Introduction: The Serology section of the Microbiology Department performs Rubella immunity screening mainly from antenatal patients and staff health clinics, with a small number of tests being performed for the diagnosis of Rubella infection. Since the implementation of the NSW Department of Health Circular 2003/91 “Occupational Screening and Vaccination against Infectious Diseases”, the number of requests for Measles, Mumps and Rubella IgG tests has increased dramatically.

Objective: To compare the results obtained for Rubella IgG using 3 automated immunoassay platforms: Vidas (BioMerieux), Vitros ECI (Ortho Clinical Diagnostics), and AxSym (Abbott Diagnostics), with the objective to determine which method is the best in our laboratory to provide accurate results with efficient workflow.

Methods: One hundred and seventy two samples comprising two panels:

1. Stored sera, which had previously given results obtained using the Vidas/MiniVidas of <25 IU/mL.
2. Newly received samples, including both routine screening and diagnostic tests.

All specimens were tested on all three platforms.

Results: The correlation of results is currently underway.

Discussion: The analysis of results will be discussed

Conclusion: The decision on which platform best suits our laboratory for Rubella IgG testing in the future will be revealed.
EARLY ANTIRETROVIRAL THERAPY (ART) AND TREATMENT INTERRUPTION IN HIV-1 INFECTION: THE IMPACT ON THE IMMUNE RESPONSE, VIRAL FITNESS AND VIRUS CONTROL

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Introduction: To investigate the impact of structured treatment interruption on viral fitness and the development and potentiation of a neutralising immune response in acute HIV-1 infection.

Methods: Twenty acutely HIV-1 infected subjects on ART in the PULSE study were studied. Subjects were fully adherent to ART for up to one year, with undetectable viral loads (VL) prior to interruption. Treatment was resumed if VL exceeded 5,000 copies during interruption. Plasma and serum samples were taken at Baseline (BL) and throughout the trial.

Results: Using an established PBMC based neutralisation assay, BL plasma from sixteen subjects inhibited replication of the CCR5-using Clade B reference isolate HIV-1MBC925. Autologous virus was successfully isolated from BL serum in fifteen of the twenty subjects. There was a significant increase in neutralisation of the reference isolate (seven subjects) and autologous virus (five subjects) using plasma isolated during interruption compared to BL. However, there was no correlation between neutralisation and subsequent virus control upon interruption. We were unable to isolate virus from BL samples for five subjects and the replication capacity of isolates from six subjects was slow. Of these eleven subjects, eight contained virus replication upon interruption.

Conclusion/Discussion: There was no correlation between virus neutralisation in vitro and virus control upon interruption. As the observed neutralisation of autologous and heterologous virus replication was unexpected, we are investigating complement in subject plasma and natural killer cells in donor PBMCs mediating antibody-depandent cell-mediated cytotoxicity as possible causes. There was a correlation between viral replication phenotype and virus control upon interruption. Using real time PCR, we are examining early virus replication kinetics as a measure of viral fitness.

These results will provide important insight into the impact of early ART and treatment interruption on viral fitness, the immune response and the importance of initial viral phenotype as a clinical marker for virus control.
THE USE AND INTERPRETATION OF QUALITY CONTROL IN SEROLOGICAL TESTING

Brooks ZC

Introduction
Each numerical result from quality control (QC), proficiency testing or patient samples falls into one of three categories: the number is right, wrong, or “close enough to right”. If patient values are right, or “close enough to right”, scientists and clinicians are able to draw correct conclusions and ultimately treat patients appropriately. If numbers, or the interpretation based on those numbers, are “wrong”, then consequences of this failure can negatively impact patients, laboratories and the overall health care system. Changes in the accuracy or precision of an analytical process can cause an unacceptable portion of “wrong” patient results to be reported. If such a change occurs, QC sample values should mirror changes in patient results and alert the laboratory scientist through failures of QC rules. A well-designed QC strategy will maximize the probability of detecting significant changes in the system, while generating a minimal number of false positive flags. When the overall mean and standard deviation of QC values are compared to the “right” number (true value) and a defined range of what is “close enough to right” (performance standard, allowable error limit or TEa), QC data can also quantify total error (TE) and determine the overall fitness of a method to fulfill its clinical purpose.

Objectives
1. To assess various sources of “close enough to right” (TEa limits) for HIV, HBsAg and RubellaG
2. To assess selected NRL data to determine which, if any, of these TEa sources are consistently attainable
3. To determine if changes in patient data are indeed reflected by changes in QC samples
4. To model QC strategies that would detect significant changes in method accuracy or precision
5. To assess if the Total Error of each method consistently fell within various TEa limits

Methods
1. An online project group consisting of Zoe Brooks, Wayne Dimech and Marilyn Fleming exchanged data and concepts at http://www.zoebrooksquality.com/harmonize. We evaluated TEa limits based on (1) Delta values, (2) a portion of the expected patient population (Tonk’s rule), (3) estimates from professionals in the field, (4) the difference between the lowest confirmed positive and the cutoff and (5) state of the art.
2. We examined >18,000 patient and >600 QC results for HIV, HBsAg and Rubella G from a single NRL laboratory from the entire year of 2004, and >1,000 recent QC results from an additional 5 to 6 NRL laboratories. In each case, a single QC sample was used throughout the study period, while reagent batches changed frequently.
3. Daily patient medians for 2004 were graphed with individual QC results to observe similarities and differences in shifts and trends, especially when reagent batches changed.
4. Quality control strategies were recommended for each laboratory/batch based on how close the observed QC values fell relative to the limits of what is “close enough to right”.
5. Total Error was calculated as [|Bias| + (1.96 SD)] for each laboratory/ reagent combination, and compared to various sources of TEa limits to assess the overall fitness of a method to fulfill its clinical purpose.
*Software employed included EP Evaluator™, Quality Advisor© and MS Excel™.

Results
Observed method performance usually fell within TEa limits that were based on a combination of (a) the difference between the lowest confirmed positive and the cutoff and (b) 5 state of the art.
QC strategies can reliably detect significant change in method accuracy and precision – if QC samples reflect changes in patient samples.

Conclusion/Discussion
Regular comparison of the overall mean and standard deviation of QC values to the “right” number (true value) and a defined range of what is “close enough to right” can reveal instances when a method may fail to fulfill its clinical purpose. A well-designed QC strategy will maximize the probability of detecting significant changes in the system, while generating a minimal number of false positive flags.
**Introduction:** The National Serology Reference Laboratory (NRL) provides Nucleic Acid Testing (NAT) for blood-borne pathogens. The NRL utilises Polymerase Chain Reaction (PCR) to test for the presence of HIV-1, HIV-2, HTLV-I, HTLV-II and Hepatitis C nucleic acid. Conventional PCR is optimized for maximum sensitivity and as a result the amount of the final product does not necessarily reflect the number of copies of DNA present in the original sample. In contrast, Real Time PCR measures the kinetics of DNA amplification. Consequently, the results are quantitative when compared with standards. The NRL is currently validating the use of Real Time PCR in conjunction with an automated nucleic acid extraction method for routine diagnostic use.

**Objectives:** To develop a Real Time PCR multiplex to detect simultaneously HIV-1 and/or HIV-2 DNA, which matches the sensitivities of the assays currently in use.

**Methods:** Nucleic acid extraction was performed using the Roche MagNA Pure LC DNA Isolation Kit. Real Time PCR was carried out using Stratagene’s MX3000P. Primers and Taqman probes were chosen for their ability to detect a wide range of subtypes (Yun Z et al., 2002). Separate Real Time PCR assays were developed for both HIV-1 and HIV-2. These assays included an internal control using cellular primers and probes for the human albumin gene (Desire N, 2001). Experiments with primers and probes, as well as PCR conditions were carried out to optimise for maximum sensitivity, specificity and reproducibility in each assay. HIV-1 and HIV-2 standards (ABI) in serial 5-fold dilutions were used to generate standard curves, calculate amplification efficiency values and to determine the detection limits. Following the optimisation of each single-analyte assay, primers and probes for HIV-1, HIV-2 and the human albumin gene were combined in a multiplex assay. Adjustments to the reagents and PCR conditions were made to maintain the optimum levels of specificity and sensitivity.

Initial testings of the multiplex assay was performed using whole blood samples from 206 HIV-1 sero-negative individuals and 26 HIV-1 positive individuals who were on anti-retroviral therapy and therefore expected to have low viral turnover rates.

**Results:** The HIV Real Time PCR multiplex successfully amplified and detected the HIV-1 and HIV-2 standards. Amplification efficiencies values of 84.6% - 109.7% (HIV-1), and 81.5% - 98.2% (HIV-2) were achieved. The R² values for the HIV-1 standards were 0.986 – 0.999, and were 0.981 – 0.999 for HIV-2. In 25 runs, the multiplex assay had a lower detection limit of 1.92 HIV-1 DNA copies per 5µl in all 25 runs, while the HIV-2 DNA detection limit was 0.38 copies per 5µl in 24 of the 25 runs.

No reactivity was detected in the 206 sero-negative samples; the presence of extracted DNA was verified by the detection of the internal control gene in all samples. Successful HIV-1 amplification and detection was achieved for 21 of the 26 HIV-1 positive samples. The 5 undetected samples were also determined as negative by the NRL’s current in-house DNA PCR.

**Discussion:** Individuals on anti-viral therapy may have low or undetectable viral loads, as well as associated lower integrated DNA levels (Sarafianos SG et al., 2004; Tubiana R et al., 1999). This may account for the results in the 5 samples that were not detected in the Real Time PCR multiplex. Development of the HIV Real Time PCR multiplex at the NRL appears to maintain our ability to detect HIV-1 DNA. Furthermore, the Real Time PCR multiplex successfully amplified and detected the viruses of interest in a fraction of the time required by conventional PCR. The NRL will examine the multiplex on a larger population, including HIV-2 positive samples as well as HIV-1 positive samples where subjects are not being treated with anti-retroviral therapy.
PRE-MARKETING EVALUATION OF ARCHITECT SYPHILIS ASSAY – A PROSPECTIVE COMPARISON WITH THE MUREX ICE ASSAY

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Introduction
Syphilis, caused by the spirochaete Treponema pallidum, can be transmitted sexually or through blood transfusion. The seriousness of this disease is the high risk of complications and congenital infections. Laboratory diagnosis is largely based on serological tests. EIA assays are widely used as the screening Assay for Syphilis because it is highly sensitive and can easily be automated. This study compares the performance of Architect with the routine Murex Ice Assay (both Abbott EIA) for the detection of IgG and IgM antibodies (together) to Treponema pallidum.

Sample Selection
471 samples with syphilis antibody requested were collected prospectively over two weeks. First consecutive 385 samples were tested by both assays. For the remaining samples (frozen), Murex Ice assay was performed first. 50 antenatal samples and 36 samples that were reactive by Murex Ice assay were selected for Architect evaluation.

Materials and methods
Testing algorithm was followed as below:
Murex Ice assay was performed using the Tecan-BEIIP combination. Initial reactive results were repeated in duplicate. Rapid plasma reagin test and Treponema Pallidum Particle-Agglutination were performed on repeat reactive samples. Fluorescent Treponemal Antibody absorption Test was used for resolving the discrepant results produced between Murex and TPPA. Architect results were only repeated on samples giving discrepant results between Murex and Architect.

Results
408 samples were negative in both assays. 63 samples were reactive in the Murex assay but only 52 in the Architect assay. 54 samples (including all 52 Architect reactive) met the criteria for true positives. Sensitivity, specificity positive predictive values and negative predictive values for each assay were: Murex 100%, 97.8%, 85.7%, 100% and Architect 96.3%, 100%, 100%, 99.5%.

Conclusion
Two assays gave comparable results although Murex was marginally more sensitive and Architect was marginally more specific. The advantages of use of a grey zone for the Architect assay will be discussed.
**HETEROLOGOUS TITRES IN THE LEPTOSPIROSIS MICROSCOPIC AGGLUTINATION TEST**

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**Introduction:** Sera tested by the microscopic agglutination test (MAT) are usually (not always) positive to other serovars in the same leptospiral serogroup as the infecting serovar. Heterologous titres, to serovars in other serogroups, can confuse diagnosis. Many canine sera tested recently by the University of Guelph have had positive titres for serovar Autumnalis, usually associated with titres to other serovars. The Guelph laboratory, using one Autumnalis culture, obtained an Autumnalis titre of 2560 for a sample positive for serovar Hardjo, but a negative result using a culture sourced elsewhere.

**Objectives:** To investigate heterologous titres in the leptospirosis MAT, and their possible significance for the diagnosis of leptospirosis in Canadian dogs.

**Methods:** Heterologous titres were studied in 13,757 results from four rounds of the International Proficiency Testing Scheme for the Leptospirosis MAT. These were reported by 85 laboratories, testing 12 rabbit antisera to serovars within 10 serogroups.

**Results:** There were 1400 positive titres (defined as ≥80): 645(92%) of 700 to homologous serovars, 319 (81%) of 394 to other serovars within the serogroup and 436 (3%) of 12,663 heterologous. Heterologous titres to serogroups Australis, Autumnalis, Bataviae, Icterohaemorrhagiae and Pomona, being most frequent, were further analysed. The highest rates of heterologous titres (>10%) were for: serogroup Bataviae used to test samples positive for serogroup Tarassovi (91%) and Sejroe (11%); serogroup Autumnalis with serogroups Grippotyphosa (49%), Pomona (42%) and Australis (26%); serogroup Australis with serogroups Autumnalis (26%) and Pomona (18%), serogroup Icterohaemorrhagiae with serogroup Canicola (10%), and serogroup Pomona with serogroup Sejroe (17%).

Heterologous titres to serogroup Autumnalis varied between rounds: 4% in Round 2 but 83% in Round 3 with antiserum to serovar Grippotyphosa, and 77% in Round 3 but 3% in Round 4 with antiserum to serovar Bratislava; they were largely with serovar Autumnalis, not other serovars. In Round 3 (2004), 56 participants reported 24 heterologous titres to serovar Autumnalis when testing an antiserum to serovar Pomona: these titres were higher and were reported more frequently in North American than European laboratories.

**Conclusion:** Heterologous titres to serovar Autumnalis were unusually high in 2004, perhaps reflecting a common origin for cultures used in North America. Cultures of different origins may produce different results in the MAT.
INTRODUCING THE NEW ROCHE ASSAYS FOR HUMAN PAPILLOMAVIRUS (HPV) TESTING

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Cervical cancer is the second most common malignancy in women worldwide with 288,000 deaths annually (WHO statistics, July 2004). In 1996, both the World Health Organization/European Research Organization on Genital Infection and Neoplasia and the NIH Consensus Conference on Cervical Cancer recognized HPV infection as the primary cause of cervical cancer (99.7% prevalence).

Approximately 40 HPV genotypes are specific to the anogenital epithelium; 13 of these are currently classified as carcinogenic (high risk genotypes) and are the primary cause of cervical intraepithelial neoplasia (CIN) and cervical cancer. Sexually transmitted infection with HPV is extremely common, with estimates of up to 75% of all women experiencing exposure to HPV at some point. The majority of HPV infections clear spontaneously. However, women with persistent high risk HPV infection constitute the true high-risk group for cervical cancer, and will benefit most from early viral detection and clinical surveillance for cytological abnormalities.

The new Roche assays for HPV DNA testing are qualitative in vitro tests that utilize PCR and nucleic acid hybridization for the detection of HPV DNA in clinical specimens. In both assays, the ß-globin gene is isolated concurrently and functions as an internal control to assess the adequacy of the patient sample. In addition, the ß-globin gene also controls for extraction and amplification for each individually processed specimen.

