BLOOD/TISSUE SCREENING

THE VALUE OF VOLUNTARY QUALITY ASSESSMENT SCHEME.
Prof. Zhu Yong Ming, President, Shanghai (Red Cross) Blood Centre

BLOOD/TISSUE SCREENING

NUCLEIC ACID TESTING AT THE AUSTRALIAN RED CROSS BLOOD SERVICE

Brown S
Australian Red Cross Blood Service, Adelaide, South Australia

Introduction: On the 7th June 2000 the Blood Service began screening blood donors for HIV-1 and HCV by NAT with a Novartis Diagnostics HIV-1/HCV multiplexed assay on a Novartis semi-automated system.
On the 5th July 2010, the Blood Service implemented the fully automated Novartis Procleix TIGRIS System with an assay that now also included HBV detection. The introduction of the HBV NAT not only allowed the detection of acute cases of HBV prior to HBsAg reactivity, but it also provided the opportunity to detect a class of chronic infections with undetectable HBsAg known as Occult Hepatitis B Infections (OBI). The use of these two assays and testing platforms has allowed a comparison of system performance and NAT yield (NAT Pos/Serology Neg) donation detection.

Methods: In the 10 years from 07-Jun-2000 until 04-Jul-2010, the Blood Service screened 11.28 million blood donations for HIV-1 and HCV RNA by a combination of IDT (Individual Donor Testing) and PDT (Pooled Donor Testing) using the Novartis Procleix HIV-1/HCV Assay on the Novartis semi-automated SAS and eSAS Systems. From 05-Jul-2010 to 19-Jun-2011 the Blood Service had screened 1.28 million blood donors by IDT using the Novartis Procleix Ultrio Assay on the fully automated Procleix TIGRIS System. The five testing sites in Perth, Adelaide, Melbourne, Sydney and Brisbane collected weekly testing data on system performance and reactive donations which was then collated centrally.

Results: The three main system performance measures monitored were Initial Reactive Only (IRO) Rate which is a measure of false reactive tests, Invalid Test (IT) Rate which measures failed tests on otherwise valid assay runs, and the Failed Run (FR) Rate which measures assay runs with no valid test results. The two Novartis NAT systems produced system performance and NAT Yield detection figures as indicated below.

System Performance	IRO Rate	IT Rate	FR rate
Procleix HIV-1/HCV Assay:	0.13%	0.22%	1.77%
Procleix Ultrio Assay:	0.05%	0.17%	3.32%

NAT Yield Donations (NAT Pos/Serol Neg):	HIV-1	HCV	HBV
Procleix HIV-1/HCV Assay:	3	24	n/a
Procleix Ultrio Assay:	1	1	28

Conclusions: The two test systems did display some differences in operational performance with the HIV-1/HCV assay on the semi-automated system achieving a more favourable Failed Run Rate, while the Ultrio assay on the TIGRIS system produced a more favourable Initial Reactive Only Rate. The inclusion of HBV detection in the Ultrio assay enabled the detection of 28 HBV positive donations that would have been missed by routine serology alone. Despite the higher Failed Run Rate of the TIGRIS system compared to the previous platform, the improvement to the IRO Rate, the ability to adopt a 100% IDT strategy for NAT, and the advantages of HBV DNA screening has shown the introduction of TIGRIS to be of significant benefit to the Australian Red Cross Blood Service.

BLOOD/TISSUE SCREENING

ANALYSIS OF REACTIVITY ON THE ABBOTT PRISM CHEMILUMINESCENT IMMUNOASSAYS DEMONSTRATES BOTH INSTRUMENT-RELATED AND BIOLOGICAL FALSE REACTIVITY AND HIGHLIGHTS PERFORMANCE DIFFERENCES BETWEEN ASSAYS.

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Introduction and Objectives. The Australian Red Cross Blood Service (Blood Service) screens all donations for hepatitis B surface antigen (HBsAg), and antibodies to human immunodeficiency virus types 1 & 2 (anti-HIV-1/2), hepatitis C virus (anti-HCV) and human T-cell lymphotropic virus types I & II (anti-HTLV-I/II) using the Abbott PRISM HBsAg, HIV O Plus, HCV and HTLV-I/HTLV-II chemiluminescent immunoassays (ChLIAs), respectively. In addition, during the study period all donations were screened by nucleic acid testing (NAT) for HIV-1 RNA and HCV RNA on the PROCLEIX HIV-1/HCV TMA assay (Gen-Probe/Novartis Diagnostics). In this study we analysed the reactive results for all four PRISM ChLIAs to (i) estimate the proportion of reactive results that were instrument-related and the proportion due to biological false (non-specific) reactivity, and (ii) compare the performance characteristics of the four PRISM ChLIAs with respect to instrument-related and biological false reactivity.

