THE ROLE OF INFECTIOUS MARKER TESTING IN ASSURING THE SAFETY OF BLOOD AND TISSUE THERAPIES – WHY WE NEED QUALITY ASSURANCE

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The past 25 years have seen a remarkable turn around in the relative risks posed by single or small pool blood and tissue products compared to manufactured large pool derivatives such as plasma derivatives. As viral inactivation processes have been introduced in manufacture, the risks of pathogen transmission by large pool products has been largely obviated from known and emerging pathogens. At the same time, the limited capacity of such processes for viable cell and tissue products has placed the onus of safety on donor selection and, most importantly, infectious marker testing. This has generated a sophisticated regulatory, policy and industry infrastructure which has ensured that such tests are developed and placed in the appropriate part of the safety paradigm. The crucial role of these tests requires constant assurance of their quality and field performance; such assurance is best provided by systems which act in parallel, but independent from, the system delivery bodies themselves. This presentation will review the development of screening tests, their application in recent blood and tissue safety issues and the role of quality assurance schemes in ensuring that these tests deliver accurate and consistent results.
ASSURING QUALITY: THE UK APPROACH
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Bevan, V.
Microbiology / virology laboratories in the UK respond to a number of drivers to assure the quality of the work undertaken. Within an overall quality assurance framework, the Health Protection Agency (HPA) assumes a leadership and influencing role in clinical diagnostic and public health laboratories. The talk will describe how the Evaluations and Standards Laboratory (ESL) provides a service to labs in the UK and more widely in Europe and globally on many aspects of quality, focussing on three aspects: the development of national standard methods, assessment of medical and other healthcare related devices, and the use of internal quality control programmes for virology / serology.

National Standard Methods, comprising standard operating procedures (mainly for bacteriology), clinical testing algorithms (mainly for virology / serology), overarching syndromic algorithms, and guidance notes are designed to guide clinical diagnostic microbiology and public health laboratories in their laboratory testing regimes. The collection of over 200 National Standard Methods have been drawn up under the curatorship of the HPA over the last ten years by multi professional working groups from professional organizations and laboratory networks from throughout the UK. National Standard Methods are well referenced, regularly updated and represent a good minimum standard for laboratories to comply with; they also help with complying with accreditation requirements. The documents undergo wide consultation with >1000 password holders worldwide and are freely available via the Internet www.evaluations-standards.org.uk.

The presentation will also describe how the HPA-Microbiological Diagnostics Assessment Service assesses the performance of microbiological in vitro diagnostic devices (IVDDs) and associated equipment used to diagnose and manage infection. The UK approach to evaluating medical devices will be described including some of the issues and dilemmas currently facing microbiology / virology in the UK.

Another of our remits is developing quality control programmes and supplying quality control reagents to help laboratories monitor the performance of their kits and equipment. The benefits of such a system will be presented, highlighting some interesting findings showing the value of the monitoring.
The Dengue fever virus belongs to the *Flaviviridae* family of viruses and is one of several arbo- or insect borne viruses.

Dengue fever today is the leading cause of viral disease carried by a mosquito vector. Globally Dengue fever has been spreading since World War II for a number of reasons including the spread of the insect vector, changing water and land management practices and changing travel patterns. Within Queensland outbreaks declined after World War II but, for North Queensland have increased and occurred seasonally over the last 2 decades. Outbreaks are generally traced back to travelers as the point source and controlled through strict insect control measures.

The virus is not endemic to Australia as it does not persist in the mosquito population in between outbreaks although the mosquito capable of transmitting Dengue, *Aedes aegypti*, is distributed at least as far south as Mackay.

While there are no reported cases of Dengue transmission from blood transfusion within Australia there are two reasons to suggest that Dengue may be associated with transfusion transmission. First there was a case report of Dengue transmission from a blood transfusion for a Hong Kong blood donor in 2002. Second, transfusion transmission is now well documented in the USA for West Nile virus, a related Flavivirus.

In 2003 a comparatively larger outbreak of Dengue in Cairns, prompted temporary local restrictions on the use of cellular blood products which has implications for blood product management should Dengue become endemic in future. This prompted the ARCBS to embark upon a study asking whether North Queensland donors are being exposed to Dengue fever virus which is non-endemic and to other known endemic insect borne (Arbo) viruses.

As part of the study blood samples were collected from donors during Dengue outbreak periods over 2003 and then 2005/2006. Samples from 2005/6 were tested in 16 donation member pools for arboviral IgG/ IgM antibodies using EIA techniques at the Queensland Health Virology Laboratory. The IgG EIA tested for Dengue I to IV, Murray Valley Encephalitis, Japanese Encephalitis, Kunjin, Stratford, Edge Hill and Kokobera. Due to the complexity of arboviral serology the IgG reactivity cannot be resolved and it is noted that the last 3 viruses while endemic do not have any known clinical significance.

As part of an multi-regional collaborative study the samples from 2003 were tested on a prototype NAT assay in research and development phase by Gen-Probe Incorporated in San Diego. Samples from blood donors from the Dengue endemic regions of Brazil and the Honduras were also tested.

The antibody testing on blood donor samples indicated that 77% (276/358) of donation pools had arboviral IgG antibody reactivity indicating past exposure to a range of arboviral agents.

Six (1.6%) pools were reactive for IgM antibody showing specificity for: Kokobera virus, n=4; Stratford virus n=1; and Kunjin virus n=1. No pools showed reactivity for any Dengue IgM antibodies.
No samples have demonstrated presence of Dengue viral RNA by NAT. However the Gen-Probe NAT systems did detect the presence of Dengue virus in blood donors from the Dengue endemic regions of the Honduras and Brazil at rates of 0.3% and 0.06%.

In conclusion international collaborative studies have demonstrated proof in principle that NAT systems can detect Dengue infected donors. However within Australia there is no evidence to suggest blood donors have donated following recent exposure to Dengue. The risk of a donor being infected may be expected to increase according to the number of clinical cases.
THE NEW ZEALAND BLOOD SERVICE EXPERIENCE USING AUTOMATION FOR NUCLEIC ACID TESTING

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Screening of blood donors for evidence of Transfusion Transmitted Infections at the New Zealand Blood Service (NZBS) is performed at two Donation Accreditation Laboratories; Auckland and Christchurch. The laboratories began Nucleic Acid Testing (NAT) for human immunodeficiency virus type 1 (HIV-1) and hepatitis C virus (HCV) in November 2001. The method selected was the Chiron Procleix HIV-1/HCV assay, using the Chiron enhanced semi-automated modular system (eSAS). This became a mandatory test at NZBS, with all donations being screened. The majority of the donations were tested in pools of 16, some selected samples were individually tested.

The semi-automated modular system was extremely reliable, with very low false reactive, invalid and failed run rates at both sites. There were limitations though, the system was labour intense, and operators needed to be highly skilled and well trained to achieve consistent results.

In 2005, NZBS evaluated the Procleix ULTRIO assay (which includes screening for hepatitis B virus DNA, as well as HIV-1 and HCV RNA), and determined that the addition of HBV testing was desirable due to the prevalence of HBV in the New Zealand population. However, for maximum sensitivity individual donor testing (IDT) was recommended, but this was not seen as being practical using eSAS.

Recently, Chiron has released the Procleix TIGRIS system, a fully automated testing platform for screening with Procleix ULTRIO. IDT is now feasible due to the high throughput of the system, and the reduced ‘hands on’ time requirements. NZBS has recently installed and validated one TIGRIS system at each of the Donation Accreditation laboratories. Validation of the TIGRIS/ULTRIO combination included analysis of false reactive, invalid and failed run rates, as well as determining the 95% detection limits for HIV-1, HCV and HBV using dilutions of WHO Standards. Operator requirements, equipment reliability, throughput and workflow were also analysed to determine whether any changes to the current timeline, from collection of the donation to its availability for release to a patient, were likely.

Initial workflow assessments showed that significantly less ‘hands on’ time was required to run the TIGRIS. This allows staff to perform other tasks in the laboratory at the same time as running TIGRIS. It was also found that in some instances the release of NAT results would be delayed by using TIGRIS/ULTRIO compared to what was achieved using the eSAS/HIV-1/HCV assay, partly due to the nature of testing on an automated platform.

Overall, the TIGRIS/ULTRIO combination provides a fully automated solution for HCV/HIV-1 RNA and HBV DNA testing, which requires less ‘hands on’ operator time, and allows the individual testing of larger numbers of samples than the previous modular platform. Data on false reactive, invalid and failed run rates, as well as equipment reliability is still being collected, and an update will be presented at the workshop.
NUCLEIC ACID TESTING (NAT) SCREENING FOR TISSUE DONORS AT THE NRL.

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Introduction: The NRL is a TGA licensed laboratory which screens blood and tissue donors for HIV-1 and HCV nucleic acid. The NRL currently provides NAT screening for cord blood donors, as well as for cadaver donors. This assay is also used to confirm NAT positive, antibody negative samples (NAT yield) from the Australian Red Cross and New Zealand Blood Services. The NRL selected the Roche AmpliScreen tests as its NAT screening assays because they were the only assays on the Australian market that has been registered for NAT screening of cadaver samples.

Materials and Methods: The COBAS AmpliScreen HIV Test v1.5 and AmpliScreen HCV Test v2.0 are qualitative \textit{in vitro} diagnostic tests for the direct detection of Human Immunodeficiency Virus Type 1 (HIV-1) and Hepatitis C Virus (HCV) RNA in human plasma. These assays use Polymerase Chain Reaction (PCR) nucleic acid amplification and nucleic acid hybridisation for detection. The tests are intended to screen samples of plasma for the presence of HIV-1 and HCV RNA in donated blood and plasma, including plasma which are intended for further manufacture and in either single donations or in samples that have been pooled using approved pooling algorithms.

Results: Since the NRL commenced testing in November 2004, the laboratory has tested 507 samples for cord blood donors, 198 samples for cadaver tissue donors, and 24 confirmatory tests for the Australian and New Zealand blood banks. None of the samples from cord blood or tissue donors has been NAT reactive. The NRL has used the Roche AmpliScreen assay and confirmed the presence of HIV-1 or HCV RNA for six NAT yield samples sent by blood service laboratories.

Discussion: In less than 3 years, a total of 708 living and cadaver donors were screened, minimising the risk of infection via donation.
THE UTILIZATION OF QC DATA ACCUMULATED IN BLOOD TESTING PROCESS CONTROL IN BTS

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Objective: Using the SPC methods and tools, looking back and analyzing the data of quality control accumulated before in blood screening laboratory, detecting the performance of process and looking for improvement opportunity.

Design: Defining main three steps of analyzing process: Identifying testing process through the collection of QC data, analyzing testing process through fixing up QC data and improving testing process through the utilization of QC data. Using visual representation such as graphs and charts to clearly understand how a particular process or operation actually does work. Histograms are particularly appropriate for displaying the distribution of data and control chart is for reviewing the trend in a process. The parameters used in process analysis such as $\bar{X}$, CV, Cpk, Range, UCL, LCL, LSL are quantitative indicator during the data analysis, which can be used to assess synthetically the capability of blood screening process.

Results: Sample selection for these charts from accumulated QC data should be taken around the same time, from the same machine, and with the same raw materials to control for additional variation. A histogram can be used to display whether the distribution in data set is normal or single-peak symmetrical distributed. If the chart is skewed to the right or left, or bimodally distributed, it is necessary that much more data should be collected. Control charts or run charts, can be excellent tools to monitor the stability of testing process. A control chart is used to distinguish variation in a process over time. Variation can be attributed to either random or system causes. The trend and breadth of graph represent the accuracy and precision. The mean of QCS ( $\bar{X}$ ) should be sensitive to any deviation and Cpk should be larger than 1.33 in a good condition. In EIA assay, LSL of weak positive QCS should be set to 1.00 and its LCL should be higher than LSL.