**AMPLICOR HPV Assay** detects 13 high risk HPV DNA genotypes: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. In non-clinical performance evaluations the sensitivity and specificity were 96.1% and 95.6% respectively, and the lower limit of detection was 60 copies/ PCR for all high risk genotypes.

**LINEAR ARRAY HPV Genotyping Assay** identifies 37 individual high-and-low risk anogenital HPV DNA genotypes: 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73 (MM9), 81, 82 (MM4), 83 (MM7), 84 (MM8), IS39 and CP6108. In non-clinical performance evaluations, the sensitivity and specificity were 96% and 99% respectively, and the limit of detection (95% positive hit rate as predicted by probit analysis) varied from 30 copies/ PCR (Genotype 16) to 480 copies/ PCR (Genotype 31). The assay demonstrated excellent reproducibility and genotype inclusivity.
National Serology Reference Laboratory, Australia

**Introduction:** Dried blood spots (DBS) provide a convenient method for blood sample collection in many settings where the prevalence of infection with hepatitis C virus (HCV) is increasing. Consequently, anti-HCV antibody assays are required that produce reliable results using samples derived from DBS.

**Methods:** Paired plasma samples and whole venous blood spotted onto Schleicher & Schuell cards were collected. Anti-HCV reactivity was determined by testing plasma samples. A direct comparison of the performance of two commercial enzyme immunoassays (EIA) using DBS samples was performed using 14 DBS samples, and the assay that provided the greatest dynamic range was used for further evaluation. The optimum buffer for the elution of samples from DBS was selected. Seventy five anti-HCV reactive and 108 anti-HCV non-reactive DBS with paired plasma samples were compared using the modified commercial EIA.

**Results:** There was a significant difference between the S/Co ratios of anti-HCV reactive and non-reactive DBS eluates in this assay (p < 0.001). The delta value for anti-HCV reactive DBS was 5.89, and was -6.64 for anti-HCV non-reactive DBS. These delta values indicate that separation of each sample population from the assay cutoff is greater that 3 standard deviations of the mean of the log S/Co ratio. The modified assay was found to have an estimated sensitivity and specificity of approximately 100% for detecting anti-HCV antibodies in DBS.

**Conclusion:** This study demonstrated the feasibility of using DBS eluates in a modified commercial EIA for the detection of antibodies to HCV. The use of DBS will enable the collection of data for epidemiological and screening purposes both in the field and in under resourced settings.
QuantiFERON®-TB Gold TESTING IN A CLINICAL SEROLOGY LABORATORY

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Introduction: The Mantoux skin test (utilising purified protein derivative (PPD)) for the detection of latent Mycobacterium Tuberculosis infection presents many limitations. Requirements of the Mantoux test include highly skilled personal to perform test and patient compliance with follow up interpretation. False positive and negative results occur and cross-reactions with nontuberculous mycobacteria (NTM) are an issue. The recently developed QuantiFERON®-TB Gold (Cellestis Australia) Assay has many advantages when compared to the Mantoux test. The assay is more specific, not affected by BCG or the majority of NTMs, it claims superior sensitivity (89%), incorporates a mitogen control, requires a single patient visit, is safe and has a rapid turnaround time of 24 hours.

Objectives: Review results for samples submitted to Sullivan Nicolaides Pathology (SNP) from November 2003 to May 2005 for QuantiFERON®-TB Gold Assay and test performance in a clinical laboratory servicing a wide geographical area. Review and analyse reasons for test referral and examine more closely patients demonstrating unusual profiles and those with a positive interpretation.

Methods: Lithium heparin samples were processed as per instructions in the QuantiFERON®-TB Gold Assay. Plasma was harvested from whole blood after being incubated with TB-specific antigens and any Interferon-γ (IFN-γ) produced was quantified by ELISA. Results were calculated utilising specific software supplied by Cellestis and then reported as negative, positive or indeterminate. Clinical information was obtained from the request slip and a phone questionnaire was conducted with the requesting doctor. These results were then correlated with other laboratory information including Mantoux testing and culture for Mycobacterium species.

Results: Results were obtained for 351 patients (365 samples). The assay was negative for 88% (309/351) of patients, positive for 9.7% (34/351), indeterminate for 2% (7/351) and unsuitable for one patient. Mitogen levels ranged from 0 to 322 IU/mL and were <0.5 in 9 patients of which 2 had antigen levels ≥ 0.35. The nil response (result from unstimulated cells) ranged from 0 to 32.7 IU/mL with 91.5% of patients demonstrating a level <0.7IU/mL and 2.6% >4IU/mL. An abnormally high nil response (32.7 IU/mL) was observed in a patient on high dose prednisone and was unsuitable for interpretation. During the testing period several runs were rejected due to slope or co-efficient data failing the kit criteria.

Discussion: Overall, the results obtained using the QuantiFERON®-TB Gold Assay provide a clear and definitive interpretation in comparison to the Mantoux test. The exception is indeterminate patients who are incapable of generating detectable levels of IFN-γ. The manufacturer indicates that high levels (>4 IU/mL) of IFN-γ in plasma from unstimulated whole blood occurs in 0.5% of patients and levels of <0.7IU/mL in 97% of patients evaluated. Data from SNP did not support this with high levels of IFN-γ observed in 2.6% of patients. Logistically there are some areas of concern with the assay. In a laboratory servicing all states of Australia, the complex requirements for collection, delivery, incubation and processing can be problematic. A recently released alternative sample collection and processing method (“In-Tube”) may be a suitable alternative due to improved processing procedure. The non-standard loading format for the ELISA is essential for compatibility with the assay specific software but inconvenient when processing smaller batches. All laboratory issues of failed runs or batch specific problems have been dealt with rapidly in a professional and practical way, with excellent and effective support from the manufacturer (Cellestis).

Conclusion: The QuantiFERON®-TB Gold Assay is a suitable alternative to Mantoux testing in the majority of cases due to reduced turn around time, minimal patient interaction and compliance, increased specificity and ease of interpretation. The alternative “In-Tube” method may reduce some processing and training issues in the future.
Introduction:
Typical human antibody responses to HIV-1 infection are unable to neutralise virus efficiently, clear the infection, or prevent disease progression. However, more potent neutralising antibodies may be capable of playing a pivotal role in controlling HIV replication in vivo. PEHRG214 is a polyclonal caprine antibody raised against purified HIV-associated proteins, such that epitopes that are immunologically silent in humans may potentially be recognised in another species. It has been administered safely with minimal side effects to HIV-infected individuals in Phase I clinical trials (Dezube et al, 2003; Pett et al, 2004).

Objectives:
To assess the potency, breadth of action, and mechanism of action of PEHRG214 antibody using neutralisation and virion lysis assays.

Methods:
Virus neutralisation was tested in PHA activated PBMC as these cells are susceptible to all isolates. The role of complement in virus neutralisation was also assessed in these cells. Representative viral isolates from clades A, B, C, D and CRF01A_E were tested. The role of antibody plus complement in virion lysis was also tested. The target proteins for PEHRG214 activity were investigated using flow cytometry and by adsorption of anti-cell antibodies from the antibody cocktail.

Results:
The antibody potently neutralised a diverse range of primary HIV-1 isolates, encompassing clades A to E and both CCR5 and CXCR4 phenotypes at concentrations similar to that observed for HIVIG, a reference pooled polyclonal anti-HIV-1 neutralising antibody. Neutralisation was enhanced by the presence of human complement. PEHRG214 also induced complement-mediated lysis of all HIV-1 isolates tested, and recognized or cross-reacted with a number of host cell proteins. Virion lysis was abrogated by adsorption with T and/or B cells expressing GPI-linked proteins, but not by GPI-deficient B cells or red blood cells.

Conclusions:
Hence, PEHRG214 was found to potently neutralise and lyse HIV-1 particles and was complement dependent. Adsorption using B or T cells indicated the involvement of antibodies against cellular proteins or host cell protein mimicry by the virus. By targeting host-cell proteins present in the viral envelope, which are present among all strains tested, PEHRG214 potentially opens up a highly novel means of eliminating circulating virus in HIV-1 infected individuals being mindful of using a goat IgG-based immunotherapy.
Estimating Uncertainty: a nuisance or a tool to improve quality?

Most laboratories are estimating uncertainty not because they want to, but because they have to. The requirement is very firm in ISO 17025 and somewhat weaker in ISO 15189, but generally it is somewhat unwelcome in all laboratory settings. Laboratories are told it is needed because clients might ask for it (no one ever does), or it is needed when reporting conformance to a specification limit (not common in medical laboratories), but there is little other justification provided for what can be an extraordinary effort. This presentation makes the case that estimates of uncertainty, or rather the process of developing the estimates, can actually be very useful quality improvement tools. Whether one develops the estimates with the “bottom-up” approach or the “top down” approach, one still must list the components of uncertainty and their approximate magnitude. This listing of components forces the laboratory to consider each measurement procedure carefully, including sources under the control of the laboratory such as technician differences and environmental conditions. If an result is produced, this listing can be a “road map” for root cause investigations; if the uncertainty is too large, this shows the laboratory the best place to direct resources to lower the uncertainty.
TRANSIENT HEPATITIS C ANTIBODY IN ACUTE Q FEVER INFECTION

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Introduction: It is well documented that a variety of infections cause the appearance of false positive IgM antibodies in assays for diseases other than the infecting agent. False positive Q Fever IgM antibodies are seen in a variety of infections such as Hepatitis A, CMV, EBV, Leptospirosis and Brucella. The presence of false positive Hepatitis C antibody is not well documented. Q Fever and Hepatitis C infection normally present different risk factors and clinical presentation and therefore confusion with diagnosis is uncommon.

Objectives: Present serological results from a patient with evidence of seroconversion for Hepatitis C and Q Fever. Investigate other possible cases of cross reaction between Hepatitis C and Q Fever.

Materials and Methods: Case 1, a 66 year old female from a rural town presented with vague illness, fever and anorexia. Risk factors for the patient included exposure to containers from a farm and also young adult children diagnosed with Hepatitis C. Serological testing was performed on initial samples for Hepatitis A, B and C, Q Fever and Leptospira. Subsequent Hepatitis C and Q Fever testing was performed on a further 5 samples over a 151 day period (days 0, 6, 13, 27, 41,151). Hepatitis C PCR and RIBA testing was performed on day 6 and 27 respectively.

Screening for potential Hepatitis C cross reaction in acute Q Fever infection was performed on serial samples from 7 other cases of acute Q Fever.

Hepatitis A serology was performed on the Abbott Axsym, Hepatitis B serology on the Abbott Architect, Hepatitis C serology on the Abbott Architect and the Murex EIA, and Hepatitis C PCR by Roche Amplicor HCV method. Q Fever testing included IgM EIA (Panbio) and CFT testing utilising Virion/Serion antigen.

Results: Hepatitis A, B and C serology was negative on the initial sample for case 1. Q Fever IgM and phase 2 CFT was detected on the second sample 6 days later. A third sample demonstrated Hepatitis C antibodies in both the Architect and Murex assays and also a greater than four fold rise in Q Fever Phase 2 antibodies. Retrospective testing of initial samples indicated that Q Fever antibodies were not present on the initial visit and that Hepatitis C remained negative until the third sample on day 13 when antibodies were detected by both assays. Hepatitis C PCR (day 6) was negative and RIBA (day 27) indeterminate. The final sample at day 151 was negative on the Hepatitis C architect assay and remained Q Fever IgM positive and Phase 1 and 2 CFT positive. Hepatitis C serology performed on serial samples from 7 other acute Q Fever cases was positive in 1/7 cases (Case 2). In case 2 Hepatitis C antibodies were detected by the Murex Assay on the second sample in a series of 6 collections and coincided with a four fold rise in Q Fever Phase 2 CFT antibody.

Discussion: The appearance of antibodies to both Hepatitis C and Q Fever, combined with the risk factors, delayed the diagnosis of Case 1. Subsequent RIBA testing, as recommended by the NRL Hepatitis C testing protocol, failed to clarify the Hepatitis C status of this patient. It was not until day 151 when both Hepatitis C antibody assays were negative, that a clinical report discounting Hepatitis C infection could be issued. The testing of a small panel of acute Q Fever samples indicated that transient false positive Hepatitis C antibodies may not be a rare occurrence.

Conclusions: The appearance of transient Hepatitis C antibody in acute Q Fever is probably uncommon however in rare cases where multiple risk factors are involved transient levels may lead to confusion in diagnosis.
DEVELOPMENT OF A RUBELLA IMMUNOGLOBULIN G (IGG) ASSAY* ON THE VITROS™ ECIQ WITH INTELLICHECK™ AUTOMATED CONTINUOUS RANDOM ACCESS IMMUNOASSAY SYSTEM.

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Objectives. We have developed a rapid, fully automated assay for Rubella IgG in human serum and plasma with the VITROS™ ECIQ Immunodiagnostic System with Intellicheck™ Technology. Intellicheck™ Technology provides sample quality and assay processing verification checks to ensure quality of result. The objective of this study was to describe and verify its performance.

Materials and Methods. The VITROS Rubella IgG assay uses a conventional anti-globulin assay design. The patient sample is first incubated with an Assay Reagent in a rubella antigen coated well. Patient anti-rubella immunoglobulins bind to the antigen on the well. After a wash cycle to remove unbound materials, a Conjugate Reagent, containing horseradish peroxidase (HRP)-labeled mouse monoclonal anti-human IgG antibody is added, followed by a further incubation. HRP conjugate binds to any human IgG captured on the well. After another wash cycle to remove unbound HRP conjugate, Signal Reagent (containing the Enhanced Chemiluminescent substrate) is added and the well is read. The light signal and amount of HRP conjugate bound is directly proportional to the concentration of anti-rubella IgG present in the sample. Time to first result is 32 minutes.

The assay is calibrated using 3 liquid Ready to Use calibrators with a range up to 350 IU/mL with reference to the WHO 1st IS. The cut off has been set at 10 IU/mL. Calibration stability is more than 4 weeks. Total CVs ranged from 6.1% to 11.0% in a precision study conducted according to NCCLS guideline EP5-A. No significant interference was detected from bilirubin, hemoglobin, or triolein. Samples positive for RF, HAMA, and ANA also showed no interference.

The data demonstrated a good agreement between the VITROS anti-Rubella IgG assay and a commercially available comparative assay was obtained. A panel of 393 patient samples from a variety of clinical categories was measured. The following specificity and sensitivity was achieved:

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The specificity and sensitivity of the anti-Rubella IgG assay was 98% (132/135) and 100% (244/244), respectively.

Conclusion. The VITROS ECIQ anti-Rubella IgG assay combined good analytical and clinical performance with the operational simplicity of a rapid automated continuous random access immunoassay system.
DEVELOPMENT OF A NEW HEPATITIS B SURFACE ANTIGEN ASSAY WITH IMPROVED SENSITIVITY TO WILD TYPE AND VARIANT HBsAg FOR USE ON THE ORTHO CLINICAL DIAGNOSTICS VITROS® ECiQ IMMUNODIAGNOSTIC SYSTEM.

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Objectives: There are increasing concerns about the contribution of variant HBsAg to vaccine escape, immune prophylaxis failure, therapy and false negatives in serological HBsAg detection for diagnosis and blood screening. The group specific “a” determinant region (124-147) attracted our attention because evidence of mutations in the region is found in 10-20% of cases in some geographical areas and with long-term chronic carriers or those undergoing treatment. Our objective therefore was to increase the ability of the assay to detect as wide a range of HBsAg variants as possible and in addition, as variant HBsAg normally co-occurs in patients or donors with wild type HBsAg, to increase the sensitivity of the test to wild type HBsAg.