Methods. According to the Blood Service viral testing algorithms, donor samples that test initially reactive (IR) on the PRISM ChLIAs are retested in duplicate. For this study, if both duplicate retests were non-reactive, the PRISM result was referred to as initially reactive only (IRO) and no further testing was performed if the sample was also non-reactive on the NAT assay; if one or both retests were reactive, the PRISM result was referred to as repeatedly reactive (RR) and supplemental/confirmatory testing was performed to determine a final status. Samples that tested repeatedly reactive on any of the PRISM ChLIAs but negative by appropriate supplemental/confirmatory testing, and non-reactive by the NAT assay, were referred to as biological false reactive (BFR). All PRISM ChLIA IRO and BFR donor screening test results (both initial and duplicate repeat results) for the period from 1 July 2007 to 30 June 2010 were included in the study; results for samples that were reactive on supplemental/confirmatory testing (indeterminate or positive) were excluded. In addition, from the study period, a group of randomly selected non-reactive routine screening test results for each PRISM ChLIA were used as a comparative control group. Test results were expressed as sample to cutoff ratios (s/co).

Results. (i) The proportion of IR results that were RR after retesting was significantly less ($p < 0.05$) for the HBsAg ChLIA (31.9%) compared to the three antibody ChLIAs, HIV O Plus (58.1%), HCV (60.1%) and HTLV-1/HTLV-2 (43.5%). Of the three antibody ChLIAs, the proportion of IR results that retested as RR was significantly less ($p < 0.05$) for the HTLV-I/HTLV-II ChLIA compared to the HIV O Plus and HCV assays.
(ii) For the PRISM HBsAg ChLIA, the s/co ratio distribution of the non-reactive retest results for IRO samples was similar to that for the randomly selected control group of non-reactive routine screening test results. However, for the HIV O Plus, HCV and HTLV-I/HTLV-II ChLIAs, the s/co ratio distributions of the non-reactive retest results were significantly higher ($p < 0.05$) compared to the respective randomly selected control groups of non-reactive routine screening test results.
(iii) For each PRISM ChLIA, IR results for IRO samples were compared to those for the BFR samples. For the HBsAg ChLIA, the s/co ratio distributions of IR results was not significantly different between the IRO and the BFR sample groups; in contrast on the HIV O Plus, HCV and HTLV-I/HTLV-II assays the IR s/co ratio distribution of the IRO samples was significantly lower ($p < 0.05$) than that for the BFR samples.

Discussion and Conclusion. The results of this study indicate: (i) Samples that tested IR on the PRISM ChLIAs but were non-reactive on repeat testing with relatively low s/co ratios (similar to the non-reactive control group) most likely represent instrument-related false reactivity; IR samples that retested as non-reactive but with relatively high (close to 1.0) s/co ratios, along with those that tested RR, most likely represent biological false reactivity (ie non-specific cross-reactivity). (ii) For the HBsAg ChLIA, most IR results appeared to be instrument-related while the IR results on the HIV O Plus, HCV and HTLV-I/HTLV-II ChLIAs represented a combination of instrument-related and biological false reactivity. (iii) Over 50% of samples with IR results on the HBsAg and HTLV-I/HTLV-II ChLIAs and approximately 40% on the HIV O Plus and HCV were non-reactive when retested. Therefore, the s/co ratio of IR results is not a specific indicator of the retest result and samples that test IR should be retested in duplicate. Only samples that test RR should be subject to confirmatory testing. (iv) Fully automated immunoassay systems do not completely eliminate instrument-related reactive results.

