Conclusion:

Swab samples are a valid sample type for the delivery of CTNG and HSV-1/2 NAT EQAS. Swabs reflect a sample type encountered in clinical diagnostic laboratories. Swab samples can now be considered in the design of future CTNG and HSV-1/2 NAT EQAS.

WHAT HAPPENS WHEN AN EXPECTED NEGATIVE QA SPECIMEN RETURNS A POSITIVE RESULT?

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Introduction/Objectives: The detection of Hepatitis B surface antigen (HBsAg) and subsequent confirmation via neutralisation (the diagnosis of Hepatitis B) is a basic function in today's busy pathology laboratory and there are numerous assays and methods available. The use of quality control ensures that these tests are performing according to the manufacturer's specifications; however the use of quality assurance can give a 'snap-shot' of performance using the same specimen over different assays and methods. The RCPA Serology Quality Assurance Program provides six Hepatitis surveys in a year containing 12 specimens in total, for Hepatitis B markers. A review of the latest survey revealed an interesting case where the majority (56%) of participants from one user group returned results inconsistent with most other participants.

Methods: The second Hepatitis B survey for this year (H2:2012), was reviewed and both specimens were pre-issue tested and intended to be negative for HBsAg. For one of the specimens (2D), 245 participants returned results, with 221 (90%) returning the expected negative HBsAg result using a variety of assays. Thirty nine participants used the ABBOTT ARCHITECT HBsAg Qualitative II assay with 22 returning an equivocal (6) or positive result (16). Only seven of these participants used supplemental and/or confirmatory testing, all returning negative results. The remaining 17 participants, reported negative results with high negative result S/CO values. An investigation of the specimen, in collaboration with the report reviewer and assay manufacturer, was undertaken to identify inconsistencies or sources of the unexpected results.

Results/Discussion: The specimen constitution was reviewed and found to be a pool of four samples which individually tested negative for HBsAg, HCV Ab and HIV Ag/Ab. Three of the samples were negative for all Hepatitis B markers, whilst the fourth sample had an anti-HBs level of >1000 mIU/mL. Specimen preparation included filtration (0.2µm) and the addition of 0.05% Bronidox.

All participants using this assay were from laboratories outside Australia, where this assay has already been released for use. The assay is pending Therapeutic Goods Administration (TGA) approval for entry onto the Australian Register of Therapeutic Goods. At the request of the manufacturer, 10 aliquots of the QA specimen have been supplied to enable further investigation. A case study scenario was also issued to participants to ascertain how laboratories would investigate such an issue.

Conclusion: The RCPA Serology QAP and Abbott Diagnostics recommend re-mixing of QAP specimens and microfuging for 10 minutes prior to testing; failure to do this may result in a false reactive result, further recommendations include retesting initially reactive specimens in duplicate.

As a participant it is important to review your laboratory's QA results to ensure performance is in line with other users. QA may also be a valuable tool for manufacturers, even though the primary focus is not to assess assay performance. An essential part of the RCPA Serology QAP's role is to help identify and investigate these types of issues.

NOVEL COST-EFFECTIVE APPROACH FOR PROFICIENCY TESTING: THE USE OF DRIED BLOOD SPOTS AND DRIED TUBE SPECIMENS

Moore, C - CDC

Proficiency Testing (PT) is a valuable tool for assessing quality in clinical and public health laboratories and a requirement for laboratory accreditation. Cost of participation and sample instability are obstacles to implementing PT in countries with limited resources. The International Laboratory Branch (ILB) within the Division of Global HIV/AIDS at the Centers for Disease Control and Prevention is distributing low cost, stable dried blood spots and dried tube specimens to countries to meet their quality assurance needs. Programs are available for HIV serology and molecular diagnostics. A PT program for the detection of TB using the GeneXpert will be launched in the fall of 2012. ILB PT programs offer 2-3 test events per analyte per year. Participants receive individual and summary reports for each test event. Countries will ultimately take ownership of the manufacturing and distribution of PT samples as part of the implementation of their national laboratory strategic plans focused on achieving laboratory accreditation.

IMPLEMENTING A NEW DRIED BLOOD SPOT TESTING ALGORITHM TO DETERMINE THE PREVALENCE OF HIV AND HCV AMONG INJECTING DRUG USERS

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Introduction:

The Australian Needle and Syringe program survey (ANSPS) provides serial point prevalence estimates of human immunodeficiency virus (HIV) and hepatitis C (HCV) amongst injecting drug users. Historically St Vincent's Centre for Applied Medical Research and the NSW State Reference Laboratory for HIV at St Vincent's Hospital have successfully analysed 34,874 dried blood spot (DBS) samples for the ANSPS. Recent withdrawal from market of the commercially available Genetic Systems HIV-1 rLAV ELISA prompted the selection, optimization and implementation of a replacement screening ELISA and associated ANSPS DBS testing algorithm.

Objectives:

To successfully update an established testing algorithm that has been recinded, enabling improved efficiency and accuracy of dried blood spot analysis for HIV and HCV antibody prevalence studies.

Methods:

Feasibility testing of two commercially available enzyme immunoassay's

Paired DBS and EDTA plasma samples (n=40), were analysed to assess specificity/ selectivity and concordance between sample collection methods. The Murex (Diasorin, Italy) 1.2.0 and Genscreen (Biorad, France) HIV1/2 ELISA were initially chosen for evaluation based on kit availability, assay processing time and recommendation through the Centers for Disease Control and Prevention (CDC) external quality assurance program for neonatal surveillance for HIV. Neither kit had been validated for using the alternate DBS specimen type, Manufacturer's IFU protocol and quality control was adhered to with an additional pre assay step for dbs elution in kit specific specimen diluent.

Optimisation of Murex 1.2.0

To improve signal to noise ratio an in-house preparation of PBS milk blocking buffer replaced kit specimen diluent in the DBS elution step. Additionally the DBS eluent volume added to sample diluent was increased to meet serum concentration equivalence as indicated by the manufacturer. As per the first method paired dbs and EDTA plasma samples (n=16) were assayed alongside CDC quarterly external QAP controls (Proficiency Testing Program for Anti-HIV-1 in DBS).

Results summary:

Both commercial ELISA kits were 100 % concordant between DBS and EDTA plasma known positive samples however a significant reduction in specificity/ selectivity for known negative samples was observed. Assay carryover was detected with screened samples on the Genscreen (Biorad) HIV1/2 ELISA (n=19) suggesting a high background possible due to interference of the DBS eluate sample, which was considered undesirable.

The optimized Murex 1.2.0 ELISA increased assay performance in terms of specificity/ selectivity significantly. Subsequent participation in the CDC DBS external QAP returned 100% concordance with expected results.

The modified elution protocol was also applied to HCV testing using the Monolisa anti-HCV plus version 2 EIA, Bio-rad. All kit controls and in house DBS controls passed assay validation criteria. The HCV prevalence rate for 2011 ANSPS samples was reported as 53% which was in agreement with the 2010 prevalence rate of 53%.

Conclusions:

The optimised Murex 1.2.0 ELISA is a suitable screening method replacement to the Genetic Systems HIV-1 rLAV to determine the prevalence of HIV and HCV among injecting drug users.

In order to further enhance the ANSPS testing algorithm a validation is currently being undertaken for detection of Hepatitis C RNA. This will enable surveillance of active HCV infection, treatment response and genotype data to be included in future surveys.