Materials and Methods: Over 60 monoclonal antibodies were characterized based on their capture and detection binding patterns of mutant HBsAg and wild type antigen. Improvements were assessed by the detection of earlier seroconversion patient panel samples and by an enhanced analytical sensitivity for wild type, subtypes and recombinant mutants. Conjugation of monoclonals was by standard procedures and the solid phase employed used an indirect streptavidin coating with biotinylated capture antibodies. This procedure should enable more rapid test improvement in the future should a new HBsAg variant emerge which requires an additional antibody.

Although recombinant mutant panels may not be a realistic clinical test of variant HBsAg detection, they are useful for test development. A wide range of artificial mutants was generated particularly from the second loop region (137-147) that had the greatest effect on conformation. The first loop region (124-137) was also studied and an antibody was identified which, working with the existing antibodies used in the test, improved detection of mutant panels known to have variations in this or nearby areas. Tests employed the standard VITROS ECiQ System with Intellicheck Technology™ process control and the normal HBsAg reagents except for the enhanced solid phase and conjugate for the same incubation times, which can allow testing of over 90 samples per hour.

Conclusions: In terms of analytical sensitivity with the formulations chosen, the sensitivity of the prototype VITROS ECi HBsAg test was improved by 14% for subtype ad and 13% for subtype ay compared with the current ECiQ assay using BBI Panel PHA807. For adw, ayw and adr subtypes the improvement was 14%, 15% and 57% respectively. For HBsAg showing variation in or around the first loop with various recombinant materials the sensitivity was improved by 11% for some of the mutants to over 100% for one mutant. For the second loop variants there were considerable increases in sensitivity for all mutants with the average final sensitivity of 0.14 ng/ml. All commercial seroconversion panels could be detected at the same or an earlier time-point compared with the existing assay. The assay was shown to be robust to different sample collection devices and carry-over. Specificity on 5000 donors was >99.95% and the total precision with a control giving a result of S/CO of 1.5 was less than 6%.
CHARACTERISATION OF THE HUMORAL IMMUNE RESPONSE TO PRIMARY RUBELLA INFECTION

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Introduction: Primary rubella infection in the first trimester of pregnancy can result in congenital rubella syndrome in the fetus, the most common symptoms of which are blindness, mental retardation and deafness. Consequently the diagnosis of recent rubella infection during pregnancy is important. An assay to distinguish between the immune response generated by recent exposure and the immune response existing as a result of past exposure or immunization is necessary. Claims are made that commercially available enzyme immunoassay (EIA) based avidity assays distinguish between low avidity (indicative of recent infection) and high avidity antibodies. The immunological principles behind these avidity assays have never been comprehensively investigated. It has been demonstrated that a peptide analogue of the E1 Rubella glycoprotein can be substituted for viral lysate in diagnostic assays.

Aim: To characterize the maturation of the humoral immune response to Rubella in recently infected individuals using isotype specific EIAs and avidity assays and to correlate the results with the relative affinity constants calculated using Surface Plasmon Resonance (SPR).

Method: Seroconversion panels consisting of sequential samples obtained from four recently infected individuals were assayed for antibody isotype reactivity to either viral lysate or a synthetic peptide derived from the immunodominant region of the Rubella E1 glycoprotein. The avidity index (AI) of the antibodies to the lysate or peptide was calculated as the proportion of high avidity antibody in the sample remaining bound in the EIA following incubation with 8M urea. A BIACore™ 2000 biosensor (BIACore AB, Uppsala Sweden) was used to measure the interaction of the serum antibodies with the E1 peptide. The binding data was evaluated with BIAlEvaluation version 3.2 software and relative affinity constants were calculated.

Results: The proportion of high avidity total IgG and IgG1 antibodies detected using the viral lysate EIA increased rapidly following infection, reaching a maximum between an estimated 20 to 80 days following symptom onset. The proportion of high avidity IgG3 and IgA increased more slowly while the proportion of high avidity IgM remained low. The immune response to the E1 peptide matured rapidly with little IgM present in the first specimen of three of the four panels while total IgG and IgG1 responses were already present in all specimens. BIACore™ analysis of the seroconversion panels revealed that the overall binding of the antibodies specific for the E1 peptide was high and increased only slightly in affinity with time.

Conclusion: The immune response to rubella infection consists of an initial low affinity peak of IgM reactivity followed by transient peaks of low avidity IgG3 and IgA reactivity. The predominant response is an IgG1 response which gradually increases in affinity with time. The 8M urea incubation used in the avidity assay appears to eliminate the detection of the early low affinity IgM, IgA and IgG3 reactivity, explaining the principal underlying the commercial avidity assays. The AI measured in the avidity assay and the affinity measured using the BIACore™ of the immune response to the E1 immunodominant peptide matured too rapidly to provide a means of identifying recent infection but may be useful for routine diagnosis.
Stability Testing and Interpretation: RCPA Serology Quality Assurance Program

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Introduction: Stability testing of QA specimens is an essential component of an effective QA Program and ensures the integrity of specimens at the time of testing and the ensuing test results. Participants receive specimens for the entire year in one dispatch at the beginning of the year and are instructed to freeze the specimens until they receive the appropriate questionnaire. Therefore, this is replicated for transit stability testing.

Objective: The objective of assessing stability of samples is to estimate the extent to which test results of samples decrease over time duration between sample creation and testing. This provides the testing laboratory with reassurance as to the integrity and reproducibility of any QA specimen tested, and the issuing QA laboratory with reassurance that all results are comparable between laboratories.

Method: A protocol that assesses stability over time has been designed to reflect firstly, the maximum duration that samples could be in transit before either testing or freezing, and the results still considered valid, and second, it will identify any non-linear time-effects, if such effects are non-linear.

A test-retest variability range is calculated based on the test-retest data of the 10 baseline samples. The test-retest range is defined as the mean ± 2 standard deviations, and should contain 95% of all sample results. Raw test results at each day are then plotted by day. The test-retest variability range gives a visual impression of both whether the overall test values are increasing or decreasing over time, or if the test-retest variability is increasing.

Results: Varying time patterns are produced that are specific to each analyte. The day that is represented by the unstable data point represents the time frame that participants must receive specimens before they become unstable and impair the integrity of participant results.

Conclusion/Discussion: Transportation times are monitored for both international and domestic couriers to ensure that all specimens are delivered to participants in a timeframe that is within established stability timeframes for each individual analyte. This procedure and practice satisfies the stability requirement for specimen integrity of QA specimens from the Serology QAP.

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Introduction: West Nile virus (WNV) entered the United States in 1999 and has spread to most U.S. states, Canada and Mexico. WNV infection usually presents as a mild self-limiting condition but in 1% of infected individuals it has emerged as an important cause of neuroinvasive disease. Because viremia usually persists for only a short period, serology has become an important tool for detecting WNV infection, and the availability of FDA-cleared assays has given laboratories the capacity to meet the demand for testing. However, with WNV becoming endemic, it has been observed that the classical marker of acute infection, IgM, may persist over months. This can confound data where a sample is positive for both IgM and IgG and the determination of a causal link between the serological data and the clinical presentation is problematic. We present data on the use of IgG avidity testing as a tool to resolve conflicts in data arising from the detection of both WNV IgM and IgG.

Methods: Sequential plasma samples were collected from blood donors testing positive for WNV by a Transcription Mediated Amplification (TMA) assay. Thirteen (13) seroconversion panels with TMA positive (index) samples were assayed for IgG and IgM antibodies using FDA-cleared assays (Panbio). All IgG-positive samples were retested in the presence of a proprietary buffered avidity reagent (Panbio). For each positive IgG sample, the results with and without avidity reagent were compared and used to generate an Avidity Index. Samples were also tested by WNV and St Louis encephalitis (SLE) PRNT (CDC, Colorado, USA).

Results: On the basis of the IgG and IgM profiles over time for the 13 individuals, the panels were classified as representing either an “acute” or “secondary” response. The “acute” samples had a typical IgM response followed by an IgG response. The “secondary” samples presented with an earlier and marked IgG response followed by a weak IgM response. This latter pattern was considered to be consistent with an anamnestic response associated with previous exposure to WNV or another flavivirus. The three panels exhibiting a “secondary” profile demonstrated an “original antigenic sin” phenomenon to SLE virus by PRNT, documenting previous SLE exposure. When the IgG avidity profiles were plotted for the panels from individuals with an “acute” profile, all samples exhibited a low Avidity Index (less than 40%) for the first 20 days post index (testing TMA positive). After approximately 40 days post index, the Avidity Index rose to over 60%. For the “secondary” samples, all positive IgG samples had an Avidity Index of ≥ 59% regardless of the days since index.

Conclusion: Avidity testing of positive IgG samples where a subject presents with a positive IgM and positive IgG response appears to be of utility in the differentiation of recent acute infection from persistent antibody and/or an anamnestic response.
THE USE OF DELTA VALUES IN CHARACTERISING ASSAY PERFORMANCE DURING PRE-MARKET EVALUATIONS OF NEW DIAGNOSTIC TESTS

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Introduction

In Australia, the National Serology Reference Laboratory (NRL) has responsibility for pre-market evaluation of diagnostic tests for HIV and HCV infection. Many of these assays are already in use overseas. Traditionally diagnostic tests are evaluated in terms of simple qualitative measures of sensitivity and specificity. However, in the course of evaluating a number of enzyme-linked immunoassays (ELISA) it became clear to the NRL that a more statistical approach to assessing performance of quantitative and semi-quantitative assays was required. The delta value ($\delta$), which takes into account the spread of results and the distance between the means of the negative and positive populations from the set cut-off, was subsequently incorporated into NRL's evaluation testing strategy.

Objectives

To describe the use of delta values in assessing assay performance, in particular its role in distinguishing between HIV-1 and HIV-2 sensitivity.

Methods

Sensitivity, specificity and $\delta$ are calculated for each quantitative and semi-quantitative diagnostic test undergoing pre-market evaluation.

The delta values are calculated using the formula: $\delta = \frac{\text{mean log}_{10}[S/Co]}{\text{SD log}_{10}[S/Co]}$

where S/Co = sample : cut-off ratio or Index value and SD = standard deviation. Separate negative and positive delta values are calculated for negative and positive populations. A $\delta$ value > 3 (indicating that the mean of the log S/Co is more than 3 SDs from the cut-off) indicates adequate separation between the cut-off value and the values obtained for the positive or negative values. For positive values it means that 99.7% of values are more than 3SD removed from the cut-off, and there is less than a 0.3% chance of producing a false negative result.

When evaluating an anti-HIV assay that yielded two false negative HIV-2 results, sensitivity and $\delta$ values for HIV-1 (n = 205) and HIV-2 (n = 27) were compared. A further analysis of HIV-2 results for the 25 commercially acquired HIV-2 panel samples (containing one of the two falsely negative samples) and the one falsely negative diagnostic sample was also undertaken. All 26 samples had been tested in nine different assays.

Results

The estimated sensitivity for the anti-HIV-1 positive panel was 100% with a 95% confidence interval (95%CI) of 97.7 – 99.9, and a positive $\delta$ of 19.6. By comparison, the estimated sensitivity for the anti-HIV-2 panel was 92.6% (95%CI 74.2 – 98.7) with a positive $\delta$ of 1.4. The calculated positive $\delta$ for the other eight assays testing the same samples was > 3 for each assay (range 5.2 – 91.7). The manufacturer provided details of a further 100 anti-HIV-2 samples that had been tested in the assay undergoing the evaluation. Despite the assay achieving 100% sensitivity in this population, an NRL analysis of the raw data showed a positive $\delta$ of 2.2 (c/f a positive $\delta$ of 1.4 obtained by NRL).

Conclusions

In these analyses $\delta$ values confirmed the initial findings of low sensitivity for the anti-HIV-2 component of an assay. The results led the manufacturer to reformulate the assay. They further justified NRL's decision to include $\delta$ value calculations with sensitivity and specificity calculations in pre-market evaluations of diagnostic tests and demonstrate the valuable role played by NRL's evaluation programmes.

THE MEDICAL DEVICE INCIDENT REPORT INVESTIGATION SCHEME (IRIS) OF THE THERAPEUTIC GOODS ADMINISTRATION (TGA)

Therapeutic Goods Administration, Canberra, Australia

Introduction:
The aim of the Medical Device Incident Report Investigation Scheme (IRIS) is to improve the quality and the safety of medical devices, through the investigation of adverse events associated with those devices and the dissemination of appropriate information. Pathology assays, known to regulators as in vitro diagnostics devices (IVDs), are considered medical devices and are included in this scheme.

Methods:
Users of medical devices are encouraged to report incidents or difficulties associated with their use. Incidents that have caused, or could have caused, an injury to the patient or the device user should be reported. In Australia, reporting of such events is mandatory for the medical device industry. In addition to reporting safety concerns, everyone is encouraged to report any concerns about the quality and efficacy of medical devices.

A panel of scientific, engineering and clinical experts assess all reports. The panel determines whether the report needs further investigation by the TGA, and if so recommends what level of investigation will take place and assigns it to the most appropriately qualified investigator. The investigator liaises with the company and the reporter during the investigation, and at times, other international regulatory bodies. If necessary, testing of the device may also be conducted.

Both the reporter and the device supplier are informed of the outcome of the investigations.

Results:
Over the past five years the scheme has received 3000 reports of problems with medical devices. This has lead to 159 recalls, 100 safety alerts, 200 product improvements and the publishing of 80 articles in the TGA News and other publications. It should be emphasised that these results relate directly to investigations into reports of events and complaints received by the IRIS. The results do not include, for example, voluntary recalls by manufacturers that are reported directly to the Recalls Section at the TGA.

Conclusions:
The statistics demonstrate considerable achievements by the IRIS in the improvement of the safety and performance of medical devices supplied in Australia and abroad. The IRIS is unique amongst global adverse event reporting schemes in regards to its approach to risk assessment, investigation and feedback. The system relies on the input from the users of medical devices, not only in terms of reporting the initial concern or event, but also during the investigations and implementation of the measures that will prevent or alleviate the hazards resulting from medical device events and malfunctions.
Evaluation of Hepatitis B surface antigen assays performed on the Abbott Architect and Bayer ADVIA Centaur analysers and detection of HBsAg mutants in a local population.

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An evaluation of the Abbott Architect and Bayer ADVIA Centaur HBsAg immunoassays was performed on a series of samples originally screened by the Abbott AxSYM HBsAg version 2 assay. 299 HBsAg negative samples were evaluated. The Architect and Centaur both detected 1 (0.3\%) non-confirming positive sample. Neither the Architect nor the Centaur was significantly affected by cadaveric samples with both instruments detecting 1/48 (2.0\%) non-confirming positive sample. Neither instrument detected any of the 17 non-confirming positives detected on the AxSYM.

185 HBsAg positive samples were evaluated. The sensitivity and specificity of the Architect was 100\% whilst the Centaur had a sensitivity of 98.9\% and specificity of 100\%. There were 3 HBsAg positive samples that were detected on the AxSYM and Architect but were negative or gave a significantly reduced signal on the Centaur.

Sample 1: Patient on lamivudine showing decreasing levels of HBsAg over a period of time. This sample was weakly positive on the Architect but consistently negative on both the AxSYM and Centaur.
Sample 2: Patient with a P142L mutation in the HBsAg protein indicative of a HBV vaccine escape mutant. The sample was highly positive on the AxSYM and Architect, but was negative on Centaur.
Sample 3: Patient with multiple mutations in the HBsAg protein including G145R indicative of a HBV vaccine escape mutant. The sample was highly reactive on the AxSYM and Architect, but was weakly reactive on Centaur (Index 2.24).

In Limit of Detection studies, the Architect was generally positive at one dilution greater than the Centaur. In a panel of HBsAg mutant recombinant proteins, all 9 mutants were detected by the AxSYM and Architect instruments but 5 were missed by Centaur.