Conclusion: Looking back and analyzing for internal quality control data is the power of the process improvement in blood screening laboratory. The staff in laboratory should focus on the collection, fixing up and analyzing for previous QC data using various quality tools so as to improve the efficiency of internal quality control in blood screening laboratory.
EXTERNAL QUALITY ASSESSMENT SCHEME FOR NUCLEIC ACID TESTING OF BLOOD DONATIONS AND PLASMA.

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Objectives: Developments in technology in the past 10 years have enabled Nucleic Acid Testing (NAT) to be used for detecting early infection with HIV, HCV and HBV. The National Serology Reference Laboratory, Australia (NRL) conducts External Quality Assessment Schemes (EQAS) to help monitor the quality of this testing, EQA Schemes are designed to assess the integrity of an entire laboratory testing process. EQAS panel samples must be correctly identified on arrival at the laboratory; they must be tested on appropriate assays and reported without error for a satisfactory result to be obtained. The NRL provides EQAS to diagnostic and blood service laboratories world-wide. Nucleic Acid Testing (NAT) is performed using a group of highly sensitive techniques to detect specific nucleic acid fragments in clinical specimens.

Methods: The NRL distributes 3 NAT EQAS panels annually. The results presented here were generated over one year (2006). Specific objectives of the 2006 EQAS panels were to examine the:
- Potential for cross-contamination;
- Ability of laboratories to detect specimens with low viral loads;
- Performance of the assays in the detection of various HCV and HBV genotypes and HIV-1 subtypes.

Results: Forty two laboratories, from Europe (28), Asia (3), Australia (7), New Zealand (2) and the Americas (2) using nine commercial test methods and three in-house methods participated in the donor screening NAT EQAS in 2006. EQAS results identified problems in testing within laboratories, such as false positive results reported for samples that contained no viral nucleic acid. This was observed in 18 instances from the testing of 956 normal human plasma samples over 3 EQAS distributions.

HIV RNA and HCV RNA positive samples were not detected in 19 and 23 instances, respectively. While the viral loads of 21 of these samples were low (500 - 1000 copies/mL), they were greater than the detection limits of the assays and thus should have been reactive. The viral loads of the other 21 samples were extremely low (60 copies/mL).

Conclusion: By participating in EQAS laboratories were alerted to potential deficiencies in their testing processes, such as contamination and insufficient sensitivities, and can initiate necessary improvements.
Many countries have established regulatory agencies that dictate how - and often where - medical laboratory testing can be carried out and HIV is regarded as a special case for greater regulatory control in some jurisdictions. Canada has a federal health agency (Health Canada) but the Provincial Governments hold primary responsibility for delivery and regulation of health care within their provinces. This has resulted in a patchwork of rules, regulations, and recommendations at both federal and provincial levels around HIV testing. In addition, the regulations surrounding HIV testing are more stringent than those for other infectious agents. Canada has a national HIV reference laboratory but this laboratory provides reference services on a voluntary consultative basis only since it has no regulatory authority.

In order to bring some science-based consensus to HIV laboratory practice in Canada, we formed a voluntary association with a membership comprising representatives from almost all HIV testing laboratories in Canada, including the National Reference Laboratory, as well as from the Canadian blood agencies and from the federal agency responsible for regulating the import of HIV testing products. This Canadian Association of HIV Clinical Laboratory Specialists (CAHCLS) is entirely independent from all regulatory agencies as well as from the HIV diagnostics industry. Our principal goals are to share scientifically generated data and clinical experience to improve the practice of HIV diagnostics in Canada and to produce consensus recommendations that will guide the rules and regulations formulated by the responsible agencies that regulate HIV testing in Canada. CAHCLS is also working with other agencies to educate patients around the interpretation and utility of HIV tests and we have established links with agencies from other countries and regions (such as the Australian NRL) to help guide our own practices and to assist others to improve the practice of HIV diagnostics. Each year we hold a national meeting to bring our members together with government and industry representatives and experts from other countries to share data, generate new initiatives, advise the industry of our needs, and formulate consensus recommendations.
ABSTRACTS
WEDNESDAY 24th OCTOBER 2007

THE FOUNDATIONS OF HIV CAPACITY BUILDING: ARE INTERNATIONAL PROGRAMS ASSISTING IN ALL THE RIGHT PLACES?

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International HIV intervention programs have grown significantly since 2000 in the belief that such programs are essential to arrest and contain HIV epidemics in the resource poor world. These programs, conducted by international non government organisations, private foundations, multilateral and bilateral donors have stressed a range of priorities and practices and set national, regional and global targets in order to achieve universal access to HIV treatments for communities infected and affected by HIV and AIDS. Funds committed, allocated and disbursed by these programs have grown significantly. Yet, in 2007 the vast majority of people requiring treatment for HIV still have no access to anti-retroviral therapies. Further, medicines needed for treatment of opportunistic infections, reliable drug and product supply systems, pharmacy management procedures and laboratory capacity and reliability are often neglected in the rush to provide anti-retroviral drugs.

Capacity building has become the catch cry of international HIV programs as attempts are made to incorporate HIV service provision into existing health care systems. But what happens when healthcare services are incapable or simply non existent? Are international HIV interventions providing the right foundations on which capacity can be built? And how might we best measure and evaluate the success of these programs in reducing HIV prevalence and incidence?

This paper will consider these issues and take a particular look at the current situation in Papua New Guinea (PNG) PNG is the only epidemic in our region classified as generalised and is the site of Australia’s biggest HIV international program. It is vital that we understand the impacts of our HIV development efforts in this country if we are to effectively contribute to better health outcomes for the people of Papua New Guinea.
Evaluation of HIV EQAS results of HIV confirmatory testing sites by KCDC (the Korea Center for Disease Control and Prevention) in October 2006, Korea.

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Background: KCDC HIV External Quality assessment (EQAS) is one of the quality management programmes, specialized for public and private HIV test laboratories in Korea from 2005. This report presents HIV EQAS results from 17 HIV confirmatory testing sites out of 600 participants in this programme in October 2006.

Methods: HIV EQAS panel consisted of 10 undiluted plasmas which included 3 positive donors (No. 1, 3, 6), 4 indeterminate donors (No. 7, 8, 9, 10) and 3 negative donors (No. 2, 4, 5) for HIV antibody. Three out of 10 samples were positive (No. 6, 7, 8) for HIV antigen as well and the reproducibility of the panels was checked by duplicates (No. 4, 5 for HIV antibody negative, No. 7, 8 for indeterminate). The samples have been distributed to each participating laboratories and the results were reported through on-line. The result was analyzed by traditional method (95% CI), robust statistics (3Z), and Youden plots (99% coverage).

Results: All of the HIV confirmation laboratories have performed Particle Agglutination (PA), Enzyme Immunoassay Antigen (EIA Ag), EIA Antibody (EIA Ab) for screening assay and Western Blot (WB) for confirmatory testing. As for EIA test, the analytic sensitivities of HIV antigen and antibody were 93.3% and 97.5% (inconsistent percentage (2.5%) is due to indeterminate samples), and the analytic specificities of HIV antigen and antibody were 94.1% and 100%, respectively. PA and WB results were consistent with reference results in all the samples but there was a small variation to define the intensity of indeterminate samples. In the reproducibility of EIA results using 4 duplicated samples, traditional method detected 2 laboratories for HIV antigen and 2 laboratories for HIV antibody as outlier, whereas robust statistics method showed better outlier detection which was 5 laboratories for HIV antigen and 4 for HIV antibody. The outliers detected by Youden plot were differentiated to six systematic and two random errors.

Conclusion: HIV results for all HIV EQAS samples were consistent with the reference result in PA, WB assay and EIA (only strong HIV positive samples). The majority of outliers of EIA in traditional method and robust statistics were due to inconsistency of indeterminate samples in two laboratories, which was turned out systematic/random error. Therefore, the design of reliable EQAS based on various and specified titers of HIV panels and advanced statistical methods for data analysis are pre-requisite to improve the quality of HIV laboratories.
THE ABSURDITY AND EVIL OF HIV DENIAL

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Since human immunodeficiency virus (HIV) was discovered in the early 1980s, and shown to be the agent responsible for AIDS, a huge volume of research has been described in the scientific literature. This volume is explained by the dimensions of the HIV and AIDS epidemic, which is responsible world-wide for about 3 million deaths annually. It may therefore seem curious that there are organised groups of people who deny the existence of HIV, or deny the role of HIV as a cause of AIDS. One such denial organisation – which describes itself as “the Perth Group” - had its day recently in an Australian court.

The case was Regina versus Parenzee. Andrew Chad Parenzee was convicted in South Australia in 2006 of three counts of endangering life. Parenzee had been accused of having unprotected sexual intercourse with three women, at a time when he knew himself to be infected with HIV, despite having been advised that by doing so he risked transmitting the virus to his sexual partners. Two representatives of the HIV denial group were called as expert witnesses by Parenzee’s legal team, when in the Court of Criminal Appeal he sought permission to appeal on the grounds of a miscarriage of justice. Parenzee’s barrister argued that, at the time of the original trial, the defence was unaware that there was a genuine scientific controversy about the existence of HIV, the reliability of diagnostic tests for HIV, and whether HIV was sexually transmissible. It was argued that, had the defence been able to produce evidence of this controversy, then the jury might not have found Parenzee guilty. Parenzee did not succeed in his application. The Director of Public Prosecutions produced eight highly qualified expert witnesses, to advise that HIV undoubtedly causes AIDS and can be transmitted sexually. The two witnesses from “the Perth Group” of HIV denialists were discredited, and the court did not accept their status as expert witnesses. Parenzee has since been sentenced to nine years in gaol.

Apart from “the Perth Group”, there are other organisations around the world that deny HIV as the cause of AIDS. HIV denialists may passionately argue their case against the overwhelming body of scientific evidence and opinion, although not all denialists have identical beliefs. The motives for HIV denial may not be obvious, and could have psychological or political elements. The techniques of HIV denialists include selecting pieces of evidence that appear to support their case while ignoring the great bulk of evidence to the contrary. Furthermore, denialists often, through ignorance or perhaps even wilfully, misinterpret the evidence on which they rely. Further they may bend the evidence to meet the needs of their arguments and often use these arguments to support their crusade of sorts.

Numerically, there are very few HIV denialists. However, they cannot be safely disregarded, because they may have a disproportionate influence. If individual patients can be persuaded that HIV does not cause AIDS, or that antiretroviral drugs are ineffective, then these patients may spread the disease to others, or fail to accept necessary medical intervention. (It is of significance that the defence team in the Parenzee case advised the defendant to cease his antiretroviral therapy and he did.) There is greater risk if HIV denialists achieve political influence, as they sometimes do. A notable example was Thabo Mbeki, President of South Africa to whom the Perth Group achieved advisor status. Mbeki’s lack of acceptance of HIV has had a profound role in allowing the epidemic to reach the proportions now present in the South African population. Thus, the case reported represents a dangerous situation which is founded in crusade psychology rather than in scientific evidence.
WHY QC IS SO IMPORTANT IN RESOURCE POOR SETTINGS

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Quality assurance is sometimes thought of as somewhat onerous add on to our core business of performing and reporting laboratory tests. It is so much a part of the laboratory process however, that it is difficult for us to imagine what our work would be like without it. Laboratories in resource poor settings do not often have access to quality assurance programs and comply variably with internal quality control processes. Some clinical case histories will be presented that highlight the problems that can arise from the lack of QA/QC. In some cases incorrect laboratory diagnoses are more harmful than NO laboratory diagnosis. Quality should be first and foremost in our thinking about laboratories in resource poor countries. Donor agencies and external experts must be proactive in ensuring laboratories have access to QA programs. Where this can not be assured then choice of test menu must take into account the potential sources of error. Simple point of care tests with inbuilt QC may be preferred over low-tech, low cost solutions that rely on high levels of training and interpretation.
AN INVESTIGATION OF ABERRANT HIV EXTERNAL QUALITY ASSESSMENT SCHEME (EQAS) RESULTS

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Introduction: The National Serology Reference Laboratory, Australia (NRL) coordinates an External Quality Assessment Scheme (EQAS) for HIV. The panels are distributed three times yearly to laboratories in Australia and internationally. The first panel distributed for 2007 (HIV Serology EQAS Panel ID 2007/Apr/03) consisted of five samples that were negative and five samples that were positive for anti-HIV. All ten samples were negative for HIV p24 antigen. One participant (Concord Hospital - SSWAHS) obtained reactive results for all ten samples in the EQAS panel (some low and some high) using the Abbott Architect HIV antigen/antibody CMIA. The pattern of results was suggestive of sample carry-over on the Architect instrument. The instrument had recently undergone scheduled preventative maintenance.