The evaluation demonstrated that the Centaur did not adequately detect two HBsAg escape mutants from a local group of HBsAg positive patients.
EVALUATION OF THE CAVIDI EXAVIR™LOAD QUANTITATIVE HIV RT LOAD KIT AS AN ALTERNATIVE HIV VIRAL LOAD MONITORING TEST FOR USE IN RESOURCE-CONSTRAINED SETTINGS

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Introduction
The widely used molecular biology based viral load assays for monitoring HIV are not technically or economically feasible in most resource-constrained settings. Thus there is an increasing need for less expensive and simpler tests to monitor HIV disease progression in resource-constrained areas to allow for the appropriate use of antiretroviral therapy.

Objective
We have evaluated version 2 of a low cost manual reverse transcriptase assay, ExaVir™ Load assay CavidiTech AB (HIV RT) and compared it to a commercially available HIV RNA assay that quantifies viral load to assess the potential use of the RT assay to monitor HIV infection in resource-constrained settings.

Method
Frozen plasma samples from HIV infected individuals previously quantified for HIV RNA using the COBAS Amplicor HIV-1 Monitor assay, ultra-sensitive preparation (RT-PCR) were retested for HIV RT activity.

Results
The HIV RT assay showed good sensitivity with detectable HIV RT in 93% of samples (n=121) with HIV RNA >1,000 copies/ml and 73% (n=33) between 401-1000 copies/ml. A positive association was found between the HIV RNA copies/ml and HIV RT copies/ml equivalents (r=0.96; n=182). A decrease in association was observed when samples ≤2000 copies/ml were analysed (r=0.35; n=89). Ten samples were tested on the same HIV RT assay using plasma input volumes of 1ml (recommended), 0.5ml and 0.25ml and compared them to HIV RNA. All dilutions differed from the matched HIV RNA test by <0.32 log₁₀. The HIV RNA results for each patient were reproducible using different volumes with variation for all patients <0.42 log₁₀ (Fig. 1)

Conclusion
The HIV RT assay showed good association with the RT-PCR assay, and has sensitivity approaching that of RT-PCR. The HIV RT assay was reproducible using smaller sample volume making it useful for paediatric testing.

Fig. 1

![Graph showing HIV RNA and HIV RT results for different volumes](image-url)
Quantification of human immunodeficiency virus type 1 (HIV-1) RNA is the "gold standard" marker for monitoring disease activity in patients receiving highly active antiretroviral therapy (HAART). However, cost, assay complexity and equipment requirements render the customary monitoring technologies not suitable for most resource-limited settings.

The ExaVir® Load is an EIA-based method that determines viral load by measuring activity of the HIV Reverse Transcriptase (RT) enzyme. The assay is simple, robust and requires only standard EIA equipment and the ExaVir® Load start up equipment.

The NRL, in collaboration with WHO, has assessed the performance of the Cavidi Tech ExaVir® HIV viral load assay, which offers a potential solution to providing viral load estimates in resource-limited settings.

Several performance characteristics of the Cavidi ExaVir® Load assay were determined:

Reproducibility was assessed by testing replicates of 6 samples with viral loads ranging from 2,000 – 85,000 copies/mL. The means, standard deviations and coefficients of variation were calculated.

Sensitivity was estimated in 65 specimens positive for various subtypes of HIV. Thirty-five samples were plasma drawn from infected individuals; thirty were cultured HIV-1 isolates spiked into normal human plasma.

Linearity was determined by testing 5 replicates of each member of a six-member dilution series. Regression analysis was used to estimate linearity.

Assay specificity was estimated in 115 anti-HIV negative specimens.

All specimens gave valid results. Between run variation was <7% for each of the samples in the reproducibility panel giving quantifiable results. Detectable RT activity was present in 91% and 99% of samples with HIV RNA >1,000 copies/mL and >10,000 copies/mL respectively. All but one cultured isolate of HIV subtype D gave results above the assay's limit of detection. Regression analysis gave an r² value of 0.99. Twenty-one of the sero-negative samples gave low viral loads (<1,000 copies/mL).

The ease of use and the performance characteristics of the Cavidi ExaVir® Load assay make it an appropriate assay for resource-limited settings. The occurrence of false positive results precludes its use for any purpose other than HIV therapeutic monitoring.
THE EPIDEMIOLOGY OF SEXUALLY TRANSMITTED INFECTIONS IN AUSTRALIA; IMPACT OF DIAGNOSTIC TESTING

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Introduction

In Australia, more than 38,000 cases of bacterial sexually transmitted infections (STIs) were reported in 2003 (30,193 chlamydia cases, 6611 gonorrhoea and 1768 syphilis). STIs are a major public health concern as they are common; can result in serious complications and sequelae including pelvic inflammatory disease, infertility, ectopic pregnancy and cervical cancer; have substantial economic consequences; and a number of STIs have been shown to facilitate the spread of HIV. After years of decline, notification rates of many STIs have recently increased substantially in Australia.

Methods

To describe the epidemiology of STIs in Australia data will be presented from passive surveillance, behavioural surveys, research and testing data.

Results

Since 1997, national notification rates of chlamydia have more than tripled from 50.1 per 100,000 in 1997 to 160.7 per 100,000 in 2003. Over the same period the total number of chlamydia tests conducted has increased considerably from 21,246 tests in 1997 to 55,600 tests in 2003 (Victoria data only). The number of chlamydia notifications and the number of tests were highly correlated. Prevalence surveys have shown that women aged less than 25 years of age, street sex workers, men who have sex with men (MSM) and indigenous Australians are at greatest risk of chlamydia.

Reported national rates of gonorrhoea have also increased from 25.8 per 100,000 in 1997 to 34.6 per 100,000 in 2003 with MSM and indigenous Australians shown to have the highest rates.

The notification rate of syphilis has increased from 6.9 per 100,000 in 1997 to 8.9 per 100,000 in 2003 with MSM and indigenous Australians also shown to have the highest rates. There has been a marked recent resurgence of syphilis in Victoria and NSW among MSM, to levels not recorded since the early 1980s, while behavioural surveys have demonstrated that the proportion of MSM reporting unprotected anal intercourse has increased significantly.

Discussion

The changing epidemiology of most STIs in the Australia probably reflects changes in sexual behaviour and increased testing and utilisation of more sensitive diagnostic techniques. The notification rates reported here are obtained from passive surveillance, which is based largely on reporting of symptomatic cases and likely to represent only a small fraction of all prevalent infections. To help understand the impact of testing on STI rates, The Victorian Department of Human Services plans to implement a sentinel surveillance system in late 2005. Future control of STIs will require a comprehensive approach involving primary and secondary prevention methods.
THE CORE DILEMMA OF HEPATITIS B DIAGNOSIS.

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Introduction:

Hepatitis B is usually diagnosed based on the pattern of serological markers but this can be complicated by hepatitis B virus (HBV) variants. Atypical presentation or absence of serological HBV markers such as hepatitis B core antibody (anti-HBc) can delay laboratory diagnosis and make patient management difficult. Here we present 8 cases of newly diagnosed hepatitis B infection in which there was delayed presentation or absence of anti-HBc.

Methods:

Samples referred to Gribbles Pathology for Hepatitis B serology were initially tested on the Bayer ADVIA Centaur for Hepatitis B surface antigen (HBsAg), antibody to Hepatitis B surface antigen (anti-HBs), Hepatitis B core total antibody (anti-HBc Total) and Hepatitis B core IgM antibody (anti-HBc IgM). Confirmatory testing of HBsAg specificity was performed using BioMerieux Vidas HBsAg Ultra kit. Hepatitis Be Antigen and antibody was preformed using BioMerieux Vidas HBeAg/Ab kit. Further anti-HBc Total testing was performed using the BioMerieux Vidas HBC Total II kit.

Hepatitis B DNA was measured using real-time PCR referenced to the WHO Standard 97/746I. HBV genotype was determined by sequencing of the HBV polymerase gene after PCR amplification and the core gene was similarly sequenced after amplification of the core gene. HBV genotype and unique HBV mutations were identified using a web based program, SeqHepB (www.seqvirology.com/genome7/index.htm). This program analyses HBV DNA to determine HBV genotype, to identify key mutations associated with antiviral resistance as well as other clinically important HBV variants by comparing the input sequence data with known HBV reference sequences.

Results:

Eight patients initially presented who were HBsAg reactive. HBeAg was present in 5 of the 6 patients. Anti-HBc Total was not detected using the Bayer ADVIA Centaur and BioMerieux Vidas HBC Total methods. Anti-HBc IgM and anti-HBs were not detected using the Bayer ADVIA Centaur assays. Seven patients had detectable levels of HBV DNA. On subsequent testing two patients were shown to have seroconverted, some two months after their last negative assay, developing both anti-HBs and anti-HBc Total. Six patients on follow-up remained HBsAg and HBV DNA positive but no anti-HBc Total was detected. All patients were asymptomatic with normal or mildly elevated LFTs.

Four of the anti-HBc negative and HBV DNA positive samples were genotyped and had the core region sequenced to determine if there were mutations in the core promoter or other mutations which could be responsible for the unusual serology. Two viruses were HBV genotype A, one genotype B and the other genotype C, reflecting the ethnicity of the patients. Although mutations were found in the core region and some of these resulted in amino acid changes, no changes correlated with a loss or reduction of core protein synthesis or with previously described significant mutations.

Conclusion:

Laboratory diagnosis of HBV infection and exposure is reliant on the detection of serological markers for HBsAg and anti-HBc. Changes in the core region of the viral genome may contribute to the inability of current serological assays to detect antibody produced by patients infected with HBV. Atypical patterns in the presentation of these markers can lead to misinterpretation of a patient’s status unless detailed serological testing and molecular methods are used to confirm or exclude HBV infection. Samples presenting with atypical serology should be followed up to determine if the cause is related to a virus variant.
GENITAL HERPES: DIAGNOSIS AND DISEASE

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Introduction: Genital ulcers disease is commonly caused by herpes simplex viruses (HSV). Once infected recurrence of virus shedding occurs frequently and often without symptoms. Most HSV infection is transmitted by asymptomatic carriers who may or may not be aware of their status and in turn the first noticeable symptoms of the disease may not be noticed for some time after infection.

Objectives: This paper will review the natural history of the infection, serological methods for investigating the prevalence of HSV infections and viral diagnostic methods eg real time PCR for detecting viral shedding and typing of HSV and the issues that the results of these investigations commonly raise.

Results: Crude IMVS HSV 1 and 2 sero-prevalence and HSV isolation data for the period 2003-2005 have been reviewed. HSV 1 and HSV 2 was detectable in 15.4% and 16.9% of genital swabs respectively but HSV 1 predominated in non anogenital areas. HSV 1 was more commonly isolated than HSV 2 in genital swabs from patients less than 30 years of age but the ratio reversed in older patients. HSV 2 seroprevalence rates increased markedly in the 30+ age group reaching a maximum of 40% in 40-49 year olds. In contrast HSV 1 seroprevalence ranged from 43% in the first decade to 85% in the seventh decade of life consistent with high rates of non sexual transmission early in life. Of note 1% of genital swabs (3.4% of isolates) contained varicella zoster virus.

Conclusion: Both HSV 1 and 2 are common genital infections of adults. Comparisons of local data with other published data will be presented and the implications for patient management discussed.
ANTIVIRAL RESISTANCE STUDIES OF HUMAN CYTOMEGALOVIRUS IN AUSTRALIAN IMMUNOCOMPROMISED PATIENTS

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Introduction: Human cytomegalovirus (CMV) infects up to 90% of the population but is generally asymptomatic in immunocompetent people. However, CMV infection and disease are significant causes of morbidity and mortality in immunocompromised patients, such as transplant recipients receiving prophylaxis and human immunodeficiency virus (HIV)-positive individuals with acquired immunodeficiency syndrome (AIDS). Since the introduction of antiviral therapy, morbidity and mortality due to direct CMV infection and disease has decreased. However, emergence of CMV antiviral resistance plays a significant role in disease progression in immunocompromised patients who have received antiviral therapy. Previous rates indicate that 30% of patients treated for greater than two months will develop CMV antiviral resistance.

Objectives: This study aimed to determine the incidence of antiviral resistance in Australian immunocompromised patients. Additionally, the sensitivity of PCR-sequencing protocols and thus the clinical applicability of such methods was evaluated.

Methods: Specimens from patients with clinical evidence of CMV antiviral resistance, that is, an increasing viral load or progression of CMV disease despite antiviral treatment, were collected and amplified. A new PCR-sequencing based protocol, which allows the genotypic detection of all known and potential antiviral resistance mutations, was subsequently optimised using limit of detection analysis. Two regions of the CMV UL54 DNA polymerase and one region of the CMV UL97 protein kinase from clinical specimens were amplified by nested PCR. The increased sensitivity and amplification of all relevant domains leads to greater accuracy in detecting CMV antiviral resistance, which has been previously underreported due to only screening UL97 protein kinase. Sequencing analysis of each PCR amplicon was done to detect known antiviral resistance mutations and to identify previously unrecognised antiviral resistance mutations.

Results: Genotypic resistance testing is rapid and sensitive, detecting down to 10 target gene copies per reaction. PCR-sequencing analysis of specimens from patients with clinical evidence of CMV antiviral resistance identified antiviral resistance mutations in UL54 DNA polymerase and UL97 protein kinase in 6/17 (35%) patients after six months of antiviral therapy. GCV resistance UL97 protein kinase mutations in clinical specimens were detected at amino acid codons 460 (M460V), L595S, A594V and a deletion of codons 592-597 was also observed. While mutations in the same region have been observed, the UL97 deletion 592-597 has not been previously detected. Two known UL54 mutations were detected, one that confers resistance to PFA (T700A) and one that confers GCV + CDV resistance (K513N). Additionally, the PFA resistance UL54 DNA polymerase mutation (T700A) was not detected until 16 months after a four week course of PFA therapy ended and a GCV resistance UL97 mutation, L595S, in the same patient was no longer detectable, despite continuing GCV treatment.

Conclusions: Genotypic screening for antiviral resistance is a useful and sensitive method which can detect all known resistance mutations. Using this method, 35% of Australian specimens screened from at-risk patients contained one or more antiviral resistance mutations. Of these, most had received long-term antiviral therapy indicating that extensive antiviral therapy is a significant risk factor for development of antiviral resistance. Additionally, resistance mutations can persist in the absence of the antiviral to which the mutation emerged despite treatment with an alternative antiviral. Detection of cross-resistance is also clinically important when evaluating future antiviral therapy. Genotypic screening for CMV antiviral resistance will increase knowledge of patient risk and assist in forming more valid therapeutic algorithms.
TRANSPLACENTAL TRANSMISSION, AND FETAL INFECTION, OF VIRUSES IN STILLBIRTHS AND MISCARRIAGES

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Introduction: Vertical transmission of viruses and other infections can occur during pregnancy (transplacentally) or during delivery. These infections can cause a wide range of congenital abnormalities in the infant, including hearing loss and developmental delay, as well as pre-term delivery and fetal death. A combination of molecular and serological techniques is necessary to determine women at risk of intrauterine transmission of viruses. CMV specific IgG avidity allows increased accuracy in estimating the timing of primary infection in pregnant women, while molecular methods such as \textit{in-situ} PCR increase understanding of the passage of virus across the placenta and in the fetus.

Objectives: A study is being conducted to determine the prevalence of, and the molecular events associated with, transplacental transmission of pathogenic viruses in stillbirths and miscarriages. This study involves the detection of viruses in human placental and fetal samples.

Methods: Women who have had a stillbirth or miscarriage were asked to participate in this study by donating samples for serology and molecular testing. Blood samples were tested using CMV IgG, IgM and IgG avidity. Three multiplex PCR (mPCR) reactions were also developed for screening all donated samples. The mPCRs screened for human cytomegalovirus (CMV), human herpesvirus-6, -7, -8, HSV-1, -2, varicella zoster virus, \textit{Toxoplasma gondii}, parvovirus B19, rubellavirus, enteroviruses, hepatitis C virus and lymphocytic choriomeningitis virus. \textit{In-situ} PCR was then performed on samples with detectable viral DNA on mPCR testing, to further identify which cell types were infected.

Results: To date, the validated mPCRs have detected the presence of CMV in 6/15 stillbirth/miscarriage cases, and 30/94 tissue samples. CMV has been detected in all tissues studied excluding mesentery, rib and trachea. Placentae from infected mothers showed that predominantly syncytiotrophoblasts were infected, although vascular, supporting and endothelial cells were all infected.

Conclusions: Based on previous serology findings, women at all stages of gestation can be at high risk of intrauterine transmission of CMV. Women infected with CMV during late gestation are more likely to transmit the virus, so failure to detect seroconversions in late gestation women may result in failure to detect infected, asymptomatic neonates. mPCR and \textit{in-situ} PCR have shown that CMV infects all tissue and cell types, although surprisingly some tissues from stillborn infants have been shown to be uninfected. The study will ultimately provide the basis for future treatment and care of mothers and babies at risk of congenital infection.
AN INTRODUCTION TO THE RISK-BASED CLASSIFICATION SCHEME FOR IN VITRO DIAGNOSTIC DEVICES (IVDs) UNDER THE PROPOSED IVD REGULATORY FRAMEWORK

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Introduction: The Australian community has an expectation that all therapeutic goods in the market place, including laboratory tests such as IVDs, are safe and of high quality, meeting standards equivalent to those of comparable countries. All Therapeutic Goods in Australia are regulated by the Therapeutic Goods Administration (TGA). At the present time, the level of IVD regulation in Australia is very limited, with only tests for Human Immunodeficiency Virus (HIV) and Hepatitis C Virus (HCV) undergoing extensive pre-market performance evaluation. All other IVDs undergo a limited level of regulatory scrutiny, or are exempt from entry onto the Australian Register of Therapeutic Goods (ARTG). In January 2002, the TGA initiated development of a new regulatory framework for IVDs that is harmonised with international best practice.

A New IVD Regulatory Framework: It is proposed that the new regulatory framework will capture all IVDs, including commercial and in-house assays, though regulatory requirements may be different. All IVDs will be required to demonstrate conformity to a set of essential principles. The essential principles provide the measures for safety and performance of all IVDs and will be set out in the regulations. There will be two types of essential principles: general principles, which are relevant to all medical devices, including IVDs, and specific principles, which will apply only to IVDs. Only IVDs that conform to both sets of principles will be able to apply for inclusion on the ARTG. The extent of TGA regulatory oversight of IVDs will be commensurate with the risk posed by the particular IVD as determined by its risk classification.

Risk-Based Classification: The principle of risk-based classification is commonly applied in regulatory systems as a way of mitigating risks inherent in a product by establishing tighter regulatory controls for products identified as carrying a higher risk burden. The risk classification will be based on the intended purpose of the IVD. “Intended purpose” is the objective intent of the manufacturer regarding the use of a product, process, or service as reflected in the specifications, instructions and information provided by the manufacturer. The new regulatory framework will encompass a rules-based risk classification system for all IVDs and it will be the manufacturer’s responsibility to classify their IVD into a particular risk class by following a set of pre-determined rules.

It is proposed that there will be four risk categories under the new framework with an increasing level of regulatory scrutiny commensurate with risk class as detailed below:

- **Class 1** no public health risk/low personal risk;
  Manufacturers will be required to notify the range of IVDs manufactured and to self-declare that their manufacture complies with the essential principles
- **Class 2** low public health risk/moderate personal risk;
  Manufacturers will be required to make a pre-market application to the TGA and include evidence that the IVD design supports the use of the IVD in the way proposed by the manufacturer. Manufacturers will be required to have quality management systems in place and have their systems certified by the TGA, unless other acceptable certification is in place.
- **Class 3** high personal risk/moderate public health risk;
  The TGA will perform an assessment that all the documentation is present and appears to have been assembled with sufficient rigour, but will not normally evaluate the material in depth. However, the TGA would retain the right to question any deficiencies or discrepancies noted. Manufacturers will be required to have quality management systems in place and have their systems certified by the TGA, unless other acceptable certification is in place.
- **Class 4** high public health risk
  The TGA will carry out a full pre-market evaluation including performance testing. The manufacturer will be required to submit a detailed application relating to the design and manufacture of the IVD. Manufacturers will be required to meet manufacturing standards and have their quality management systems certified by the TGA, unless other acceptable certification is in place.

This poster will present an introduction as to how the new IVD framework will provide a risk-based approach to the regulatory control of IVDs for supply on the Australian market.
QUALITY CONTROL PROGRAMME FOR NUCLEIC ACID SCREENING IN BLOOD SERVICE LABORATORIES.

Jardine D, Dent B, Read S and Dax EM

Background: Recent developments in technology have seen the introduction of Nucleic Acid Testing (NAT) to screen for early infection with HIV or HCV in blood donors. The National Serology Reference Laboratory (NRL) has implemented a quality assurance (QA) programme for blood service laboratories using these technologies. QA methods used by the NRL include Quality Control (QC) and External Quality Assessment Schemes.

Ten laboratories participate in NRL’s QC programme, performing nucleic acid screening with the Chiron TMA Multiplex assay (TMA). The performance of individual laboratories, luminometers and TMA reagent batches, was determined by analysing data reported exported from EDCNet.

Materials and Methods Participants were supplied with three QC samples (HCV RNA: 380 geq/ml, HIV RNA: 250 geq/ml and normal human plasma) to monitor run-to-run precision of the TMA assays. The two RNA positive samples were produced by the Viral Quality Control Laboratory (now Acrometrix VQC) in The Netherlands and were secondary working reagents calibrated to the WHO International Standards for HIV and HCV. QC samples were run as a Go/No Go controls in every assay run and the results submitted to the NRL through the NRL’s on-line QC interface, EDCNet (www.nrlqa.net). This application allows laboratories to assess run performance immediately. Data were exported from EDCNet and analysed using SPSS (SPSS Inc. Chicago, Ill) Version 12.1. Analysis of variance (ANOVA) was used to compare TMA reagent batches, laboratories and luminometers.

Results Between September 2001 and June 2005 participants submitted in excess of 30,000 QC sample results from a number of QC sample batches. Analysis of the data from the QC sample batches hivspy004 and hcvspy004 showed that from 12,221 results in 6110 assay runs, 13 (0.21%) were invalidated on the basis of a nonreactive QC sample result. Analysis of the data from the subsequent QC sample batches showed that 0.05% and 0.07% of assay runs were invalidated on the basis of a nonreactive QC sample result, respectively. Inter-laboratory precision, estimated by coefficients of variation (CV), ranged from 10.92 - 15.05%. Significant reagent batch to batch variation was detected. However the significance was attributed to the minimal within reagent batch variation seen with the TMA assay. It was assessed not to be of practical significance. In no case did the lower 95% CI of S/Co for the positive QC samples drop below 6.9, which is well above the assay’s positive cut-off of 1.0.

Discussion. Blood service laboratories have used EDCNet to monitor the precision and accuracy of results in ‘real time’ since 2001. The proportion of assay runs that were invalidated by laboratories because of negative QC sample results was minimal. Variation in results between luminometers and between TMA reagent batches whilst statistically significant, were not of practical consequence.
THE IMPORTANCE OF MONITORING FALSE REACTIVITY RATES IN PRISM ASSAYS AT BLOOD SERVICE LABORATORIES

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1National Serology Reference Laboratory, Australia and 2 Australian Red Cross Blood Service, on behalf of the Virus Serology & Biochemistry Team

Introduction: The National Serology Reference Laboratory, Australia (NRL) provides a Specificity Monitoring Programme to five Australian and two international blood service laboratories screening with the anti-HIV, anti-HCV, anti-HTLV, and HBsAg PRISM ChLIA. This programme monitors the rate of false reactivity in donor specimens over time by assessing the initial and repeat reactor rates of the PRISM assays. The initial reactor rate (IRR) is calculated as the proportion of negative samples that were reactive when initially tested. The repeat reactor rate (RRR) is the proportion of presumed negative samples that were reactive when initially tested and, when retested in duplicate, one or both retests are reactive. A sudden increase in false reactivity may indicate a problem batch or indicate a problem occurring within a laboratory. The difference between the initial and repeat reactor rate is a measure of technical error. Increases in the IRR prompts increased repeat testing which in turn increases costs to the blood service. Increases in the RRR will result in increased deferral of donated blood, which impacts the blood supply.

Method: Laboratories reported the number of specimens screened, the number of initially and repeatably reactive specimens and the number of confirmed positive specimens for each week by assay and PRISM reagent lot (batch). The study period examined was between week 27, July 2004 and week 26, June 2005. An estimate of the numbers of donations discarded per 100,000 was calculated and the estimated cost of discarding these donations was also calculated. The overall IRR and RRR were examined for each PRISM assay from data submitted by the five Australian Red Cross Blood Service Laboratories (ARCBS). The IRRs and RRRs for each laboratory were graphed as a rolling average of three week periods and examined for sudden increases in reactivity.

Results: An estimated total of 239 specimens per 100,000 donations was discarded during this period because of false reactivity in an antibody screening test. The cost of discarding RR donations was estimated to be 5.3 times greater per 100,000 donations for the HIV assay and 3.0 times greater per 100,000 donations for the HCV assay than for donations discarded because they were falsely reactive in the HBsAg assay. The reactor rates of the PRISM HIV assay were higher than the rates observed for the other assays. A sudden increase in the repeat reactor rate of the PRISM HBsAg assay between week 27, 2004 and week 24, 2005 was observed for one Australian laboratory. Increases in reactor rates due to reagent batch were not observed in any laboratory.

Conclusion: Increases in false reactivity observed in the PRISM assays at blood service laboratories, increases the amount of testing performed at the ARCBS, increases the cost of testing and reduces the number of donations available for transfusion medicine. The Specificity Monitoring Programme identifies sudden increases in IRR and/or RRR which can be a prompt for a laboratory to investigate its testing processes for any causes of such an increase.
DETECTION OF RUBELLA IgM FROM DRIED VENOUS BLOOD SPOTS USING A COMMERCIAL ENZYME IMMUNOASSAY

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Introduction
In countries with limited infrastructure and laboratory capacity, collection and transport of venous blood samples may be difficult. The use of dried blood spots for the detection of rubella IgM antibodies can aid rubella control programs which are important in the goal of measles elimination through immunization strategies of many developed and developing countries that use a combined measles-rubella vaccine.

Objectives
To adapt a commercial enzyme immunoassay (EIA) for the detection of rubella IgM in dried venous blood spots (DVBS).

Methods
We used 60 DVBS, prepared at the time of venous blood collection, from enhanced measles/rubella surveillance program in Victoria and 28 DVBS prepared using donor red blood cells spiked with serum, which had been tested as part of a rubella outbreak in a Pacific Island nation. The kit manufacturers protocol was adapted to include variations in incubation times and washing procedures.

Results
Optical densities were corrected for kit variation as recommended by the manufacturer but no further adjustment was needed to compare serum and DVBS results. The sensitivity of the DVBS compared with serum for the categorization of rubella IgM as positive or negative was 96.7%(95% CI, 83.3%-100%) and the specificity was 100%(93.7%-100%).

Conclusion
We conclude that DVBS can be used to detect rubella IgM using a modified protocol in a commercial EIA. This EIA is widely used in diagnostic and reference laboratories.
DONORS WITH NON-SPECIFIC REACTIVITY IN SCREENING AND CONFIRMATORY ASSAYS: A CASE OF COLLATERAL DAMAGE

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The aim of this presentation is to present an overview of some of the problems and challenges created for blood services by donors who give non-specific reactivity in screening and confirmatory assays. As well, some of the approaches to managing these issues will be described.

In developed countries where blood services use a combination of sensitive serological and NAT screening assays, the risk of transfusion transmitted infections is now very low. Although serological assays also have excellent specificity, in low risk populations such as blood donors, a number of non-specific reactive results are still generated. Donors with non-specific reactivity in screening and confirmatory assays pose a number of issues and challenges for blood services. At a time when blood services are finding it difficult to maintain sufficiency of supply, non-specific reactivity in screening assays is a source of product and donor loss.

The primary strategy for reducing donor non-specific reactivity (biological false reactivity - BFR) in screening immunoassays (IAs) is to select assays with high specificity. Recent analysis of ARCBS donors who give BFR results on antibody screening assays has shown that most continue to give BFR results at subsequent donations. Data from this analysis has been used to develop procedures for managing donors with BFR results.

It is now well established that most donors who give indeterminate results on anti-HIV and anti-HTLV immunoblots (IBs) represent non-specific reactivity rather than “true” antibody. ARCBS has been able to significantly reduce the number of non-specific indeterminate results in blood donors by reducing the number of donors requiring IB testing. This has been achieved by the use of a sequential IA strategy whereby donors who are reactive on a primary screening IA are further tested on a secondary IA. Only donors who are reactive on both IAs are subject to further testing by IB. This has resulted in an improvement in the specificity of the ARCBS testing algorithms without loss of sensitivity.

Anti-HCV indeterminate results pose an additional challenge to blood services due to the natural history of HCV. Several lines of evidence now suggest that a proportion of donors with anti-HCV indeterminate/HCV RNA negative results may represent previous exposure to HCV. Clarification of anti-HCV indeterminate results and the management of these donors remain an issue for blood services.
External Quality Assessment Scheme for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* nucleic acid testing

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Introduction: Participation in the NRL’s External Quality Assessment Schemes (EQAS) for *Chlamydia trachomatis* (*C. trachomatis*) and *Neisseria gonorrhoeae* (*N. gonorrhoeae*) nucleic acid testing (NAT) contributes to assuring the quality of laboratory performances. By comparing results in a series of samples with reference results, the entire testing process can be assessed.

Objectives and Methods: Objectives of this EQAS in 2004 were to assess 1) the sensitivity of *C. trachomatis* detection by challenging assays with samples spiked with low-level, quantified amounts of antigen, 2) *N. gonorrhoeae* assay reproducibility by challenging with identical samples, 3) an element of assay robustness, the potential for cross contamination between samples, by inclusion of samples with high-levels of antigens alternating with negative samples, and 4) the possibility of cross-reactivity in *N. gonorrhoeae* assays by challenging with samples spiked with a strain of *Neisseria subflava* (*N. subflava*).

Results: There was inconsistent detection of *C. trachomatis* at low antigen concentrations. Nearly 20% of laboratories failed to consistently detect *C. trachomatis* at 1,000 genome equivalents/mL. All laboratories demonstrated reproducible detection of *N. gonorrhoeae* at the concentration equivalent to 100,000 colony forming units/mL. One incident of sample cross contamination occurred. Several (3 of 23) commercial *N. gonorrhoeae* assay users reported initial cross reactivity when testing samples spiked with *N. subflava*.