Objective: To determine the cause of the aberrant HIV EQAS panels, results obtained by Concord Hospital.

Methods: Three principal measures were taken to investigate the aberrant results at Concord Hospital:

- Panel samples from Concord Hospital were swapped with those from another laboratory participating in NRL’s HIV EQAS;
- NRL provided three additional panels A, B and C to Concord Hospital, that were constructed so that results of their testing would confirm whether or not carry-over was occurring. In Panel A the negative samples’ IDs were 1-5, the positives’ 6-10. Panel B, was constructed to have two aliquots of the same negative sample following each of three of the positive samples. Concord Hospital was instructed to test the samples in Panel A and then Panel B in order and in the same “run”. Panel C was a replicate of EQAS panel 2007/Apr/03.
- Service personnel from Abbott examined the instrument on two occasions.

Results: The results of testing of Panels A and B confirmed sample carryover:

- When negative samples preceded positive samples, negative results were obtained for the negative samples
- When negative samples followed positive samples, the negative samples were falsely positive and there was a dilution effect observed in the results for sequential negative samples.

Following continued aberrant results for negative samples in Panel B, Abbott service personnel replaced the sample probe and a valve in the wash station of the Architect instrument. Concord Hospital then tested Panel C and obtained results that agreed with the reference results for the panel.

Discussion: Concord Hospital services a population with a low prevalence of HIV and relies on participation in EQAS to confirm its ability to detect anti-HIV positive samples. An investigation revealed that an instrument related problem was causing sample carry-over. The problem was corrected after the sample probe and a valve in the wash station were replaced. Before this, a scheduled service to the Architect instrument had been carried out but it was not detected that sample carry-over was occurring.

This case study highlights:

- The importance of EQAS participation in providing samples not routinely encountered;
- That scrutiny of EQAS results provides an early warning mechanism to detect problems;
- The importance of equipment maintenance;
- The importance of ensuring the recommended maintenance is performed;
- The importance of a planned approach to troubleshooting.
DETECTION OF LABORATORY ERRORS THROUGH QUALITY ASSURANCE

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Introduction / Objectives: Quality assurance (QA) is a means for verifying the accuracy of testing procedures, the results that they produce and the interpretation of those results. The objective of an external QA Program is to provide a measurable “snapshot” of a laboratory’s performance, including the pre-analytical, analytical and post-analytical phases that can be compared to the performance of peers using the same methodology. By providing a series of “snapshots” over time (comprised in a module), a laboratory is able to identify their errors as random or systemic and evaluate their overall performance.

Methods: Survey reports are constructed to include an assessment of qualitative results, use of expired kits, transcription errors or confusing specimens (so that results of 2 specimens are reversed), and use of units that are incorrect or inappropriate for the specimen values and/or methodology. Clerical errors are sometimes included to assess the pre-analytical phase of the testing process. Quantitative results are evaluated for the benefit of participants but are not included for scoring.

Results: Reports from 2006 were evaluated to gain an indicator of overall error rates. Approximately 20% of participants did not detect clerical errors throughout the year in various surveys. Inconsistencies in reporting were highest with rubella (25% for Survey 1, reducing to 15.5% for Survey 4) with Hepatitis B close behind (10.5% for two surveys in 2006). Transcription errors / reversal of specimens are at high level with an average of 2% in various surveys throughout the year. Up to 3% of participants use expired kits.

Discussion: While there is an emphasis on establishing if results are consistent / inconsistent with consensus of ≥80% of participating laboratories; it is essential that laboratories address other aspects of the testing process that are detected through QA. It appears that some participants do not read survey reports as the same error is repeated throughout the year. It is essential that participants respond to the information produced by a QA Program to ensure that the problem is in fact a “snapshot” rather an indication of ongoing problems.

Conclusion: Ultimately, each of these errors impacts on results produced for a patient who has an expectation of accuracy in their clinical assessment. QA is integral in identifying errors but ultimately, it is the responsibility of laboratories to use the detection of errors through QA to improve performance; thus ensuring patients and their health practitioners receive data that is accurate and error free.
QUALITY CONTROL IN A LOW COST HIV VIRAL LOAD ASSAY

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Introduction
HIV antiretroviral therapy is becoming more widely utilised in resource-limited settings, therefore the use of viral load testing to monitor HIV is more widespread. Factors that bear on the appropriateness of particular viral load assays for resource constrained settings include cost, technical complexity and quality control (QC). We have introduced a QC sample (Cavidi 002) provided by NRL into a regional laboratory in Suva, Fiji, in conjunction with initiation of the ExaVir Load assay (Cavidi AB). This assay is a cheaper and easier alternative to the RNA based viral load assays.

Methods
Whilst training laboratory staff in Fiji on the use of the ExaVir Load assay all trainees (and the trainer) included at least one QC sample in their assay. Results were compared to results from an established laboratory in Melbourne, Australia using EDCnet.

Results
Nine samples were tested in Fiji on four assays each with a different operator. Three were training assays in which the scientists had not previously performed the particular test. One was a demonstration assay performed by an experienced operator. The data were compared with results from an experienced laboratory in Australia using the same QC sample (n = 13 runs).

There was no difference between the mean value for the two laboratories (3.95 log\textsubscript{10} copies/ml equiv) with a standard deviation of 0.05 and 0.07 and % CV of 11.5 and 15.8 in Melbourne and Suva respectively. The largest difference between the trainer and trainees’ results in Fiji was 0.12 log\textsubscript{10} and the largest difference between the results in Fiji and Melbourne was 0.22 log\textsubscript{10}. The differences seen here might be easily accounted for by assay variation.

Conclusion
This is the first time this QC sample has been compared between laboratories. We found that there was very little difference between the QC results obtained from Melbourne and Fiji., indicating that the assay is robust and that the training provided was sufficient for this assay.
AN INTERNATIONAL EXTERNAL QUALITY ASSESSMENT FOR HIV-1 GENOTYPIC ANTIRETROVIRAL RESISTANCE TESTING

Land S\textsuperscript{1}, Sayer D\textsuperscript{2} and Dax EM\textsuperscript{1} on behalf of the Laboratories participating in the EQAS for HIV Genotyping.

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Introduction: An External Quality Assessment Scheme (EQAS) for HIV-1 Genotypic antiretroviral (ARV) testing (Genotyping) was commenced at the National Serology Reference Laboratory, Australia (NRL\textsuperscript{TM}) in June 2000. The objective of the EQAS was to optimise the quality of sequencing techniques and standardise outcomes of Genotyping to facilitate meaningful and confident comparison of results between laboratories. Genotyping is a multilayered test that involves genetic sequencing of the viral genome, detection of drug resistance mutations (DRMs) that confer ARV resistance, and the interpretation of ARV resistance. Laboratories differ with respect to the molecular and interpretation techniques used to perform the testing. Furthermore, mixed virus populations, called nucleotide mixtures (NMs), can complicate the interpretation of test results. In early EQAS, nine Australasian laboratories demonstrated improved testing outcomes by increased detection of DRMs and NMs, and agreement in the interpretation of ARV resistance. The results of four EQAS tested by up to 16 Australasian and international laboratories are presented.

Methods: Laboratories performed Genotyping using their standard protocols. EQAS samples were plasma from infected individuals, or plasma spiked with cultured virus. A consensus sequence was constructed by aligning the nucleotide sequence determined by each laboratory and choosing the result of the majority. Nucleotide sequence agreement, detection of DRMs and NMs at positions in the genome associated with ARV resistance, and interpretation of ARV resistance were analysed.

Results: A high level of agreement (>98%) at the nucleotide sequence level was demonstrated by most laboratories. Consistent with previous EQAS exercises, the majority of laboratories maintained their level of detection of DRMs (approximately 90%) but varied in their detection of NMs. Detection of NMs correlated with detection of DRMs ($p < .01$). Early results suggest that detection of DRMs may be influenced by the proportion of DRMs present NMs. Agreement in the interpretation of resistance to 16 ARV drugs was maintained at 92% when the same interpretation system was used by all laboratories. However, EQAS demonstrated that interpretation of resistance is effected by suboptimal detection of DRMs and NMs.

Discussion: The inception of the NRL’s EQAS for HIV Genotyping correlated with an increase in Australasian laboratories’ abilities to detect ARV-associated mutations and improved agreement in their interpretation of ARV resistance. Subsequent EQAS have assessed the sequencing quality of participating laboratories and shown the laboratories have maintained a high quality in their testing outcomes. Elements of the testing process, such as suboptimal detection of DRMs and NMs, were shown to affect the outcome of Genotyping. Therefore, the EQAS has detected deficiencies that could impact on standardisation of test outcome across laboratories. Many laboratories that have consistently participated in this EQAS for Genotyping have demonstrated that they have maintained and, if necessary, improved the quality of their testing.
ABSTRACTS
WEDNESDAY 24th OCTOBER 2007

OCCUPATIONAL HEALTH & SAFETY – CASE STUDY REVIEW

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Introduction/Objectives: In 2006, the RCPA Serology Quality Assurance Program (SQAP) introduced a ‘Specimen Free’ Module. The objective of this module was to present scenarios and case studies to facilitate group discussions at a laboratory level, in the absence of actual testing specimens. The results are peer reviewed and generally graded satisfactory or unsatisfactory. Laboratories can use these reports to make comparisons and discuss procedures, algorithms or other issues with staff.

Methods: The Occupational Health & Safety (OH&S) case study included two different photographs over two surveys. The first was titled ‘OH&S - Laboratory Scenario’ and the second ‘OH&S - Office Scenario’. Participants were asked to review each photo and identify the OH&S issues; list potential problems associated with each issue and provide a resolution for each issue.

Results: 18 participants responded to the ‘Laboratory Scenario’ photograph. Overall, there were 22 OH&S issues identified. 100% listed the misuse of safety glasses, no gloves, unfastened lab coat and the presence of food and drink in the laboratory. 89% participants reported the untied technician’s hair and the overflowing bin as issues. 6% of participants listed issues including lab chair with wheels, non-hazardous waste in biohazard bin and personal items in the laboratory.

20 participants responded to the ‘Office Scenario’ photograph, with 18 OH&S issues identified. 100% listed the used gloves on the desk, 90-95% indicated specimens in the office, height of books and access to the work area as issues. 20% of participants did not suggest the wearing of a lab coat in the office as an issue. General clutter was also listed as an important issue.

Potential problems reported for both scenarios included the risk of contamination, manual handling injuries, reduction of ergonomic efficiency and trip hazards. In both cases the resolutions of these issues were satisfactorily described, essentially including education and training of staff on OH&S issues.

Discussion: The majority of participants identified greater than 10 issues for both photographs with many participants identifying additional issues. Most responses were appropriate for the concerns raised and some responses highlighted critical issues. Generally the resolution of the identified issues was appropriate.

Conclusion: It was encouraging that the majority of participants reported the OH&S issues, potential problems and resolutions in these cases. However, the omission of several important issues by some participants highlights the need for this opportunity to participate in this type of review to compare and discuss issues with staff members as an adjunct to existing education and training in the laboratory.
HAZARD IDENTIFICATION, RISK ASSESSMENT AND CONTROL MEASURES IN A DIAGNOSTIC LABORATORY.

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National Serology Reference Laboratory, Australia.

The Victorian Occupational Health and Safety Act 2004 (Act no. 107/2004) states that an employer’s general duty of care is to “provide a workplace that is safe and without risk to health”. A diagnostic laboratory, by its very nature, is an extremely hazardous environment, one where it is impractical if not impossible to eliminate all risks. So how do employers and staff ensure that they are working in a safe environment without risk to health?

In addressing this challenge, the NRL is using a systematic approach that has been derived from AS/NSZ 4360:2004 Risk Management, to help identify hazards within the workplace.

A hazard is any situation that has the potential to cause injury or adversely affect the health of a worker. Hazard identification is the process used to identify ALL possible hazards in the workplace where an injury, illness, disease or loss of income or productivity may occur. Once a hazard has been identified, a risk assessment must be conducted to determine the likelihood that workers may be exposed to any of these negative outcomes. All practicable measures for reducing such outcomes in the workplace are then identified. Once controls have been implemented, it is essential to measure and continually review the measures in order to ensure their effectiveness.

The workplace will never be free of risks. However by conducting regular risk assessments and implementing controls, risks in the workplace will be managed as far as reasonably practicable. Organisations that manage risk effectively and efficiently are more likely to achieve their objectives but not at the cost of the health and well being of their staff, and they can expect to do so at lower overall cost.
THE HUMORAL IMMUNE RESPONSE – IMPLICATIONS FOR SEROLOGY

Sewell WA

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During an immune response, IgM is the first immunoglobulin isotype to be secreted. IgM is largely produced by cells within the secondary lymphoid tissues such as the lymph nodes and spleen. Marginal zone B cells are important producers of IgM, and can secrete IgM in a T cell dependent or T cell independent fashion. The strength of individual IgM antigen-binding sites (affinity) is low, but the presence of 10 antigen-binding sites in each IgM molecule compensates for the low affinity, by increasing the overall binding strength (avidity) between IgM and antigen. IgM producing cells are short-lived, making the IgM response transient and therefore useful in detecting current or recent infection. However, the low affinity of individual antigen-binding sites in IgM is associated with polyreactivity, or a capacity to bind to multiple different antigens. This contributes to non-specific findings in diagnostic serology. Further problems with IgM assays arise because in some instances the IgM response can be prolonged. In these cases, IgM is less useful in identifying recent infection.

During an immune response, activated B cells enter the germinal centres in lymphoid tissues, where they undergo class switch recombination and somatic hypermutation. These two processes are closely linked, they both involve changes to the DNA of the immunoglobulin genes, and they both depend on the presence of follicular helper T cells. Class switch recombination results in the replacement of the IgM heavy chain with that of other isotypes. In somatic hypermutation, point mutations are randomly inserted into the variable genes of the heavy and light chains. If the mutations cause B cell surface immunoglobulin to bind antigen with higher affinity, these cells proliferate faster and dominate the immune response. The resulting clones populate the bone marrow as long-lived plasma cells. Thus later in an immune response, circulating antibody becomes dominated by high affinity IgG, and such antibody can be detected for many years.

Assays of circulating IgG antibodies therefore typically assess established rather than recent immune responses. Unlike IgM, IgG antibodies are not polyreactive and binding is usually specific. Thus IgG assays are less confounded by non-specific binding than are IgM assays. However, binding to related antigens can occur with IgG, and such binding is not necessarily predictable. There are 4 IgG subclasses, of which IgG3 is the first to be produced in an immune response. Selective detection of IgG3 responses may be useful in the diagnosis of recent infection. There are a variety of other strategies to manipulate IgG assays, in order to make them useful in the assessment of recent infection.
ABSTRACTS
THURSDAY 25th OCTOBER 2007

THE USE AND MISUSE OF SEROLOGY ASSAYS IN THE DIAGNOSIS OF ACUTE INFECTIONS

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Infectious disease serology has a long and distinguished history beginning in 1901 with von Behring’s work on tetanus and diphtheria. From this point on bacterial serology became an increasingly important diagnostic tool and with the advent of antibiotics its use accelerated. Viral serology has a much shorter history of less than 60 years. But with developments in cell culture techniques and the subsequent improvements in antigen production, viral serology has out-stripped its bacterial cousins.

The development of the Enzyme Linked Immunosorbent Assay (ELISA) technique has seen the greatest expansion of serology in its entire history. This technique has allowed us to detect antigen and antibody. It has allowed us to determine antibody class quickly and easily. It has increased sensitivity to an extreme level – approaching that of molecular techniques. The great advantages of ELISA can also be its greatest disadvantages. It can give us the greatest sensitivity and specificity of all the serologic techniques but do we really want or need this level of detection. Do we use it appropriately, do we understand the real limitations and the effects (positive and negative) this has on our ability to diagnose recent infection and determine immune status. What effect can these issues have on disease notifications? Is ELISA the most appropriate technique to use? Is the theory of the immune response consistent with the reality of the immune response as detected by serologic techniques?

In this discussion we will answer these questions and explore whether we really use serology appropriately or whether we misuse it.
ATTENUATED OR ATYPICAL HIV-1 INFECTION – HOW DOES THIS HELP IN DIAGNOSIS AND A BETTER UNDERSTANDING OF VIRAL PATHOGENESIS?

McPhee, DA1,2,3,4,9, Ashton, L7, Birch, C4, Bowden, F4, Churchill, M4, Crowe, S4,6, Dax, E1,2, Deacon, N4, Doherty, RG1, Dyer, W6, Gelgor, L7, Gorry, P2,4,6, Greenway, A4, Gust, I6, Kemp, B4, Learmont, J6, Padiglione, A4, Sullivan, J6, Verity, E4, Wesselingh, S4,6, Wilson, KC4, Wilson, KM1,2, and Zotos D1.

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Background: One of the important aspects that have improved the understanding of the HIV-1 infection process and diagnosis of infection is from the study of atypical cases. Over the years in of studying HIV-1 pathogenesis, several attenuated or atypical infections have been identified and provided insights into the infection process. The outcome of HIV-1 infection of individuals may result in a lack of detection or unusual results utilizing standard serological assays. Identification of such individuals and resolution of errant results was the primary goal for each of these investigations.

Methods and Results: Several cases are reported from studies over the last 18 years. The first case was a homosexual man with Kaposi’s sarcoma (KS) that was consistently HIV negative by standard tests. Upon investigation this individual had detectable antibodies to an immunodominant epitope in gp41 and to a second regulatory HIV-1 protein, Nef. The subject’s consistent positive reaction against gp41 and Nef with our in house EIAs and recent epidemiologic analyses of the incidence of KS pointed to HIV infection, all be it defective. This highlighted improved diagnosis using immunodominant peptides. The second case was a cohort of individuals infected via transfusion with what transpired to be an attenuated viral HIV-1 strain. These individuals were infected for up to 27 years (Long Term Non-Progressors; LTNP). Detailed study of the antibody responses indicated these to be varied and very slow in development. This study highlighted the delay in development of antibody responses due to a highly attenuated virus infection and the host’s genetic makeup. Slow progression of infection was only observed upon detection of a viral load. Due to the varied deletions in the nef gene and lacking material after initial infection sero-archeology was used to define better the regions in the nef gene that were originally present. The third example was an individual severely affected clinically with an HIV-like illness but no seroconversion detected by standard serological techniques over a period of 18 months. Retrospectively, investigations showed this subject was infected with a highly attenuated HIV-1 strain. Seroconversion was eventually observed but this case highlighted the importance of the HIV antigen-antibody combination assay in diagnosis.

Discussion: This presentation summarizes some very interesting cases that have lead to major contributions to better understanding the HIV-1 infection process. For improved HIV-1 diagnosis the implementation of synthetic peptides derived from highly antigenic sites proved useful. For early or unusual diagnoses, the use of new technologies, such as viral nucleic acid and/or antigen detection, improve detection. The four generations in the development of more sensitive and specific HIV immunoassays have built on examples such as these. Our improved understanding of the development of antibody responses in very slow infections emphasized the importance of the host’s genetic makeup in influencing the outcome of infection. In addition, these studies showed that potent, broadly neutralizing anti-HIV antibodies and robust CD8+ T-cell responses to HIV infection were not necessary for long-term control of HIV infection for some members, and were not sufficient to prevent viral evolution, augmentation of pathogenicity and eventual progression of HIV infection for others in the cohort of LTNP. Despite this, differences in the development of antibody responses have been dissected to reveal more potent immune responses due to a delayed infection process. All these studies have assisted in a much better understanding of HIV-1 infection, diagnosis and viral pathogenesis.
ENHANCED LABORATORY SURVEILLANCE OF ACUTE HIV-1 INFECTION IN INNER SYDNEY.

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Objectives: To determine the effect of qualitative nucleic acid screening on the yield of acute HIV infection detection in an inner Sydney population with generally, an over representation of risk factors associated with HIV acquisition.

Methods: Routine clinical diagnostic samples referred for HIV testing that screened negative by a fourth generation HIV-1/2 antibody/antigen combination enzyme immunoassay (Abbott Architect, IL, USA) were combined in pools of 6 samples each. Samples were included if they were referred from high HIV case load primary care general practitioners from individuals who had consented to HIV testing. Pooled samples were tested for HIV-1 RNA using a qualitative nucleic acid amplification assay (NAAT) designed for blood and tissue donor screening. (Ampliscreen HIV-1, ver 1.5 NJ USA). Pool member samples were retested individually if the pool tested positive for the presence of HIV-1 RNA to identify the infected sample.

Results: A total 1836 HIV-1/2 Ab/Ag seronegative samples in 306 pools were screened using NAAT over a 4 month period. One pool tested positive for HIV-1 RNA. Individual panel members were re-tested in single and the reactive sample was identified as from a 41 year old homosexual male presenting with a non-chlamydia, non-specific urethritis and underwent routine testing for HIV. The sample was negative for HIV-1 p24 antigen EIA, HIV-1/2 3rd generation EIA and HIV-1 western blot. A follow-up sample collected 3.3 months later confirmed the individual had seroconverted with reactivity to all viral proteins on HIV-1 western blot, 3rd generation HIV-1/2 antibody EIA, 4th generation Ab/Ag EIA and HIV-1 proviral DNA. HIV-1 p24 antigen EIA was negative.

Conclusions: The inclusion of HIV nucleic acid testing in a testing strategy increases the identification of acute cases of HIV-1 infection and provides an important means of enhanced surveillance of HIV incident infection and opportunities for prevention.
PROSPECTIVE ESTIMATION OF INCIDENCE RATES IN NEWLY DIAGNOSED CASES OF HV INFECTION AT ST VINCENTS HOSPITAL SYDNEY 2005-2007

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

A number of immunoassays have been developed which distinguish between individuals with established HIV infection and those that have been infected within the previous six months. In 1997, Janssen et al (CDC USA) developed the ‘detuned’ or ‘Less-sensitive’ ELISA by modifying a first generation lysate EIA for HIV-1 (infection within <176 days). More recently the BED HIV-1 IgG incidence EIA (Calypte Biomedical, USA) estimates incidence by measuring HIV-1 specific IgG with total IgG (infection within <153 days). These testing strategies are known as STARHS (serological testing algorithms for recent HIV seroconversion). It is well known that these assays have limitations including overestimation of HIV incidence in populations where non-B subtypes of HIV-1 are prevalent, individuals with advanced HIV infection and individuals who have received long term antiretroviral therapy (ART) and virologically suppressed.

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

<table>
<thead>
<tr>
<th>Year of diagnosis</th>
<th>2005 (n=289)</th>
<th>2006 (n=230)</th>
<th>2007 (n=147)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Detuned</td>
<td>BED</td>
<td>Detuned</td>
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<tr>
<td>Incident HIV infection</td>
<td>46.3%</td>
<td>37.9%</td>
<td>42.3%</td>
</tr>
<tr>
<td>Established HIV infection</td>
<td>53.7%</td>
<td>62.1%</td>
<td>57.7%</td>
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NB: 2007 figures represents the first 6 months of newly diagnosed cases.

Over the three years, the ‘detuned’ OTC assay (44.9%) estimated the number of individuals with incident HIV infection on average 5.7% higher than the BED assay (39.2%). Further analysis of discordant results (n=29) revealed 20 (69%) of samples misidentified as incident infections by ‘detuned’ OTC assay were correctly identified as established infections by the BED EIA (low CD4+ count or reported date of diagnosis >1 year). A further 5 (17%) samples were identified as established but classified as incident by the ‘detuned’ LS-OTC EIA. These samples were from patients with recent laboratory confirmed seroconversion and the difference may highlight the different cutoffs of the 2 assays (153 vs 176 days). The remaining 4 samples could not be confirmed due to absence of followup or supplemental test results.