Discussion: EQAS contributes to quality assurance by providing a retrospective analysis of laboratory testing performance at a point in time. Its value lies in continuous participation in the scheme and depends on the results being generated in the usual way that specimens are processed. Aberrant or inconsistent results should alert the participating laboratory to review their test procedures. In addition, potential problems can be averted through reviewing aberrant results and the causes proposed for these. Ideally, participation in EQAS should be combined with daily reviews of quality control results. To this end the NRL now offers an external QC programme to laboratories performing *C. trachomatis* and *N. gonorrhoeae* NAT diagnostic testing. In the context of quality management measures, EQAS results may be viewed as an indicator of integrity of performance and external QC an indicator of run-to-run variation.
Introduction The rapid and widespread heterosexual transmission of the human immunodeficiency virus (HIV) in developing countries in Sub-Saharan Africa has been attributed, at least in part, to the high prevalence of sexually transmitted infections (STIs), in particular genital ulcer disease. All STIs are known to facilitate the transmission of HIV as a result of the increased concentration of HIV receptive cells and HIV infected cells in areas of genital inflammation and in genital secretions and exudates in persons with STIs. The World Health Organization that the annual incidence of genital ulcer disease (GUD) exceeds 20 million. In southern Africa up to a third of STI clinic attendees have GUD; this is about 10 fold more than that found in industrialised countries. In southern Africa the primary aetiologic agents of GUD among the STI clinic attendees are *Treponema pallidum*, *Haemophilus ducreyi*, and herpes simplex virus type 2 (HSV-2), whereas the L-serovars of *Chlamydia trachomatis* and *Klebsiella granulomatis* occur infrequently. The aetiology of GUD varies geographically and temporally. Studies conducted in Central Australia have shown that the main cause of GUD is *T. pallidum*; this is followed by genital herpes, and occasionally donovanosis is encountered. In order to establish the aetiology of genital ulcers a number of laboratory tests need to be carried out. Depending on the geographic location of health facilities access to laboratory services and availability of tests may be limited. Recently it has been noted that there has been an increase the reported cases of lymphogranuloma venereum in many industrialised countries, in particular cases have been occurring increasingly commonly amongst men who have sex with men.

The management of GUD depends on the aetiology of the ulcers. In the syndromic management approach recommendations for managing cases are based on the known causes and the prevalence rates of the different aetiologic agents; these vary from place to place. Where laboratory services are available appropriate use of laboratory tests may be made. The main constraints in diagnosing the cause of GUD are the availability of tests that need to be carried out.

In this paper an overview is given of the causes of GUD, their clinical manifestations and the laboratory tests that need to be performed, together with possible treatment regimens that may be used. The problems in making a diagnosis are highlighted.
A Combination Immunoassay for the Detection of Hepatitis C Virus (HCV) Antibodies and Antigen on the Abbott ARCHITECT Instrument


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HCV exposure in blood donors is serologically determined by the detection of anti-HCV antibodies. However, a “window” period of 30-70 days after exposure exists where specific antibodies to HCV antigens are not detected using commercially available immunoassays. Nucleic acid testing for the detection of HCV RNA or antigen testing for the detection of HCV core protein has resulted in dramatic reductions in the pre-seroconversion window period. We have developed a prototype combination assay for the simultaneous detection of both anti-HCV antibodies and HCV core antigen. This chemiluminescent, magnetic microparticle-based immunoassay is compatible with the Abbott ARCHITECT® instrument. The antibody component of the assay captures human anti-HCV specific antibodies utilizing microparticles coated with HCV recombinant antigens, which are then detected with an anti-human IgG specific monoclonal antibody conjugated to a chemiluminescent compound. The antigen element of the assay utilizes an anti-HCV core monoclonal antibody coated on microparticles to capture core antigen. The captured core antigen is then detected using anti-HCV core specific monoclonal antibodies conjugated to the same chemiluminescent compound. The specificity of this assay was determined to be 100% upon testing a population of 458 random volunteer blood donors. Assay sensitivity was determined by testing 9 commercially available seroconversion panels that were initially HCV nucleic acid negative. In each panel tested, a positive result in the combination assay was obtained prior to detection of antibody alone, resulting in an average reduction of the window period by 34 days. In addition, positive results were obtained for 41 of 43 nucleic acid positive/antibody negative specimens (>95%), thereby exhibiting sensitivity nearly equivalent to nucleic acid testing. In conclusion, a prototype, automated HCV combination assay was developed. Results from this preliminary evaluation were comparable to that of nucleic acid testing in terms of reducing the window period from infection to detection of anti-HCV antibody.
IMPLEMENTATION OF QUALITY ASSURANCE PROGRAMS IN CAMBODIAN LABORATORIES

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Introduction
A National Laboratory of Public Health (NLPH) cooperative agreement between National Institute of Public Health (NIPH) in Cambodia and USCDC was implemented in September 2002, as part of the Global Aids Project. The funding was provided to improve the quality of laboratories supporting ART for HIV positive patients and to ensure the provision of safe blood products for transfusion.

Objectives
The objective of the continuing support is to develop the NLPH to become a high quality Reference Laboratory in Cambodia and to support the development of laboratory capacity at the provincial level through strengthening Public Health Laboratories, including those providing a blood transfusion service.

Methods
The main components of the project are external validation testing by NLPH, for 18 provincial blood transfusion centers through retesting of the blood donor specimens collected from the provinces. Direct supervision by NLPH staff of provincial laboratories and refresher training for their staff at NLPH. Introduction of IQC and EQA programs for the NLPH laboratory for Biochemistry, Hematology and Serology and Immunology. Implementation of a National EQA program for Cambodian laboratories.

Result
From the start of the project in April 2003 a great improvement has been found in the rate of false positive and negative HIV, Hepatitis B & C and Syphilis testing done on blood donors at the provincial laboratories. The number of laboratories in the scheme has increased from 12 to 18 with 3 more planned for the future. 77 number of supervision visits by NLPH staff have been undertaken since April 2003, with a gradual improvement in the quality of the laboratories. 6 number of refresher training courses has been run at NLPH since April 2003, with a marked improvement in the knowledge shown by the provincial laboratory staff. 4 EQA programs have been implemented at NLPH and continue to show good results. In 2005, with the help of the Liz Dax and the NRL in Australia a Cambodian national EQA program is currently being developed for HIV, Hepatitis B & C and Syphilis. Other disciplines will be undertaken in the future.

Conclusion
Since in April 2003 a vast improvement has been seen in the quality of the National Laboratory of Public Health in Cambodia, along with a gradual improvement in the provincial laboratories due to the implementation of Quality Assurance Programs. The full presentation will document the process undertaken to achieve these results and the difficulties encountered along the way.
Improved Identification of Acute HIV Infection with the Abbott Architect HIV-1/2 Combo Assay

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Introduction

The NSW State Reference Laboratory for HIV/AIDS services a population at heightened risk of HIV infection. The Abbott Architect Ag/Ab combo assay was evaluated as part of the registration process for TGA approval. It is well known that detection of HIV-1 p24 antigen can reduce the HIV pre-seroconversion window period by 3-5 days.

Objective

The purpose of this study was to describe the performance characteristics of the Abbott Architect HIV Ag/Ab combo assay in this laboratory as part of the registration process, highlighting the assay’s ability to detect acutely infected individuals.

Methods

Evaluation panel: 80 recently diagnosed HIV positive samples from 2004; 14 paired seroconversion panels; 5 HIV untreated seroconversion panels; 8 HIV treated seroconversion panels; HIV-1 p24 antigen dilution series (9pg/mL to 135pg/mL); 4 index specimens from patients presenting with acute HIV infection during the course of this evaluation and 500 low risk seronegative samples. The laboratory employs two testing strategies dependent on the whether a patient has been identified as being ‘at risk’ of infection (presenting with a history of a high risk encounter, acute seroconversion illness) or whether the individual is undergoing routine testing (generally ‘low risk’). The testing strategy for ‘at risk’ patients eliminates the reflexive cascade of tests and performs all assays immediately (HIV-1 antibody (Abbott AxSYM HIV-1/2 Combo, Biorad GenScreen HIV-1/2; p24 antigen (Genetic Systems HIV Ag), NAT (Roche HIV proviral DNA PCR), and BioRad HIV western blot to reduce turnaround time for a confirmed notifiable result.

Results

Of the 80 Architect newly identified positive samples all gave a higher S/CO value when compared to AxSYM. The 14 paired seroconversion panels were run across the AxSYM Combo, Genscreen HIV-1/2 Genscreen Plus, p24Ag, WB and NAT assays. One sample was negative on AxSYM Combo, Genscreen Plus and WB and had detectable p24Ag. This sample was reactive by Architect (S/CO 2.13 vs AxSYM 0.35). The 5 untreated and 8 treated seroconversion panels showed comparable reactivity on both Abbott both platforms. Of the 4 acutely infected patients all were considered negative by AxSYM criteria but were repeatedly reactive by Architect.

Conclusion

These evaluations have demonstrated Architect Ag/Ab combo assay to be a reliable, highly sensitive and specific serological assay for the detection of HIV infection. During the evaluation, a number of acutely infected individuals patients were identified that may have been missed if other Ab/Ag Combo tests were used as screening. Testing strategies incorporating direct detection of HIV-1 p24 antigen and virus specific nucleic acid assays are essential for accurate diagnosis of primary HIV infection.
BROAD NEUTRALISATION AND COMPLEMENT-MEDIATED LYSIS OF HIV-1 BY \textsuperscript{PE}HRG214, A NOVEL CAPRINE ANTI-HIV-1 POLYCLONAL ANTIBODY.

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Introduction:
Typical human antibody responses to HIV-1 infection are unable to neutralise virus efficiently, clear the infection, or prevent disease progression. However, more potent neutralising antibodies may be capable of playing a pivotal role in controlling HIV replication in vivo. \textsuperscript{PE}HRG214 is a polyclonal caprine antibody raised against purified HIV-associated proteins, such that epitopes that are immunologically silent in humans may potentially be recognised in another species. It has been administered safely with minimal side effects to HIV-infected individuals in Phase I clinical trials (Dezube et al, 2003; Pett et al, 2004).

Objectives:
To assess the potency, breadth of action, and mechanism of action of \textsuperscript{PE}HRG214 antibody using neutralisation and virion lysis assays.

Methods:
Virus neutralisation was tested in PHA activated PBMC as these cells are susceptible to all isolates. The role of complement in virus neutralisation was also assessed in these cells. Representative viral isolates from clades A, B, C, D and CRF01A_E were tested. The role of antibody plus complement in virion lysis was also tested. The target proteins for \textsuperscript{PE}HRG214 activity were investigated using flow cytometry and by adsorption of anti-cell antibodies from the antibody cocktail.

Results:
The antibody potently neutralised a diverse range of primary HIV-1 isolates, encompassing clades A to E and both CCR5 and CXCR4 phenotypes at concentrations similar to that observed for HIVIG, a reference pooled polyclonal anti-HIV-1 neutralising antibody. Neutralisation was enhanced by the presence of human complement. \textsuperscript{PE}HRG214 also induced complement-mediated lysis of all HIV-1 isolates tested, and recognized or cross-reacted with a number of host cell proteins. Virion lysis was abrogated by adsorption with T and/or B cells expressing GPI-linked proteins, but not by GPI-deficient B cells or red blood cells.

Conclusions:
Hence, \textsuperscript{PE}HRG214 was found to potently neutralise and lyse HIV-1 particles and was complement dependent. Adsorption using B or T cells indicated the involvement of antibodies against cellular proteins or host cell protein mimicry by the virus. By targeting host-cell proteins present in the viral envelope, which are present among all strains tested, \textsuperscript{PE}HRG214 potentially opens up a highly novel means of eliminating circulating virus in HIV-1 infected individuals being mindful of using a goat IgG-based immunotherapy.
CENTRALISATION OF METROPOLITAN SEROLOGY, VIROLOGY AND MOLECULAR DIAGNOSTIC SERVICES IN QUEENSLAND HEALTH PATHOLOGY SERVICE

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Introduction: Following an initiative from the Director of Queensland Health Pathology Service (QHPS) in 2002, a draft plan was developed and then a working party set up to explore options and make recommendations to rationalise the delivery of metropolitan serology, virology and molecular diagnostic (SVM) services in QHPS. The SVM Project was established with the objective of consolidating metropolitan SVM services to the Royal Brisbane and Women’s Hospital (RBWH) site. A required outcome of the overall project was improved turn around times (TAT) for SVM tests for clients statewide. A number of initiatives were implemented to achieve the outcome.

Methods: In May and June 2005 SVM staff and most of the work from the other two major metropolitan QHPS laboratories, Princess Alexandra Hospital (PAH) and The Prince Charles Hospital (TPCH), transferred to the RBWH. Three staff from PAH (including a supervisor) and one staff from TPCH transferred. Three new staff were also employed to cope with the extended hours of operation. Twenty one assays (27,000 tests per year) transferred from PAH while 20 assays (17,000 tests per year) transferred from TPCH. All referral work from the peripheral metropolitan QHPS laboratories (Caboolture, Redcliffe, Logan, Redland and QEII) transferred to the RBWH. This work was previously performed at PAH and TPCH. Some assays are performed by a different method at RBWH (eg, PCR rather than DFA). Clients were informed of these changes. New instruments were purchased to cope with the increased workload at RBWH. This included, two Roche MagNA Pure Extractors, an Applied Biosystems 7500 real time PCR system, a Corbett Rotagene real time PCR system, a Corbett CAS1200 precision liquid handling system, a freeze dryer and a storage refrigerator. The hours of operation of the SVM laboratories will be extended with the introduction of a late shift and Saturday work. To accommodate the extra staff and new equipment several existing laboratories and an old office were reorganised and refurbished. Items such as extra laboratory benches, furniture, PCs and staff lockers were purchased. Upgrading of the high volume random access analyser and several molecular diagnostic instruments (eg, to Roche Taqman 48 and LightCycler 2) is planned for the near future. The supervisory structure of SVM services was altered to better manage the changes. SVM services were split into two work units, serology and molecular diagnostics/virology, each with a supervisor and 11 staff. Staff training was a priority so that the late shift and Saturday work could be accommodated.

Results: The new centralised SVM services have been running for one month and the late shift and Saturday work remain to be implemented. There were no major issues with the transfer of staff and specimens from the other laboratories and only a few minor problems with the test method changes. Some serology testing was retained at PAH and TPCH. These tests (eg, hepatitis serology) are associated with transplantation requirements and performed on high throughput analysers (eg, AxSym at PAH). The number of assays performed at PAH dropped from 42 to 21 resulting in a 36% decrease in workload. At TPCH the number of assays dropped from 30 to 10 resulting in a 59% decrease in workload. RBWH now offer in excess of 140 assays and the workload is expected to rise to 220,000 tests per year, an increase of 25%.

Conclusions: The project was well planned and the transition occurred with only minor problems. Anecdotally TAT has improved but the full benefit of centralisation is not expected until extended operational hours are fully implemented and the upgrade of molecular diagnostic instruments is complete.
Introduction:
The National Serology Reference Laboratory, Australia (NRL) has conducted External Quality Assessment Schemes (EQAS) for infectious diseases for over a decade. In 2004, the NRL assessed the effectiveness of DigitalPT (HealthMetrix, Canada) – a web-based program that manages the administration, collection, analysis and reporting of results of EQAS.

Objectives:
To review the implementation of DigitalPT in 2005 for the NRL’s Hepatitis B, Hepatitis C, HIV-1, C.trachomatis and N.gonorrhoeae Nucleic Acid Testing (NAT) EQAS.

Methods:
In March 2005, EQAS panels were provided to laboratories enrolled in the NRL’s NAT EQAS. DigitalPT facilitated the data submission of EQAS results (either by the laboratory or NRL) and enabled laboratories to monitor their EQAS shipments, registration information, current and archived reports online in real-time.

Results:
Over 70 laboratories used DigitalPT in 2005. DigitalPT reports from the first viral EQAS were available 2 weeks after the deadline for reporting results. Laboratories could log onto DigitalPT at any time after that to view their laboratory results summary, participation statistics and archived reports.