This study shows that approximately 39% of newly identified cases of HIV are presenting with incident HIV infection. The ‘detuned’ LS-OTC assay appears to overestimate the number of incident cases of HIV by falsely classifying individuals with chronic HIV infection. These results suggest these tests may be useful for population estimates of recent infection however, use for individual patient management is not recommended. Although the number of
cases of incident infection using the BED assay is increasing it does not reach statistical significance (p = 0.157; Wilcoxon rank sum – test for trend). STARHS for newly identified cases of HIV-1 infection and provides an important means of enhanced surveillance of HIV incident infection and may represent opportunities for prevention.
Evaluation of the Abbott ARCHITECT Syphilis TP Assay: Improved Detection of Early Primary Syphilis

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The QHPS-Central laboratory performed an evaluation of the Abbott ARCHITECT Syphilis TP assay. All samples were originally screened with the Murex ICE Syphilis EIA assay. Samples initially reactive on the Murex assay were tested for RPR (BD Macro-vue RPR Card Test) and TPPA (Fujirebio Serodia TP-PA). Discordant samples were also tested for FTA-ABS (MarDx FTA-ABS).

In the evaluation, 505 samples were tested (345 Murex non-reactive, 160 Murex Reactive). 340 samples were non-reactive by both assays and 147 samples were reactive by both assays. Of the discordant samples, 13 were considered to be false reactive.

Three samples were Murex non-reactive but reactive by ARCHITECT. Two patients had repeat serology collected 1-3 months later that demonstrated seroconversion or a significant rise in the RPR titre and were reactive for both TPPA and Murex EIA. The third was from a patient diagnosed clinically as early primary syphilis. Two patients weakly reactive on the Murex assay were non-reactive on the ARCHITECT assay. Both samples were known cases of previously treated syphilis.

The sensitivity of the ARCHITECT and Murex assays was 98.7% and 98% respectively and the specificity was 98.0% and 96.9%. The Murex ICE Syphilis assay failed to detect three patients with early primary syphilis infection who were detected by the ARCHITECT assay. The Architect Syphilis TP assay failed to detect two past treated syphilis patients who were detected by the Murex assay. A possible explanation could be differences in antibody profiles between early primary and past syphilis infection, such as presence of IgM, IgG avidity and IgG subclass.

The results show that the ARCHITECT assay is more sensitive and specific than the Murex ICE assay for the detection of primary syphilis. Sensitivity at this end of the disease spectrum is particularly important in terms of treatment for the patient and infection control for possible contacts.
THE NEW DEVELOPMENTS OF HEPATITIS/RETROVIRUS INFECTIONS AND THEIR DIAGNOSIS/SCREENING ASSAYS

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Early detection of acute HIV, hepatitis B, and hepatitis C infections plays a key role in allowing for the earlier diagnosis and management of individuals in the acute (pre-seroconversion) phase of infection and in helping to prevent further transmission of these viruses. Furthermore, diagnostic accuracy demands surveillance and identification of viral variants to ensure that diagnostic assays incorporate detection of variants into their designs.

Worldwide, more than 350 million people have chronic hepatitis B. Global infection control measures (such as universal vaccination) combined with increased access to HBsAg diagnostic assays have successfully reduced the rate of HBV infection. However, the recent emergence of HBV vaccine escape mutants has proven to be a detection issue with some HBsAg diagnostic immunoassays, especially with a single monoclonal coated solid phase capture reagent and a single monoclonal detection reagent. Such assays could generate false negative results, if the sample contains a HBsAg mutation which demolish the binding epitope of the monoclonal reagent.

HIV in Australia has gradually increased over the past five years, from 656 cases in 2000 to around 930 in 2005, a 41% increase. Given that acute HIV is the most infectious, yet often missed phase of HIV infection (window period), there is a need for practical, cost effective assays that provide early detection- HIV Ag/Ab combo assays. By combining HIV antigen and antibody detection into a single assay, the window period has been further reduced by 3–5 days. An Australian experience clearly demonstrated that the HIV Ag/Ab combo assays facilitate identification of population during antigenic phase in absence of HIV antibodies. Over a span of approximately 1 year, HIV Ag/Ab combo assays have resulted identification of 27 patients that would have been missed by antibody only assays. In majority of cases, the patients were unaware of HIV exposure and would have been source of spreading the viral infection to their partners.

Detection of antibodies against HCV and/or detection of HCV RNA by nucleic acid testing (NAT) are the commonly utilized methods for detecting exposure to HCV in both blood screening and diagnostic laboratories. Stand-alone HCV core antigen tests and HCV antigen/antibody combination tests could provide an alternative to HCV NAT in blood banks where such testing has not already been implemented, and may be utilized in diagnostic laboratories to identify early HCV infection-pre-seroconversion window period.
HEPATITIS C VIRUS GENOTYPING: EVALUATION OF A NEW LINE PROBE ASSAY.

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Background: Hepatitis C virus (HCV) can be classified into six major genotypes, which have clinical significance in determining dose and duration of antiviral therapy. In Australia, the predominant genotyping assay is the VERSANT HCV Line Probe Assay (LiPA; Siemens Medical Solutions). However, due to its reliance on discriminating genotypes based on sequence variation in the conserved 5’ untranslated region (5’ UTR), it can mistype some genotype 6 samples as genotype 1. In addition, sometimes the banding pattern on the LiPA cannot be used to identify the HCV genotype. In this study, we evaluated a new generation LiPA (LiPA 2.0), which simultaneously detects sequences in the 5’ UTR and the HCV core region, to determine if it can overcome some of the deficiencies of the current assay.

Material and Methods: Several subgroups of samples were used in the evaluation and included:
   (i) Samples identified as genotypes 6c-l by core sequencing
   (ii) Samples with atypical LiPA banding patterns
   (iii) Samples with low viral load and giving suboptimal LiPA profiles
   (iv) Samples identified by LiPA as HCV genotype 1a/1b

Samples were tested by the conventional LiPA, the new generation LiPA (LiPA 2.0) and by core sequence analysis.

Results: LiPA 2.0 successfully identified genotypes 6c-l, which had previously been genotyped by the original LiPA as genotype 1. LiPA 2.0 was also able to successfully identify some genotypes which gave an atypical banding pattern on the conventional LiPA. LiPA 2.0 proved to be more sensitive compared to LiPA and therefore was able to genotype some samples with low viral load. Finally, as LiPA 2.0 utilizes the core region as well at 5’ UTR, it can discriminate 1a/1b genotype pattern on LiPA into the correct subtype.

Discussion: The combination of using the Roche COBAS AMPLICOR HCV test for PCR amplification and the current VERSANT HCV Line Probe Assay for genotyping offers great convenience. As the LiPA 2.0 assay requires an in-house reverse-transcriptase PCR and HCV RNA extraction before genotyping, it is unlikely that the LiPA 2.0 will replace the current assay. Nevertheless, our results presented here indicate that the new generation LiPA will be a useful supplementary assay for the genotyping of HCV.
COMPARISON OF THE ROCHE COBAS AMPLIPREP AUTOMATED INSTRUMENT WITH MANUAL EXTRACTION FOR HEPATITIS C VIRUS TESTING.

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Introduction: The Roche COBAS AmpliPrep (CAP) is an automated instrument for the extraction of nucleic acids from clinical specimens. Nucleic acids from the CAP can be used as templates for the Roche COBAS TaqMan 48 (CTM) and the Roche COBAS Amplicor (CAMP) amplification and detection systems. This study compared:

- CAP/CTM automated system for Hepatitis C virus (HCV) viral load measurement against manual extraction using the Roche COBAS Amplicor Monitor version 2.0 (CAM)/CAMP method.
- CAP/CAMP automated system for HCV qualitative testing against manual extraction using the HCV Amplicor version 2.0 qualitative test (HAQ)/CAMP method.

Methods: Thirty-three serum and/or plasma specimens received for routine HCV viral load testing were analysed using the CAM/CAMP method and the CAP/CTM method. Forty-five serum and/or plasma specimens received for routine HCV qualitative testing were analysed using the HAQ/CAMP method and the CAP/CAMP method. Manufacturer’s protocols were followed for all methods (Roche Molecular Diagnostics). All runs included kit controls and external quality assurance samples NRL 210 (quantitative) and 300 (qualitative).

Results: HCV viral load: The correlation coefficient (r) between methods was 0.8303. HCV genotypes 1, 3, 4 and 6 were represented. The mean interassay differences for genotypes 1, 3, 4 and 6 were 0.06, 0.06, -0.68 and –0.01 log_{10} IU/mL respectively, with HCV genotype 4 demonstrating the highest underestimation for the CAP/CTM system.

HCV qualitative: The r value between methods was 0.9364. Forty-four out of 45 samples showed concordant results. One sample from a known HCV RNA positive patient demonstrated a grey zone optical density (OD) result of 0.395 from the CAP/CAMP and a negative result (0.003) from the HAQ/CAMP.

Conclusion: The CAP/CTM combination for HCV viral load testing produced comparable results to the CAM/CAMP method. Slightly underestimated HCV genotype 4 values were determined, however only two HCV genotype 4 samples were tested. Further research is required using a larger sample number. Alternatively, assuming that viral load monitoring for patients occurs using the same testing method throughout treatment, this variation of viral load values can be minimised.

The CAP/CAMP automated method demonstrated good linear correlation with the HAQ/CAMP manual extraction method for HCV qualitative testing. The single discrepant result may be due to improved sensitivity and/or sampling on the CAP instrument.
HEPATITIS C VIRUS: CORRELATION BETWEEN QUALITATIVE PCR RESULTS AND ANTIBODY S/CO VALUES ON THE ABBOTT ARCHITECT AND AXSYM ASSAYS

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Symbion Laverty Pathology

**Aim:** To ascertain whether the hepatitis C antibody S/Co value was predictive of the PCR result.

**Method:** Data was collected over a 3 year period from all patient samples which had both HCV serology and PCR requested on the same day. The Abbott AxSYM system was used for the first 393 samples and the Abbott Architect system for the remaining 673 samples. The Roche Amplicor HCV assay was used to obtain the qualitative PCR result. All samples positive on the Abbott antibody test were also tested on the MUREX assay (Abbott) for HCV antibody. The Antibody result was reported as equivocal if the Architect or AxSYM result was reactive and the MUREX was negative.

**Results:** AxSYM: The PCR result was negative for all samples with negative or equivocal antibody results (n=108). For antibody positive, PCR negative samples (n=102), the S/Co ranged from 1.1 – 263, mean = 41, median = 20. For antibody positive, PCR Positive samples (n=183) the S/Co ranged from 22-212, mean and median = 120.

ARCHITECT: One sample with an equivocal and one sample with a negative antibody result were positive for HCV by PCR. These samples were from patients with acute hepatitis C infection. All other negative or equivocal antibody results were PCR negative (n= 115). For antibody positive, PCR negative samples (n=198) the S/Co ranged from 1.1 – 17, mean = 9. For antibody positive, PCR positive (n=358) the S/Co ranged from 5.7 – 18.8, mean = 13.

**Conclusion:** Except in rare instances of early acute hepatitis C infection, low AxSYM (< 22) and Architect (< 5) S/Co ratios are associated with negative PCR results. This information is a useful check when validating hepatitis C PCR results.
DETECTION OF AN HBsAg MUTANT S143L WITH THE BAYER/SIEMENS ADVIA CENTAUR

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Background: Mutations of the HBsAg viral genome can occur in the area of the “a” determinant loop from amino acid 125 to 146. Selection pressure from anti-HBs antibodies (vaccine, Ig, immunosuppression) can give rise to “escape” mutants. There is a variation amongst assays in their ability to detect these HBsAg mutants.

Materials and Methods: Two samples collected from the same patient 17 days apart were tested for HBsAg on the Bayer ADVIA Centaur. The initial sample was tested because of an ALT >2000 U/L. The second sample was collected as a follow up investigation of the initial HBsAg result. The initial HBsAg result was non-reactive and following discussion with the consulting gastroenterologist, an in-house investigation was initiated. A second sample was obtained and retesting of both samples for HBsAg on the Bayer ADVIA Centaur, the Abbott Architect and the bioMerieux VIDAS, HBeT and HBcIgM on the Centaur, and HBeAg and anti-HBe on the Architect was performed. Two different HBsAg Centaur reagent lot numbers were used. Both samples were referred to VIDRL for HBV viral load testing and the second sample was referred to VIDRL for HBV sequencing.

Results: When first tested, the initial sample was HBsAg non-reactive on the Bayer ADVIA Centaur and repeat tested as non-reactive. (Index 0.30 and <0.10 respectively with a cutoff of 1.00) but strongly reactive on the Abbott Architect and the bioMerieux VIDAS. The second sample was a low level reactive (Index 2.70) on the Bayer ADVIA Centaur. The initial sample was retrieved from frozen storage and retested on the Centaur and this second testing gave a low level reactive result (Index 1.36). Two different reagent lot numbers were used between the first and second tests. Both samples were strongly reactive for aHBcT and aHBcIgM on Centaur and HBeAg on Architect. HBV viral loads were also strongly positive and the HBsAg was sequenced as an S143L mutant.

Discussion: The ability of an assay to detect this type of mutation is determined by its format and which target epitopes are used for the assay antibodies. If this target is the immunodominant region of the “a” determinant loop from aa 140 to 146, then any alteration in these sequences may inhibit or prevent antibody binding and subsequent HBsAg detection. This HBsAg S143L mutant was not detectable with one reagent lot number on the Bayer ADVIA Centaur but was detected near the cutoff with a different reagent lot number. The ability of the assay to detect this type of HBsAg mutation appeared affected by reagent lot variability.
CHLAMYDIA NAAT QUALITY CONTROL AND NATIONAL EVALUATION

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*Chlamydia trachomatis* is the most common sexually transmitted infection in the United Kingdom with almost 110,000 cases diagnosed in 2005. *Chlamydia* in 16-24 year old females (1,359 cases in 100,000) and 20-24 year old males (1,070 cases in 100,000) indicates a diagnostic prevalence of greater than 1% of the UK population within these age groups. Figures like these have led to and given strength to successful programmes in the UK such as the National Chlamydia Screening Programme (NCSP) including the introduction of new technologies in selected laboratories for nucleic acid amplification tests (NAAT).

A key to the NCSP success has been attributable to its use of NAAT assays as the sole screening method. A national comparative evaluation was established using the most common NAAT assays, some results of which will be presented.

As part of continual improvement, the Health Protection Agency Quality Control Reagents Unit (part of the Evaluations and Standards Laboratory) has identified the need for the development and production of a Chlamydia NAAT QC programme to assist laboratories in the control of *Chlamydia trachomatis* screening and diagnosis using any NAAT assay. Distribution will be in the form of HPA Lenticules, giving users greater flexibility in storage, ease of use and will provide quality assurance of an assay from extraction to result.
ABSTRACTS
FRIDAY 26th OCTOBER 2007

HUMAN PAPILLOMA VIRUS: ROCHE AMPLICOR HPV TEST VERSUS DIGENE HYBRID CAPTURE 2 HPV DNA
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Background: Persistent Human Papillomavirus (HPV) infection is a necessary cause of cervical cancer, the second largest source of female cancer mortality worldwide. When program-based screening using conventional cytology (Pap smear) and HPV DNA testing are co-requested, the sensitivity for detecting high-grade cervical disease and cancer has been reported as greater than 99%. Research has shown that approximately 20% of women develop high-grade abnormalities (CIN 2/3+) within three years, if they are persistently high-risk HPV-positive when using the Pap and HPV DNA test combination. A single “double-negative” high-risk HPV DNA and Pap test combination provides better prognostic assurance against risk of future CIN 3 than three subsequent negative Pap smears in a low-risk population. HPV DNA testing is a more sensitive indicator for prevalent high-grade CIN than either conventional or liquid-based cytology (such as Thin Prep) alone; a combination of HPV DNA and Pap testing has more than a 99% sensitivity and negative predictive value for high-grade CIN. The Roche AMPLICOR® HPV Test (AMPLICOR), which is Polymerase Chain Reaction (PCR) based, offers an alternative to the Digene Hybrid Capture® 2 (hc2) test.

Materials and Methods: Routine requests for HPV DNA received by our laboratory during the period of the trial were processed in triplicate (n=122). Initial testing was performed using the Digene Hybrid Capture® 2 (hc2) High-Risk HPV Test. Subsequent testing was performed using the Roche AMPLICOR® HPV Test by two different operators, using two different DNA extraction protocols (Operator 2 used cell concentration prior to extraction) furnished by Roche Diagnostics Australia. Specimens deemed discordant between methods, namely, Digene hc2 vs Roche AMPLICOR and Roche AMPLICOR Operator 1 vs Roche AMPLICOR Operator 2 were further tested using the Roche LINEAR ARRAY HPV Genotyping Test. Those specimens for which histological testing had been performed concurrently or within one month of HPV DNA referral were also evaluated for concordance between the assays and histological findings (n=13). Several comparisons of the data from each of the assays and protocols were performed and included: Consensus results, defined as a minimum of 2 of the 3 assay (Digene/Roche AMPLICOR/Roche LINEAR ARRAY) results in agreement, %. “True Results” defined as the result interpretation matching what the assay is intended to detect, that is the 13 High-Risk HPV Genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) only.

Results: Concordance with consensus: Digene hc2 (84.30%); Roche AMPLICOR Operator 1 (92.56%) and Roche AMPLICOR Operator 2 (86.78%). Concordance with “True Results”: Digene hc2 (91.07%); Roche AMPLICOR Operator 1 (94.64%) and Roche AMPLICOR Operator 2 (91.96%). Comparison to Histological findings: Digene hc2 (75.00%); Roche AMPLICOR Operator 1 (83.33%) and Roche AMPLICOR Operator 2 (61.54%). One specimen was histologically confirmed to contain integrated HPV (by use of the p16 immunostain) but was Negative for HPV DNA by both operators performing the Roche AMPLICOR HPV Test (Digene hc2 was Positive). Suggestions from Roche Diagnostics Australia were that this specimen may have had a deletion of the HPV L1 region.

Discussion: The data obtained from these analyses suggest that the Roche AMPLICOR® HPV Test is a more sensitive method compared to the Digene Hybrid Capture® 2 (hc2) test. However, some loss of specificity was associated with the increased sensitivity of Operator 2’s cell concentration method. The rapid and sensitive Roche AMPLICOR® HPV Test has a reduced specimen requirement and may provide an alternative to the Digene Hybrid Capture® 2 (hc2) test. However, deletion of the HPV L1 region was suspected in our study, and research has found this to be a common occurrence in high-grade CIN lesions and cervical cancers and may lead to false negative results when using the Roche AMPLICOR® HPV Test.
The NRL’s SYPHILIS SEROLOGY EXTERNAL QUALITY ASSESSMENT SCHEME, 2006

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Introduction: In 2006, the National Serology Reference Laboratory, Australia (NRL) introduced an External Quality Assessment Scheme (EQAS) for Syphilis serology. The aims of the EQAS were to monitor the performance of the participants and the assays used, and also to facilitate the exchange of information between participants.

Materials and Methods: Panels of 10 coded samples were distributed to participants three times in 2006. Participants were instructed to treat all samples in the same way as routine specimens so as to reflect the day-to-day functions of the laboratory. The Syphilis Serology EQAS panels were distributed to blood transfusion services and diagnostic laboratories both in Australia and internationally. Results submitted by participants were either faxed to the NRL or entered into DigitalPT (an Internet-based application for the collection and reporting of EQAS results). Assay interpretations submitted by participants for each assay tested were compared with a reference result. Assay interpretations that did not agree with the reference results were identified as aberrant.

Results: Twenty-three participants from 12 countries sent results for the Syphilis Serology EQAS in 2006. Thirty-two different assays were used to test the Syphilis Serology EQAS panels: eight manual EIAs, one instrument-based EIA, eight RPR, ten TPHA/TPPA, two immunoblots and three miscellaneous methods. A total of 1,152 assay interpretations were submitted over the three panels. Thirty-one results (2.69%) were reported to the participating laboratories as aberrant.

Conclusion: The NRL’s Syphilis Serology EQAS provides participants with a means of assuring the integrity of their testing processes within each laboratory. Problems identified in the EQAS were reported back to the participant to enable prompt corrective action.
AN EPIDEMIC INCREASE IN INFECTIOUS SYPHILIS (IS) IN SOUTH EASTERN SYDNEY 2001 – 2007.

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Background. Following decades of decline, overall rates of syphilis infections have been rising throughout the developed world since 2000 and especially in homosexually active men (HAM). An increase in the rates of infectious syphilis in south eastern Sydney between 2000 and 2004 was reported last year* and this increase appears to have continued into 2007 as noted in this laboratory.

This laboratory provides a diagnostic service to NSW public hospitals as well as correctional health and a number of sexual health clinics in south eastern Sydney where there is a high concentration of HAM amongst clientele.

In syphilis the treponemal specific antibody tests (FTA, TPPA & EIA) may remain positive for many years after infection whereas the reagin antibody levels (detected by RPR & VDRL) fall with advancing disease and treatment and eventually become negative with time. Therefore the presence of reagin antibody suggests recent infection and the rates of patients with positive reagin antibody tests should provide an estimate of rates of infectious (primary and secondary) syphilis (IS) rather than old or treated infections.

Materials and Methods:
The results of serological tests for syphilis (STS) performed on 91,370 samples in this laboratory between 2001 and August 2007 were available for analysis. Over this period the patient mix had not changed significantly with the exception of a small increase in the number of low risk tissue donors that were screened since 2004.

Infectious syphilis (IS) for each year was defined as the presence of an RPR titre >1 together with at least one positive treponemal test. For each year the positive lists were de-duplicated on the basis of date of birth and clinic number or names.

For the purpose of comparison the rates of IS for each year represent the rate of patients / specimens tested.

Results: Between 2001 and 2004 the rates of IS rose from 0.46% to 1.60% and has continued to increase to 1.95 % for the first 8 months of 2007. Between 2001 and 2007 the proportion of males in the IS group has remained unchanged and the average age was the same (41 years).

Discussion;
Although this study has not analysed the clinical histories, or sexual practices of the IS group the increase noted between 2000 and 2004 is in keeping with that shown by public health surveillance in a major part of the population serviced by this laboratory. These data suggest that this increase in the rates of infectious syphilis in HAM in south eastern Sydney that occurred between 2000 and 2004 has continued into 2007.

Using an RPR titre of >1 may have resulted in some patients with latent syphilis being included however this should have been consistent each year for the purpose of comparison. The de-duplication would have eliminated double counting of patients previously diagnosed and retested for monitoring response to treatment.

Because the denominator used in these calculations was specimens tested and the numerator was patients the rates are certainly an underestimate. Never the less the current rate (at least 1.95 %) is alarming in the context of reflecting the results of unsafe sexual practices.
ABSTRACTS
FRIDAY 26th OCTOBER 2007

A NOVEL PCR TECHNOLOGY DESIGNED FOR DETECTION OF TARGETS WITH A HIGH LEVEL OF GENETIC HETEROGENEITY

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"Quantitative measurement of HIV levels in peripheral blood has greatly contributed to the understanding of the pathogenesis of HIV infection and has been shown to be an essential parameter in prognosis and management of HIV infected individuals. Several different technologies have been utilized for measurement of HIV-1 RNA including reverse transcription-polymerase chain reaction (RT-PCR), isothermal nucleic acid sequence-based amplification and branched DNA signal amplification. The most recent technological advance in viral load quantification has been the application of real-time RT-PCR. Real-time PCR offers several advantages over traditional end-point analysis including increased dynamic range, faster time to results, and reduced risk of carry-over contamination. Presently, the most commonly used probe format in real-time PCR tends to be TaqMan probes.