Conclusions:
DigitalPT was implemented in 2005 for the NAT EQAS. Laboratories could receive analysed results 2 weeks after the programme’s closing date – an improvement of 4 weeks over normal analysis. Further improvements are being made to the DigitalPT system before its implementation for the Serology EQAS so that laboratories receive increasingly meaningful results. The NRL plans to expand the programmes it offers to include EQAS for other analytes in 2006.
SYNTHESIS OF THE MOST RECENT EVALUATIONS OF THE INNOVATIVE MONOLISA HCV Ag-Ab ULTRA AND GENSCREEN ULTRA HIV Ag-Ab ASSAYS

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Introduction
The new and innovative Ag-Ab combined screening assays of the Bio-Rad Ultra line: Monolisa HCV Ag-Ab Ultra and Genscreen Ultra HIV Ag-Ab have been mainly developed in order to reduce significantly the Window Period for blood bank and diagnostic laboratories.

Objective
The aim of this study is to take stock of the most recent evaluations performed over the last year with Monolisa HCV Ag-Ab Ultra and Genscreen Ultra HIV Ag-Ab in the blood bank and diagnostic field.

Methods
Monolisa HCV Ag-Ab Ultra:
A synthesis of 5 posters / articles, from different authors, on evaluations of Monolisa HCV Ag-Ab Ultra kit in blood donor and in high-risk population (hemodialysed patients or HIV co infected patients) will be shown. More than 50 seroconversions and 100 HCV RNA positive/ HCV Ab negatives samples have been tested for sensitivity study. The specificity has been tested on more than 3000 samples (hospital and blood bank population).
A sensitivity study has been done on 10 commercial seroconversions to compare the performance of this assay with those of mini-pool nucleic acid testing (TMA in pools of 8 donations and Roche molecular system in pools of 24 donations) during window period.

Genscreen HIV Ag-Ab Ultra:
A comparative study regarding HIV Ag-Ab combined screening tests was performed in France, in order to compare the analytical sensitivity, the early detection with 23 seroconversions panels and the specificity with 1005 negative samples from 4 French diagnostic laboratories.
4 other specificity studies were performed in France, Germany, Poland and Sweden in order to assess accurately the routine specificity level of Genscreen Ultra HIV Ag-Ab on 25 000 negative blood bank donors.

Results
Monolisa HCV Ag-Ab Ultra:
The Monolisa HCV Ag-Ab ULTRA test reads positive on average only 5.1 days after the viral genomic diagnostic tests carried out on pooled samples. This was shown in the results obtained in the virology laboratory of the French reference centre for Hepatitis B and C in transfusion. Monolisa HCV Ag/Ab allowed to reduce the Window period HCV RNA pos /HCV Ab negative from 21.6 to 49 days depending of evaluations.
The specificity analysed in 2503 consecutive blood donations was estimated at 99.88% in blood donors and 99.5% to 100 % in hospital population.

The new Genscreen Ultra HIV Ag-Ab shows the highest sensitivity level among the HIV Ag-Ab combined screening assays and the best sensitivity level among the microtiterplates HIV Ag-Ab combined screening assays without any compromise for specificity.

Conclusion
Monolisa HCV Ag-Ab Ultra in Blood Bank: The performance of this new test makes it an interesting alternative to the viral genomic test (PCR or other techniques) carried out on pooled samples by blood banks in a number of countries and at a lower cost than the viral genome detection technique in terms of price, laboratory organisation and instrumentation.
Monolisa HCV Ag-Ab Ultra in Diagnostic Labs: Early diagnosis of HCV infection is crucial to prevent further transmission in high-risk groups and allows for a rapid treatment decision that has proven high efficacy in acute hepatitis C. For diagnosis in this high-risk population (Drug addict, HIV patient, hemodialysed patient, etc..) this new assay based on the detection of both HCV Ag and Ab reduces significantly (up to an average of 49 days) the long seronegative window period seen in HCV infection. Use of such assays in the setting of high-risk behaviours, particularly in HIV co-infected patients, allows earlier HCV diagnosis and rapid clinical management.
With excellent sensitivity and specificity performances, Genscreen Ultra HIV Ag-Ab fulfils the most demanding requirements for blood bank and diagnostic laboratories.
RELATIVE SENSITIVITIES IN THREE IMMUNOASSAYS THAT DETECT HUMAN IMMUNODEFICIENCY VIRUS (HIV) p24 ANTIGEN.

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Background: The analytical and seroconversion sensitivities of the Vironostika HIV-1 Antigen MicroElisa System, the Genetic Systems HIV-1 Antigen EIA (bioMerieux and BioRad respectively) and the Murex HIV Ag/Ab Combination EIA (Abbott Laboratories) were compared.

Method: The analytical sensitivity of each assay was determined by testing 11 serial dilutions of an international standard ranging from $1 \times 10^5$ to 3.5 pg/mL of recombinant p24 antigen. The assays were also evaluated for the ability to detect p24 antigen in 15 HIV-1 seroconversion panels.

Results: Results showed that the HIV recombinant p24 antigen analytical sensitivity of the Vironostika HIV-1 Antigen MicroElisa and Genetic Systems HIV-1 p24 EIAs performed similarly at approximately 7 pg/mL. The Murex HIV Ag/Ab Combination EIA had a limit of detection of approximately 15 pg/mL. In five of the 15 seroconversion panels tested, the Genetic Systems HIV-1 Antigen EIA detected p24 antigen in samples earlier than the Vironostika HIV-1 Antigen MicroElisa System. In two seroconversion panels, the Vironostika and Genetic Systems assays detected p24 antigen earlier than the Murex HIV Ag/Ab Combination EIA. In one seroconversion panel the Vironostika assay failed to detect p24 antigen, where the Genetic Systems and Murex EIAs had detected p24 antigen in at least 3 bleeds.

Conclusions: In the serum panels tested, the Genetic Systems EIA identified the presence of p24 antigen in samples earlier in the course of HIV seroconversion. This observation has lead to the NRL’s incorporating the Genetic Systems HIV-1 Antigen EIA into its HIV-1 testing strategy.
AGE-SPECIFIC PREVALENCE OF INFECTION WITH HSV-2 IN URBAN AREAS OF KOREA

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Introduction: Herpes simplex virus type-2 (HSV-2) infection is caused by sexual contact resulting in an STD with ulcer. HSV-2 seroprevalence may be a useful marker for sexual behavior and genital HSV-2 infection is a risk factor to increase the chance of HIV infection. We investigated on the prevalence of HSV-2 to provide a basis for the STD policy.

Methods: The subjects consisted in three groups: general population (n=1611), commercial sex workers (n=304), the HIV-seropositive (n=198), To examine HSV-2 specific antibody, HerpeSelect 2 ELISA IgG (Focus Technologies, USA) was used and followed the manual. In this cross-sectional study to investigate HSV-2 seroprevalence, we compared and analyzed general population with commercial sex workers and the HIV-infected; we classified general population into under 7 years (n=397), 7-11 years (n=399), 12-19 years (n=400), 20-29 years (n=499), 30-39 years (n=198), 40-49 years (n=126), 50-59 years (n=53) and over 60 years (n=41). We analyzed the difference of prevalence using Statistical Analysis System 9.1 for chi-square test by group, age, and sex.

Results: In general population under 20 years, HSV-2 seroprevalence was 3.0% (under 7), 3.3% (7-11 years), and 2.7% (12-19 years), so that there was no significant difference by age (p=0.9440). The HSV-2 prevalence for 20-29 years was 4.1%, but HSV-2 prevalence of over 30 years was 31.2%, remarkably higher (p<0.0001). The seroprevalence of commercial sex workers (76.4%) and the HIV-seropositive (48.0%) was significantly higher than that of general population (p<0.0001). The twenties in Korea showed lower seroprevalence (4.1%) than that of the twenties in the United States (17%), which was against our expectation

Conclusion: The results showed that the general population over 30 years old and the high risk group of STI had remarkably higher HSV-2 seroprevalence, so systematic and active prevention program, education and publicity on STI including HSV-2 would be effective for the group under 30 years. Lower seroprevalence of 20-29 year group needs additional studies including epidemiological survey, study covering more regions, with new sampling strategy.
A COMPARISON OF RESULTS REPORTED BY LABORATORIES IN THE NRL’S AUSTRALIAN AND INTERNATIONAL ANTI-HCV EXTERNAL QUALITY ASSESSMENT SCHEMES

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National Serology Reference Laboratory, Australia

Introduction: The National Serology Reference Laboratory, Australia (NRL) coordinates two External Quality Assessment Schemes (EQAS) for anti-HCV. The Australian anti-HCV EQAS is provided mainly to laboratories in Australia; the International anti-HCV EQAS mainly to laboratories in the Southeast Asian and Western Pacific regions. EQAS panels are distributed 3 and 2 times yearly, respectively.

Objective: To investigate the error rates reported by laboratories participating in the Australian and International anti-HCV EQAS when the same panels were provided to laboratories in both schemes.

Methods: In October and December 2004, anti-HCV EQAS panels consisting of the same ten samples (five of which were anti-HCV positive and five of which were anti-HCV negative) were distributed to laboratories participating in the International and Australian anti-HCV EQAS, respectively. Results were analysed and error rates calculated.

A test result interpretation was provided by each laboratory for each specimen in each assay.

An aberrant testing result interpretation was one that did not agree with the reference laboratory status.

The error rate was defined as the total number of aberrant test result interpretations, expressed as a percentage of the total number of reported test result interpretations in a given EQAS.

Results: Eight of the 100 laboratories (8%) in the Australian anti-HCV EQAS reported aberrant testing result interpretations. Sixteen of the 1353 test result interpretations reported were aberrant, giving an error rate of 1.2%. The aberrant test result interpretations were associated with four of the 14 assays used by participants: two were automated immunoassays used as screening assays while two were manual immunoassays used as supplemental assays. Two laboratories reported nine of the 16 aberrant test result interpretations (56%): five were reported by one laboratory and four by the other.

Eleven of 53 laboratories (20.8%) in the International anti-HCV EQAS reported aberrant testing result interpretations. Twenty-one of the 815 test result interpretations reported were aberrant, giving an error rate of 2.6%. Eighteen of the aberrant test result interpretations were either negative or inconclusive and associated with the anti-HCV positive panel samples 6 and 10: 10 with sample 6 and 8 with sample 10. Eleven assays were associated with the 21 aberrant test result interpretations: two automated immunoassays, three manual immunoassays and five rapid assays all used as screening assays, and an immunoblot used as a supplemental assay. Rapid assays were associated with 52.4% of the errors.

The false positive rate was 0.4% for the Australian anti-HCV EQAS and 0.8% for the International anti-HCV EQAS.

The false negative rate was 0.9% for the Australian anti-HCV EQAS and 2.3% for the International anti-HCV EQAS.

The overall error rates were 1.2% and 2.6% for the Australian and International anti-HCV EQAS, respectively.

Discussion: The overall error rate was lower in the Australian anti-HCV EQAS than for the International anti-HCV EQAS. The major factor contributing to the errors in the Australian EQAS appeared to be laboratory-related as two participants reported multiple aberrant test result interpretations. In the International EQAS, the major contributing factor to the errors was false negative test result interpretations obtained for two of the anti-HCV positive samples.
RESULTS OF THE NATIONAL HIV-SCREENING PROFICIENCY PROGRAM IN SINGAPORE IN 2004

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Background: Since 1991, National HIV Reference Laboratory in the Department of Pathology, Singapore General Hospital, has run the National HIV-Screening Proficiency Program on the behest of the Ministry of Health, Singapore. This program is funded by the Ministry of Health, and is an essential component for registration of HIV screening laboratories in Singapore. Proficiency panels are prepared and sent to participating laboratories 3 times a year (Panels A, B & C). The Reference Lab compiles and analyzes the results and provides appropriate advice and trouble-shooting recommendations for the participating. For the year 2004, 25 laboratories participated in the program and used a varying mixture of test systems comprising 3rd and 4th generation HIV antibody assays. This enabled our reference laboratory the unique opportunity to compare capabilities of assays from different manufacturers, as well as to test the variability of performance of these laboratories using assays by the same manufacturer.

Aim: The aim was to study how the various HIV antibody assays used in Singapore perform in the detection of HIV-1 and HIV-2 antibody samples of varying dilutions, as well as in the testing of replicate samples. In addition, the performances of the participating HIV screening laboratories and the effects on sample storage on optical density (OD) readings were also assessed.

Method: The results from the National HIV-Screening Proficiency Program in Singapore in the year 2004 (Panels A, B and C) were studied. Various dilutions (from 1:256 to 1:8192) of a positive HIV-1 antibody sample from a patient R were prepared at the beginning of the year and stored at -70°C. Duplicates of these dilutions were sent with each panel. In addition, one HIV-2 antibody sample diluted at 1:768 was sent with panel B, and pooled negative HIV sera were sent with every panel. The diluted HIV antibody positive samples tested reactive using the Abbott AxSYM HIV-1/HIV-2 Combo MEIA and confirmed by Western Blot before being sent to the laboratories.

Results: A total of 7 different HIV screening systems from Abbott and Roche were used by the 25 participating laboratories, with 3 systems having more than 5 users. All test systems/kits were able to detect the highly diluted HIV-1 samples except Roche COBAS CORE HIV Combo which failed to detect the samples at 1:8192 dilution. All systems but Roche COBAS CORE HIV Combo also successfully detected the diluted HIV-2 sample. From the standard deviation from mean (SD), it was observed that there was 1 laboratory showing consistent negative bias results for all samples of Panels A and C, suggesting inherent system or equipment problem. There were no significant differences in the OD readings of the same diluted samples tested over 3 panels, which indicate that there was no deterioration in the sera stored in -70°C for 9 months.

Discussion: The use of varying dilutions of HIV antibody positive serum is useful in assessing the sensitivities of HIV screening systems but has its limitations in assessing the 4th generation assays which also detect HIV antigen. Nevertheless, our study showed that most of the test assays for HIV screening in the Singapore market have the sensitivity to detect even highly diluted ‘manipulated’ HIV-1 and HIV-2 antibody positive samples, Roche COBAS CORE HIV Combo EIA proved less sensitive than the other systems in detecting low level of antibody. However, it does not translate to its failure in picking up positives in clinical samples because this study was unable to assess its antigen detecting capability.
Estimating Uncertainty: a nuisance or a tool to improve quality?

Tholen D

Most laboratories are estimating uncertainty not because they want to, but because they have to. The requirement is very firm in ISO 17025 and somewhat weaker in ISO 15189, but generally it is somewhat unwelcome in all laboratory settings. Laboratories are told it is needed because clients might ask for it (no one ever does), or it is needed when reporting conformance to a specification limit (not common in medical laboratories), but there is little other justification provided for what can be an extraordinary effort. This presentation makes the case that estimates of uncertainty, or rather the process of developing the estimates, can actually be very useful quality improvement tools. Whether one develops the estimates with the “bottom-up” approach or the “top down” approach, one still must list the components of uncertainty and their approximate magnitude. This listing of components forces the laboratory to consider each measurement procedure carefully, including sources under the control of the laboratory such as technician differences and environmental conditions. If an result is produced, this listing can be a “road map” for root cause investigations; if the uncertainty is too large, this shows the laboratory the best place to direct resources to lower the uncertainty.
DIAGNOSIS OF Q FEVER INFECTION BY PCR ON SERA DURING A RECENT OUTBREAK IN RURAL SOUTH AUSTRALIA

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Introduction: The diagnosis of Q fever in the past has largely been dependant upon serological findings. This often leads to delayed diagnosis as seroconversion may take several weeks to develop. During a recent Q fever outbreak in rural South Australia, we performed real time PCR to complement the serological tests used routinely in our laboratory. In this study, the diagnostic rate of serology was compared with combined PCR and serology.