Highly polymorphic targets, such as viruses, present a significant challenge to the design of assays that are required to provide reliable detection and quantitation. Nucleotide mismatches within the probe binding site have the potential to reduce hybridization efficiency resulting in underquantification or lack of detection. HIV-1 is characterized by a high level of genetic diversity, rendering it an especially challenging target for technologies that rely on hybridisation of specific primers and/or probes. Recombination has substantially increased the overall sequence complexity of the virus and given rise to at least 19 circulating recombinant forms (CRF) and numerous unique recombinant forms (URF). Abbott have developed a new class of probe for use in real-time PCR assays designed to detect and/or quantify genetically polymorphic targets. These partially double-stranded linear probes exhibit increased tolerance to nucleotide mismatches and are well suited for detection and quantification of targets with a high level of genetic heterogeneity. We describe some of the research that has gone into the development of this novel technology and why it warrants consideration for a wide range of real-time PCR applications and offers a robust alternative for detection of targets with a high level of genetic heterogeneity."

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DEVELOPMENT OF NEW DENGUE DIAGNOSTICS
Greg Hafner1 PhD, Vice President of Research and Development, Panbio

The prevalence of dengue has increased considerably over recent decades. In terms of morbidity, mortality and economic costs, dengue is the most important mosquito-borne viral disease in the world. 40% of the world’s population lives in areas that place them at high risk of dengue infection.

As the number of dengue infections increases, the need for efficient treatment decisions becomes more important.

Early diagnosis of dengue allows earlier implementation of supportive therapy and monitoring. This reduces the risk of complications such as dengue haemorrhagic fever or dengue shock syndrome, especially in countries where dengue is endemic.

The pan-E Dengue Early ELISA is designed for the qualitative detection of the dengue NS1 antigen in serum. It enables the diagnosis of a dengue infection from the first day a patient presents with symptoms.

Detection of dengue NS1 antigen by ELISA is a valuable procedure, as it allows detection of infection prior to seroconversion. NS1 antigen can be detected in serum from Day 1 after onset of fever and up to Day 9. This compares to IgM antibodies that are not detectable until Days 3-5.
ABSTRACTS
FRIDAY 26th OCTOBER 2007

COMPARISON OF THE NEW PANBIO EBV ONE-STEP ASSAY (PAN-E EBV VCA M-ONE) RUN BOTH MANUALLY AND ON THE TRITURUS® EIA ANALYSER.

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Introduction
Epstein-Barr virus (EBV) is a member of the family Herpesviridae and is the causative agent of infectious mononucleosis (IM) and chronic active EBV infection. EBV is also known to be associated with two human cancers, Burkitt’s lymphoma and nasopharyngeal carcinoma. It is estimated that greater than 90% of adults are seropositive for EBV. Primary EBV infection is usually asymptomatic but is the cause of infectious mononucleosis in juveniles and young adults. Viral capsid antigen (VCA) is the primary marker used for diagnosis of EBV infection. IgM can be present from 2-4 months and may persist in less than 10% cases for 6-8 months. Panbio manufactures an EBV VCA IgM ELISA (Cat. No.: E-EBV01M) and has been established in the Australian market for a considerable period. When run manually in pathology laboratories this assay performs well, however with the increase in laboratory automation the assay is frequently adapted to EIA auto analysers. Independent studies have indicated variability when this assay is performed by the Triturus® EIA analyser which can affect the performance. The root cause of this issue was isolated to the heating mechanism within the automated instrument. Panbio has produced a new one-step EBV VCA IgM ELISA (pan-E EBV VCA M-One; Cat. No.: E-EBV03M). This assay differs from the Company’s traditional EBV VCA IgM ELISA (E-EBV01M) as it incorporates a one-step sample dilution preparation and is run at room temperature making it more amenable to automation adaptation.

Here we present a comparison of the new one-step EBV VCA IgM ELISA (E-EBV03M) run both manually and on the Triturus® EIA analyser.

Methods
A population of 173 frozen retrospective clinical specimens submitted for routine EBV serology screening were used in the study. The specimens were characterised by the traditional Panbio EBV VCA IgM ELISA (E-EBV01M) performed manually. Within the population 93 specimens were characterised as serologically negative for VCA IgM and 80 specimens were characterised as serologically positive. These specimens were then tested with the new Panbio one-step EBV VCA IgM ELISA (E-EBV03M) both manually and on the Triturus® EIA analyser following the manufacturer’s recommendations. The ELISA results were compared to determine the sensitivity, specificity, and agreement of the assay relative to the characterisation.

Results
The clinical specimens were tested in the new Panbio one-step EBV VCA IgM ELISA (E-EBV03M) using manual operation of the assay. The new assay demonstrated a serological sensitivity of 100.0% and serological specificity of 97.9% when compared to the traditional Panbio EBV VCA IgM ELISA (E-EBV01M). This correlated to a relative agreement of 98.8%. When the same assay was run on the Triturus® EIA analyser it achieved a serological sensitivity of 98.8% and serological specificity of 100.0% when compared to the traditional Panbio EBV VCA IgM ELISA (E-EBV01M). This correlated to a relative agreement of 99.4%. Furthermore, the reduction in assay signals sometimes apparent with the traditional assay (E-EBV01M) on the Triturus® EIA analyser, were not evident. The relative agreement of the new Panbio pan-E EBV VCA M-One assay (E-EBV03M) when run both manually and on the Triturus® EIA analyser was 98.3%.
Conclusions
The new Panbio one-step EBV VCA IgM ELISA (E-EBV03M) is easy to use, has comparable performance to manual operation of the traditional Panbio EBV VCA IgM ELISA (E-EBV01M) and has the flexibility to be used in both manual operation and on the Triturus® EIA analyser.
POSITIVE BARMAH FOREST SEROLOGY - WHAT DOES IT REALLY MEAN?
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INTRODUCTION
Barmah Forest virus is an alphavirus. Barmah Forest disease is notifiable in New South Wales and endemic in the Mid North Coast. There have been increasing notifications of Barmah Forest to NSW Health in recent years with Barmah Forest categorised as an emerging infectious disease demanding a greater understanding. The notification case definition of Barmah Forest in NSW includes detection of Barmah Forest specific IgM and this test is the basis of a considerable proportion of all notified cases. The impact of false positive serology results on an understanding of Barmah Forest epidemiology is not known.

METHODS
All Barmah Forest notifications from the Mid North Coast to the Hunter New England Population Health unit between 1 January and 30 June 2007 were systematically reviewed. All Barmah Forest positive IgM specimens were referred to CIDMLS virology reference laboratory for confirmatory testing including a neutralizing antibody test. All cases were interviewed to ascertain the onset date and nature of their symptoms and serological results were interpreted in the context of this information.

RESULTS
Forty Barmah Forest cases were notified to the Population Health unit from the Mid North Coast for this period and preliminary analysis of available results indicate that the majority of positives could not be confirmed by neutralizing antibody testing. Synthesizing test results with clinical history suggested that most of the remaining available notified cases were not the result of recent infection.

DISCUSSION
The availability of relatively affordable rapid serological tests has simplified diagnosis in rural areas. However results must be interpreted within the performance characteristics of specific tests and should not be considered in isolation from the patient’s clinical presentation. The significance of our results for patient diagnosis, public health action and gaining a better understanding of Barmah Forest epidemiology will be discussed.
AN EBV EVALUATION: COMPARISON OF THE DIASORIN LIASON AND PANBIO ELISA

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Introduction: EBV testing remains one of few larger volume assays performed manually in our laboratory currently on the PanBio assay. In order to improve workflow an automated option was investigated: the DiaSorin Liason (distributed by Immuno) automated, random access, chemiluminescent (CMIA) system.

Method: Sequential routine patient samples on which EBV serology had been requested (n=368) were obtained and screened on both the PanBio EIA and DiaSorin Liason CMIA system for EBV Viral capsid antigen (VCA) IgG and IgM. This sample population included 14 patients acutely infected with EBV. A targeted panel of patients seropositive for either Parvovirus or Cytomegalovirus (CMV) IgM, were also screened on both systems to review potential ‘cross-reactivity’. In order to resolve results which were discrepant between the assays, patient histories, age, previous EBV results (if available), current haematology and biochemistry results, and the presence of Epstein-Barr virus nuclear antigen (EBNA) were considered.

Results: For the 368 routine samples, concordance between the two systems was 93.5% for VCA IgG, and 91.1% for VCA IgM. Based on analysis of discrepant results against probable ‘true’ status; Sensitivity for VCA IgG was 98.1% PanBio and 99.2% DiaSorin Liason. Specificity for VCA IgG was 95.7% PanBio and 98.9% DiaSorin Liason. Sensitivity for VCA IgM was 85.7% PanBio and 92.9% DiaSorin Liason. Specificity for VCA IgM was 97.7% PanBio and 97.1% DiaSorin Liason. Of the potentially cross-reactive samples (CMV; n=15, Parvovirus; n=7), 4 (26.7%) CMV IgM and 5 (71.4%) Parvovirus IgM samples were falsely positive on the Liason EBV IgM system, whereas all samples were negative for EBV IgM on the Panbio assay.

Conclusion: In our laboratory DiaSorin Liason EBV VCA testing displayed both acceptable performance for the routine screening of Epstein-Barr virus and correlation with the current EIA system in use. The assay had > 90% sensitivity and specificity for the detection of EBV VCA IgG and IgM on requests from our routine patient population. However it would be recommended that laboratories determine co-request rates for EBV, CMV and Parvovirus in order to estimate the impact of potential cross-reactivity. DiaSorin Liason would enable increased throughput and improved workflow in a large pathology practice.
ABSTRACTS
FRIDAY 26th OCTOBER 2007

EVALUATION OF THE DIESSE CHORUS AND BIOMERIEUX MINIVIDAS VARICELLA IgG ASSAYS

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Introduction: Varicella zoster virus (VZV) IgG serology testing is commonly requested in diagnostic pathology and is especially important for staff screening and potential exposure during pregnancy. Micrtitre enzyme immunoassay (EIA) based tests, commonly used in Australia, are usually batch tested resulting in turnaround times being less than desirable. In addition, testing can also take several hours to produce results.

The Diesse Chorus (distributed by Laboratory Diagnostics) and the bioMerieux miniVIDAS both provide automated qualitative assays for the detection of IgG antibodies to VZV in serum. Testing samples by both the Chorus and the miniVIDAS can be performed easily on a daily basis. Total time required for test preparation and assay duration is significantly less than that of microtitre EIA with minimal calibration and controls required due to the use of separate, self-contained, disposable testing strips.

Methods: A total of 143 serum samples that had been previously tested with the Dade Behring Enzygnost VZV IgG EIA assay on the BIO-RAD EVOLIS automated platform were selected to test with the Diesse Chorus and the bioMerieux miniVIDAS VZV IgG assays. Of the 143 specimens, 37 were part of a staff screening program, 64 were routine screens for either antenatal or reproductive investigations, 5 were from patients that had either symptoms or exposure and 37 were from post chemotherapy, renal patient screens or a variety of other conditions. An overall consensus result was determined for each of the specimens, allowing the sensitivity and specificity to be compared for each of the three assays. A specimen was categorised as positive or negative by achieving the same result from all three assays. Any specimen results that did not correspond to the majority were repeated by the discrepant method and by the Becton Dickinson VZV scan total antibody latex agglutination test. Consensus for these specimens was achieved if three out of the four methods provided the same result. Precision testing was also performed on both the Diesse Chorus and the bioMerieux miniVIDAS by repeat testing of a patient serum with known low reactivity. A commercially available quality control sample was also tested with a number of runs for both methods.

Results: The sensitivity of the Chorus VZV IgG (n=82) was estimated to be 100%, with a confidence interval (CI) of 94-100% and the specificity (n=56) estimated to be 100% (CI: 92-100%).

The sensitivity of the bioMerieux miniVIDAS (n=76) was estimated to be 93% (CI: 84-97%) and the specificity (n=56) estimated to be 100% (CI: 92-100%).

The sensitivity of the Dade Behring Evolis (n=82) was estimated to be 100% (CI: 94-100%) and the specificity (n=54) estimated to be 96.4% (CI: 87-99%).

The precision measured as a coefficient of variation (CV) for the Diesse Chorus VZV IgG assay was calculated using a low patient sample (n=13) to be 10.3%.

The precision measured as a coefficient of variation (CV) for the miniVIDAS VZV IgG immunoassays was calculated using a low patient sample (n=15) to be 8.7%.

Discussion: The results of the study show that both the Chorus and miniVIDAS each had sensitivities greater than 92% and a precision which is acceptable for routine diagnostic VZV IgG screening. Both the Chorus and miniVIDAS offer the advantages of automation and improved turn around times. In addition, the Chorus also includes VZV IgM in the test menu.
RUBELLA IGG SEROLOGY – EVALUATION OF THE ABBOTT ARCHITECT ASSAY

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Introduction: Rubella IgG screening is recommended for all antenatal patients to determine immunity levels and more recently there has been an increase in pre-work screening for employees of childcare centres and schools and for medical and nursing students. Rubella IgG testing is performed throughout Australia and New Zealand, using reagents from a number of manufacturers utilising a variety of methods. Recent RCPA surveys indicate 16 different assays are in use, with the majority using automated analysers (eg AxSYM).

At Sullivan Nicolaides Pathology (SNP), a private pathology company performing testing throughout Queensland, the Northern Territory and northern New South Wales, Rubella IgG testing is performed utilising an in-house EIA. This assay has been standardised against the WHO Proposed 3rd International Standard for Anti-Rubella Serum Human. A review of workflow for antenatal screening coincided with the recent release of the Abbott Architect Rubella IgG assay. Serology for HIV, HBV, HCV and syphilis are already performed on the Architect, therefore with the addition of Rubella to this system, all antenatal serology could be performed on one system with improved turn around time.

Objective: To evaluate the Abbott Architect assay as an alternative to the SNP in-house EIA.

Method: The Abbott Architect Rubella IgG Assay was evaluated utilising a panel of 110 samples from 109 patients with Rubella IgG levels ranging from 0->100 IU/mL on the SNP assay. The in-house assay has an immune cut-off of 10 IU/mL while the Architect has a grayzone of 5.0-9.9 IU/mL and positive cut-off of 10 IU/mL.

Results: The Abbott Architect assay demonstrated 84% sensitivity and 96% specificity when Architect equivocal (grayzone) values were regarded as non-immune. The correlation factor for regression analysis was 0.94, indicating that Architect values were slightly lower than the SNP assay. Evaluations by Abbott indicated that the Architect values were also lower than the AxSYM values with a similar regression value. If results <10 IU/mL are classified as non-immune (NCCLS document 1997), 95 out of 110 specimens (86%) were concordant between the Architect and in-house assay. Fourteen of the fifteen discordant specimens were non-immune on the Architect assay and immune on the in-house assay. Discrepant analysis suggests that the majority of these patients are likely to have low levels of rubella IgG antibody following vaccination rather than no detectable Rubella IgG antibody.

Conclusion: Although levels on the Architect assay overall were slightly lower than those on the in-house assay, the Architect assay correlation with the in-house assay showed adequate sensitivity and high specificity. The Architect assay may underestimate the level of antibody, however a recommendation to boost levels by vaccination of these patients is unlikely to be harmful and indeed may protect against rubella re-infection. The assay was determined to be a suitable replacement for the SNP assay and has been implemented for routine use.
THE EVALUATION OF A COMMERCIAL EIA FOR THE DETECTION OF LEGIONELLA PNEUMOPHILA ANTIBODIES.

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INTRODUCTION: *Legionella pneumophila* serogroup 1 has been implicated in the majority of legionella outbreaks worldwide, it is most commonly found in water storage systems where the water is stored at 25-42°C. *L. pneumophila* SG1 was the causative agent of Australia’s largest outbreak of legionnaires’ disease at the Melbourne Aquarium in 2000 resulting in 125 confirmed cases including 4 deaths. Currently at VIDRL an in-house indirect immunofluorescence (IFA) assay is used for detecting antibodies to legionella. The IFA assay includes *L. pneumophila* serogroups 1–6 as well as *L. micdadei* and *L. longbeachae*. We undertook the evaluation of a commercial enzyme immunoassay (EIA) (Vircell, Granada, Spain) for the detection of antibodies to *L. pneumophila* serogroups 1-6 IgG and IgM.

METHODS: We tested 72 serum samples, which included 21 paired bleeds showing a seroconversion or a significant rise in titre (≥ four fold rise) in our in-house IFA assay as well as 30 samples that were collected as case controls following the Aquarium outbreak. In addition another 8 patient samples were tested which had confirmed a diagnosis of Legionella by PCR. All samples were stored at -20°C. These samples were tested using the Vircell EIA IgG & IgM kit and the results compared to those obtained with our in-house IFA.

RESULTS: Of the 21 paired bleeds tested by the in-house IFA 14 were *L. pneumophila* infections and 7 were *L. longbeachae* infections. The Vircell EIA showed a seroconversion in 15 pairs and positive stationary levels for one other pair. The five paired samples that remained negative were *L. longbeachae* infections.

The 30 case control samples which all had titres <64 by our in-house IFA returned 1 equivocal index value in the Vircell EIA and 29 negative index values.

When we look at the additional 8 single bleed samples, 3 of these were positive for *L. pneumophila* and 5 for *L. longbeachae* by PCR testing. The Vircell EIA detected 2/3 *L. pneumophila* samples and 0/5 *L. longbeachae* samples. The in-house IFA detected 3/3 *L. pneumophila* and 5/5 *L. longbeachae* samples.

CONCLUSION: The Vircell EIA assay is a user-friendly assay to perform and requires a small amount of serum (5 μl). The assay was not able to detect any of the *L. longbeachae* infections. In our study we found the sensitivity for detecting legionella infections compared to our in-house IFA to be 66.7%. When we exclude *L. longbeachae* samples the sensitivity increased to 94.1%. The specificity was 97.7%.

The EIA assay could be easily automated and the subjectivity associated with reading IFA’s would be avoided. The assay would be a useful screening test in an outbreak situation.
THE ROLE OF SPECIMEN TYPE IN RESPIRATORY VIRUS DETECTION.
May ML, Then H, Byrne S, McCarthy K, Cherian S, Robson J

Sullivan Nicolaides Pathology is a large private pathology practice in Queensland providing diagnostic services to predominantly community-based practitioners across Queensland and to other Sonic Healthcare laboratories nationally. Since 2006, a multiplex PCR panel of respiratory viruses has been used to diagnose viral respiratory tract infection on respiratory tract samples with rapid antigen testing for RSV and Influenza A & B used to provide more rapid turn-around times when necessary. August 2007 saw a significant epidemic of influenza A, surpassing the total number of PCR positives in 2006 by 180% (746 vs 396). In comparison to PCR, rapid antigen testing (Directigen; Becton-Dickinson) showed a sensitivity and specificity of 69.3% and 98.8%. Nasopharyngeal aspirates are usually considered to provide better quality samples than swabs of the upper airways. Analysis performed on sample type tested, swabs (nasopharyngeal, throat, or a combination) yielded significantly more positive results than nasopharyngeal aspirates (NPA) (28.8% vs 20.6%; chi-squared; p<0.0001), with nasopharyngeal or a combination of nasopharyngeal and throat swabs having the highest yield (33% and 29.7% respectively). Lower respiratory tract samples had significantly lower rates of positivity (13.9%; p<0.001). In 2006, with a lower pre-test probability in the population, combined nasopharyngeal and throat swabs again provided significantly more positive results than NPA (17.2% vs 6.2%), and lower respiratory tract specimens performed poorly. These results have supported a shift away from nasopharyngeal aspirate collection, to swabs alone, for suspected viral respiratory tract infection.
A COMPARISON OF THREE COMMERCIAL ENZYME IMMUNOASSAYS WITH PARTICLE AGGLUTINATION FOR DIAGNOSIS OF ACUTE MYCOPLASMA PNEUMONIAE INFECTION

May ML¹, Ross P, Hunt S, Pratt K, Riley J, Hurst C, McCarthy K, Cherian S, Robson J.

A prospective trial of three commercially available enzyme immunoassays (EIA)s for Mycoplasma pneumoniae IgG, IgA and IgM antibodies was undertaken, in comparison to Particle Agglutination (Serodia Myco II). One hundred and twenty three samples from 109 patients were tested, including 16 paired seroconversions (as defined by PA) and 15 clinically defined cases. Twenty samples from adult blood donors and sixteen children were used as healthy controls. In addition, a further 42 samples from other infections were tested to determine the specificity of the assays. Results showed good performance for all three assays tested, with overall agreement with the reference method being 71.5% (Vital Diagnostics), 81.3% (AniLabsystems) and 78.9% (seroMP). All EIAs enabled better differentiation of past from acute infection than PA, with a similar ability to detect acute infection. IgA EIAs marginally improved detection of acute infection, but also increased non-specific results to a small degree. Overall SeroMP was found to give the best performance.

Introduction

Despite the increased development of in-house PCR techniques, serological diagnosis of Mycoplasma pneumoniae infections remains the current standard method of diagnosis in most centres. Many features of Mycoplasma pneumoniae infection make interpretation of serological results in isolation challenging. Firstly, symptoms of acute infection are not specific and can be similar to many other respiratory pathogens.¹ Secondly, numerous patient variables affect the performance of mycoplasma serological assays, including age, duration of symptoms at testing, and a history of prior infection.²,³,⁴ Thirdly, M. pneumoniae produces a broad immunoglobulin response against a range of different antigens, which increases the difficulty in the design of specific assays.⁵

Comparative studies suggest that EIA techniques have several advantages over traditional serological methods, in that they are more automated, less subjective in interpretation, and the identification of separate immunological classes assists with more accurate diagnosis of acute infection as opposed to residual antibody from past exposure.²,⁶

Sullivan Nicolaides Pathology is a large private pathology company providing a centralised serological diagnostic service to predominantly community medical services across Queensland, Northern New South Wales, Northern territory and Western Australia. The aim of this study was to evaluate the diagnostic performance of four different commercially available Mycoplasma EIA kits for IgM, IgG and where possible IgA in comparison to our currently used method, particle agglutination.

Methods

Patient selection

Patients who had M. pneumoniae serology requested by the referring practitioner and had positive results (PA ≥ 160) were reviewed prospectively from November 2006 to March 2007.

Description of Patient Samples

Positive: =45

10 patients with paired samples & documented seroconversion by PA.
1 patient was stored samples from documented seroconversions

4 patients with paired samples where the first sample was retrospective.

15 patients with a high clinical probability of mycoplasma infection ("Clinical Positives").

Negatives =36

20 adult controls, 16 child controls

Other illnesses =33

Documented seroconversion to other respiratory and systemic pathogens: 9 Epstein-barr virus, 6 Bordetella pertussis, 3 parvovirus, 7 influenza A virus, 3 influenza B virus, 3 Legionella pneumophila, and 2 Streptococcus pyogenes (GAS) infections.

Persistent positives =9

9 patients with persisting positive PA titres.

Definitions

Criteria for a serological diagnosis of acute mycoplasma infection on unpaired samples were decided on the basis of information provided the manufacturers (SMP-EIA, ANL-EIA) and literature review.\textsuperscript{5,6,9} These included: 1) a positive IgM or IgA on any single sample, with or without a positive IgG, or 2) an equivocal IgM or IgA on any single sample with a positive IgG. A positive or equivocal IgG alone was not considered sufficient to diagnose acute infection. On paired samples, a 1.5 fold rise in a positive IgG or a change of any of IgA, IgM or IgG from negative to positive was considered consistent with acute infection or re-infection.

The specificity of the EIA kits was assessed using sera from An additional 9 patients who had documented sustained high PA titres to mycoplasma over months to years were also included.