Methods: Fiftyfour sera from 27 patients were submitted to laboratory for Q fever antibody testing during the outbreak period. Testing for Q fever antibodies including phase 1 and phase 2 IgG, IgA and IgM were performed using the indirect immunofluorescence antibody technique (IFA). All sera were tested by two separate PCR’s, the 27kDa Outer Membrane Protein (COM1) PCR and the Insertion Sequence (IS1111) PCR. Total DNA was extracted from 200ul of serum using the Qiagen DNA blood kit. PCR was performed on 5uL of extract, Amplified for 45 cycles using the Roche LightCycler.

Results: Seroconversion (≥ a 4 fold rise in antibody between paired sera) was detected in all 21 patients that submitted two or more serum samples. Six patients submitted single samples. Four had phase II IgM titres of ≥1:1280. One had a phase II IgM titre of 1:80. One patient was antibody negative. PCR was positive in 63% (17/27) of patients. However, PCR was positive in 89% (17/19) of phase II IgM negative sera and only 9% (3/35) of phase II IgM positive sera.

Conclusions: LightCycler PCR is a useful tool for the early diagnosis of acute Q fever infection. Q fever PCR is a suitable test to use in combination with serology for outbreak investigations.
Introduction: In developing countries human immunodeficiency virus (HIV) infection remains a major public health concern and technical & financial problems limit recommended screening. Testing for HIV infection is usually performed using sensitive screening tests (EIA), with positive specimens tested by a supplemental test (western blot). Such tests are impractical for field conditions in developing countries where there is a need for a rapid, accurate and inexpensive test requiring minimal laboratory equipment to detect HIV antibodies.

Objectives: We evaluated an algorithm comprising of three rapid tests against reference strategy of three ERS assay (ELISA, Rapid, Simple) recommended for developing country for the detection of HIV antibodies.

Methods: Serum specimens were collected from 1000 consecutive suspected HIV cases at A.I.I.M.S, New Delhi. All specimens were tested for HIV antibodies by Capillus HIV-1/ HIV-2 (Cambridge Diagnostics) test. A reactive sample was again tested by Immunocomb-II HIV-1&2 BiSpot (Orgenics). The specimens found to be reactive by this test were subjected again to testing with HIV Spot (Genelabs Diagnostics). This algorithm was evaluated against reference strategy of three ERS assay utilizing three different ELISAs. The specimens found nonreactive in the screening test using Capillus test were not subjected to any further testing.

Results: Compared with the reference strategy the combination of rapid test was having the sensitivity of 99.6% and specificity of 99.9% and positive and negative predictive value of 99.6% and 99.9% respectively.

Conclusion: The reference strategy of ERS assay, are at times impractical for field conditions in developing countries as most EIA take a minimum of 2-3 h to perform, and they require access to electricity, and maintenance for sophisticated equipment. We conclude that the testing protocol using three rapid tests may provide suitable alternative to HIV antibody testing at resource poor settings as well as in emergency situation.
INTERNATIONAL COMPARISON OF PERFORMANCE OF ABBOTT PRISM ASSAYS USED FOR BLOOD DONOR SCREENING

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Background
The National Serology Reference Laboratory, Australia (NRL), coordinates a Quality Control (QC) programme for laboratories that screen for anti-HIV 1&2, anti-HCV, HBsAg and anti-HTLV I/II using the Abbott PRISM assays. Nineteen laboratories from Australia, Belgium, Canada, Ireland, Israel, The Netherlands, New Zealand, Norway, Singapore, South Africa and Thailand have submitted data for this programme.

Aims
To determine the precision and accuracy of results from laboratories. Individual PRISM instruments and different PRISM reagent lots were compared by analysing data accumulated between October 2001 and January 2005.

Methods
The multi-marker QC sample ‘PeliSpy s2058 Type 7’ (s2058), produced by Viral Quality Control, The Netherlands (now AcroMetrix-Viral Quality Control), was provided to participants. Laboratories tested s2058 in each calibration run, in addition to the manufacturer’s controls, on each sub-channel of the instrument. S2058 was used as a ‘Go/NoGo Control’ and results were required to be reactive (S/Co>1) for a test run to be valid. Data were collected and analysed using the NRL’s internet-based application EDCNet (https://www.nrlqa.net). Following submission to EDCNet, laboratories were able to compare their results with those submitted by other laboratories and investigate differences in results from PRISM reagent lots and instruments. Data for five different s2058 lots were exported from EDCNet and analysed.

Results
Nearly 95,000 results were submitted: all results were reactive (S/Co>1). Fifty of these results (0.0005%) were excluded from the analyses because they were reported from invalid test runs [pipetting, aspiration or sampling errors (n=48) or unacceptable results (n=2)]. Another 16 results were excluded because data provided by laboratories were inconsistent or incorrect.

A total of 94,189 results, reported using 332 different PRISM reagent lots (145 for anti-HIV, 79 for anti-HCV, 55 for anti-HTLV and 53 for HBsAg), were analysed. Results from PRISM HBsAg and anti-HIV showed the least variation with coefficient of variations (CV) of <10% for all s2058 lots. Results from PRISM anti-HCV and anti-HTLV produced CVs between 9.17% and 15.38% for all s2058 lots.

Data reported for s2058 lot PS030618 (n=38,662, range from 8,080 for anti-HTLV to 10,225 for HBsAg) were analysed further to review the QC performance in individual PRISM reagent lots. The HBsAg QC results showed the least variability between PRISM reagent lots with <8% Bias for the 14 PRISM HBsAg reagent lots used (Bias was calculated as the difference between the mean ratio for the reagent lot and the weighted mean ratio for all reagent lots, expressed as a percentage of the weighted mean ratio for all reagent lots). The QC results for the PRISM anti-HTLV showed greater variability between reagent lots with a single reagent lot generating a +63% Bias. The QC results for the PRISM anti-HCV showed the greatest variability within reagent lot with results from 12 of 21 reagent lots showing a CV between 10% and 13%.

Summary
In 94,255 results in a QC sample distributed to 19 laboratories the performance of the Abbott PRISM assays was found to be consistent over four assays. EDCNet was robust in supporting laboratories’ abilities to follow precision and accuracy of the assays in real time.

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NUCLEIC ACID AMPLIFICATION TESTING FOR SEXUALLY TRANSMITTED DISEASES

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Nucleic acid amplification tests (NAATs) are now becoming the method of choice for routine detection of sexually transmitted diseases. However, these tests have limitations. These include the typical problems associated with the use of NAAT protocols, such as high cost, carry-over contamination, inhibition of the reaction, high quality control requirements and the absence of antibiotic resistance data. More importantly, there are sequence-related limitations, including the generation of false-positive and false-negative results. These sequence-related issues are epitomised by the difficulties experienced with NAAT detection of gonorrhoea. In particular, false-positive results are a major consideration for \textit{Neisseria gonorrhoeae} NAATs. These primarily stem from the frequent horizontal genetic exchange occurring within the \textit{Neisseria} genus, leading to commensal \textit{Neisseria} species acquiring \textit{N. gonorrhoeae} sequences. In fact, nearly all \textit{N. gonorrhoeae} NAATs used routinely, including the Roche Cobas Amplicor and Becton Dickinson ProbeTec assays, are known to cross-react with strains of commensal \textit{Neisseria} species. As a result of these problems, \textit{N. gonorrhoeae} NAAT testing is not recommended for use on sites where commensal \textit{Neisseria} species are ubiquitous, including throat and rectal specimens. It should also be noted that some \textit{N. gonorrhoeae} subtypes may lack specific sequences targeted by a particular NAAT. Therefore, NAAT false-negative results owing to sequence variation may occur in some gonococcal populations. Most notably, the cppB gene, which is a popular target for supplementary \textit{N. gonorrhoeae} NAATs, is absent in numerous Australian gonococcal strains. Thus, the cppB-based NAATs are no longer considered suitable for routine use in some populations. Recently, our laboratory developed a new \textit{N. gonorrhoeae} real-time PCR assay targeting the \textit{N. gonorrhoeae} \textit{porA} pseudogene. To date, the results suggest the oligonucleotide targets utilised by this assay are both conserved and specific to \textit{N. gonorrhoeae}. In addition, the high specificity of the assay may facilitate routine NAAT testing of throat and rectal specimens. We are continuing evaluations of the \textit{porA} assay and have now embarked on a multi-centre evaluation to determine its suitability on populations Australia wide. Overall, the \textit{N. gonorrhoeae} species continues to present a considerable challenge for molecular diagnostics. The need for supplementary testing and the need to evaluate \textit{N. gonorrhoeae} NAATs prior to their use in any new patient population are emphasized.
MEASUREMENT UNCERTAINTY IN CLINICAL BIOCHEMISTRY

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Introduction: Measurement Uncertainty (MU) describes the metrological concept that a quantitative measurement is incomplete without full definition of the property measured and quantitation of result accuracy. The scientific principles and mathematical approaches to quantifying MU were developed largely for use by calibration and reference laboratories employing physical measurements. As a critical quality parameter of analytical methods, MU is now included in laboratory quality and accreditation standards such as ISO 17025/15189. Laboratories measuring biological substances eg medical testing, have had difficulty implementing MU and understanding its relevance to clinicians.

The Australasian Association of Clinical Biochemists formed a Working Group (WG) in 2003 to develop guidelines for applying MU in diagnostic clinical biochemistry laboratories. The WG determined that the application of MU should be of practical value, that it applies to the measurement procedure and excludes pre- and post-analytical factors, that the clinical value of MU be addressed, and that use of metrological terminology is minimised.

Biochemistry ‘analytes’ are often not capable of being adequately defined due to the limitations of the analytical principles used and the complexity of sample matrices. Similarly, many ‘analytes’ currently do not have full traceability to an SI-based international standard. Despite such constraints laboratories should identify the intended measurand, the extent of traceability and clinically relevant interferences for each routine method.

Quantitative biochemistry patient test results are clinically interpreted by comparison with fixed values eg reference intervals, previous results, clinical decision limits. In this context quantitative knowledge of the expected dispersion of the estimated result around the ‘actual’ result allows a reasonably objective judgement of the ‘distance’ of the result from the comparative fixed value. Thus the most important MU parameter is the imprecision of the test result, as calculated from the laboratories own long-term internal quality control data (+/- 1.96 (SD or CV %)). Analytical bias is relevant to MU only where clinical decision limits have been determined by methods other than the one used for the patient result. Laboratories should therefore be able to comply with ISO 15189 MU requirements using data already available in their own records.

Clinical biochemistry laboratories have used basic MU parameters (analytical imprecision and bias) for many years to define and control the key performance characteristics of their methods. These parameters can also be used to assess the ‘fitness for clinical purpose’ of their methods by applying a well accepted principle that analytical imprecision should not significantly add to the natural biological variation of the measured substance, desirably less than 0.5 CV of the intra-individual biological variation. This approach is often not physiologically or clinically appropriate and other criteria such as state-of-the-art or professional judgement may be used. Such assessments may result in method modification or change. All MU data should be readily accessible to clinical users, whilst for some tests clinical value may be added if included with individual patient reports.

The Guidelines (www.aacb.asn.au) provide a practical starting point for implementing MU in the diagnostic laboratory, whilst recognising that the recommendations will require future modification as methods change and full calibration traceability becomes more commonplace.
CHARACTERISATION OF NEUTRALISING ANTIBODY RESPONSES IN HIV-1 LONG TERM NON-PROGRESSORS AND LONG TERM SURVIVORS USING TWO DIFFERENT ASSAYS.

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Introduction: In a small proportion of infected individuals, HIV-1 appears to be less pathogenic and there is little or no apparent progression to disease. Lack of disease progression results from multiple factors linked to either the virus or the host. For some of these HIV-1 infected long term non-progressors/long term survivors (LTNP/LTS) two clearly identified factors are nef gene deletions in the virus and CCR5 co-receptor mutations (CCR5-Δ32) in the host. Longitudinal samples for these two groups were assembled from several LTNP/LTS cohorts. We have previously characterized the antibody responses to viral antigens, focusing on total IgG and IgG3 antibodies and comparing these with samples from progressors and AIDS patients. These results showed the intensity of the immune responses in HIV-1 nef attenuated subjects correlated with a detectable albeit low viral load. Consistent potent total IgG WB antibody responses were observed for the CCR5-Δ32 heterozygote subjects. The aim of this study is to compare the neutralising antibody responses in these two groups using two assays, one with primary cells and the other with a transfected cell line.

Objectives: To determine the potency of neutralising antibody responses in well defined groups of LTNP using a primary cell based assay and a high throughput stably transfected cell line.

Methods: Neutralising antibody responses were tested against ADA, a CCR5 using clade B reference isolate. For neutralisation of virus we compared the gold standard primary peripheral blood mononuclear cell (PBMC) assay with a cell line (TZM-BL) stably expressing CD4, CCR5, CXCR4 and containing an integrated reporter gene for luciferase which is under control of the HIV-1 LTR (Montefiori, 2004). The latter assay has been developed by the Vaccine Research Center, NIH as a reference for HIV-1 vaccine trial neutralising antibody response testing.

Results: The humoral immune responses differed depending on whether there was a viral attenuation or host mutation. Neutralisation of the reference clade B virus revealed a direct correlation of viral load and potency of neutralising responses for the nef attenuated subjects and little correlation to overall neutralising responses and viral load for the CCR5-Δ32 heterozygote subjects. The high throughput single round infection assay was at least as sensitive as the PBMC based assay.

Conclusion: While the intense antibody responses reflect a preserved immune response and lack of disease progression this was not always accompanied by potent neutralising antibody responses. This study suggests better preservation of the immune responses and/or impaired viral function contributes to the slow progression observed.
Quantitative in vitro Diagnostics - Validation of the RealArt™ HBV RG PCR kit

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artus is the leading manufacturer of licensed Real-Time PCR kits for in vitro diagnostics and offers the broadest product range for molecular detection of infectious diseases. The majority of artus kits have shown conformity to the European Directive for In Vitro Diagnostics (98/79 EG, IVDD), and hence acquired the CE mark.

The RealArt™ HBV PCR kit constitutes a ready to use reagent system for the detection and quantification of Hepatitis B Virus DNA. RealArt™ HBV PCR kits are available for several commonly used Real-Time PCR machines like the LightCycler (Roche), the Rotor-Gene (Corbett Research) and the ABI cyclers (Applied Biosystems).

The assay amplifies a 134 bp target in the core region of the HBV viral DNA. The amplification is monitored during the run with the use of a dual labelled oligonucleotide probe which binds to the amplified PCR product. The generated fluorescence is directly correlated to the initial amount of HBV DNA, thus a quantitative result is obtained at the end of the run. Additionally the RealArt™ HBV PCR kit includes an internal control for monitoring the extraction and amplification efficiency of the system using a separate fluorescence channel of the Real-Time PCR instrument.

The RealArt™ HBV PCR kits are validated as required for compliance to the European Directive for In Vitro Diagnostics (IVDD). Validation data obtained for the RealArt™ HBV RG PCR kit will be presented for sensitivity (3.8 IUWHO/ml), specificity (100%) and subtype performance (A-H), variability (lowest standard, Ct value: 1,29% CV) as well as linear dynamic range (4-4x10^9 IUWHO/ml).

Further, comparative data against the Roche COBAS TaqMan HBV Test for 287 clinical samples from two external validation sites will be discussed. Both assays show high correlation (R² = 0,956) with the results suggesting a higher sensitivity for the RealArt™ HBV RG PCR kit.