BLOOD/TISSUE SCREENING

NRL and ARCBS: Working together to ensure safe blood supply in Australia

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Since the development of the first antibody test for human immunodeficiency virus (HIV) in the mid-1980's, NRL has supported the Australian Red Cross Blood Service (ARCBS) in the provision of a safe blood supply for Australia. Over the years, the range of infectious disease analytes supported by NRL has expanded beyond HIV to include hepatitis B virus, hepatitis C virus, human T-cell lymphotrophic virus, cytomegalovirus and *Treponema pallidum*, as well as an increase in the types of support services offered. These support services include:

- Assistance in the commissioning of new testing platforms through the provision of bespoke validation panels designed to verify fitness for purpose
- External Quality Assessment Schemes (EQAS) for Serology and NAT that challenge the entire testing system, including a multimarker NAT scheme designed for multiplex tests such as Novartis PROCLEIX ULTRIO
- Quality Control (QC) Programmes designed to identify changes in performance of assays and instruments on a daily basis via continuous monitoring through EDCNet
- Ensuring screening of blood-borne infectious diseases is cost effective and efficacious through the Specificity Monitoring Programme
- Tertiary reference testing on difficult to diagnose samples

NRL also conducts the pre-market evaluations of *in vitro* diagnostic devices, which includes those used by ARCBS.

The NRL-ARCBS partnership has grown due to good communication and by identifying future ARCBS needs through an evidence-based approach to ensure provision of a safe blood supply for Australia.

EMERGING DISEASES - 1

WEST NILE VIRUS: THE EUROPEAN PERSPECTIVE.

Dr. Vittorio Sambri, University of Bologna, Italy

EMERGING DISEASES - 1

EMERGING DISEASES AND THE BLOOD SUPPLY – KEEPING THE ENEMY AT BAY.

Tony Kellar, Australian Red Cross Blood Service, Perth

INFLUENZA A VIRUS IDENTIFICATION AND TYPING USING SEQUENOM'S MASSARRAY IPLEX SNP GENOTYPING PLATFORM.

Jennifer Nakos[1], Cheryl Bletchly[1], David Whiley[2], Ralf Moser[3], Michael D. Nissen[1,2], Theo P. Sloots[1,2], Graeme R Nimmo[1]

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Introduction

The Sequenom MassARRAY iPLEX single nucleotide polymorphism (SNP) genotyping platform uses matrix assisted laser desorption/ionisation time of flight mass spectrometry (MALDI-TOF MS) coupled with single-base extension PCR for multiplex SNP detection.

Methods

In this study, two mulitplex influenza A (INF-A) assays designed using Assay Designer v4.0 software were evaluated. Both multiplex assays contained a redundant number of assays targeting the haemaglutinin, neuramidase and matrix genes to facilitate parallel viral typing and characterisation of particular INF-A types/strains, including the Tamiflu resistance H275Y mutation. PCR and biochemical reactions were performed according to manufacturer's instructions. Data were acquired on a Compact MALDI-TOF mass spectrometer and analysis was carried out using Typer 4 analyser (Sequenom). On the background of the 2009/2010 pandemic flu outbreak, 286 INF-A positive clinical respiratory samples were tested. These samples had previously been characterised and subtyped using standard real-time PCR and DNA sequencing methods (pandemic swH1N1 positive n= 218, seasonal H3N2 = 68).

Results

In total, 204/218 pandemic swH1N1 INFA (4 of which contained the H275Y mutation), and 65/68 seasonal H3N2 were accurately detected and subtyped by MassARRAY iPLEX. All 17 clinical samples showing negative results on MassARRAY had very high cycle thresholds in excess of 38 cycles in standard INFA real-time PCR thus representing low viral loads. Interestingly, all H3N2 positive samples were determined to be from one geographical location in Queensland.

Conclusion

In summary, the Sequenom MassARRAY iPLEX offers a robust and high throughput system for characterisation of influenza A viruses. In addition, the MassARRAY platform can readily be used to detect changes in INFA subtype epidemiology.

EMERGING AND RE-EMERGING FLAVIVIRUS DISEASE IN AUSTRALIA.

Dr. Mario Lobigs, John Curtin School of Medical Research

EMERGING DISEASES - 2

HENDRA VIRUS INFECTIONS: DIAGNOSIS AND CONTROL.
Dr. Deborah Middleton, CSIRO

EMERGING DISEASES - 2

COMPARISON OF RESPIRATORY VIRUSES DETECTION METHODS

Anna-Maria Costa, Presenting of behalf of Siemens Healthcare Diagnostics

Department of Microbiology and Infectious Diseases, Royal Children's Hospital and Royal Women's Hospital, Parkville, Victoria, Australia

The sensitivity and specificity of respiratory virus detection have improved considerably with the advent of improved extraction methods and nucleic acid amplification tests (NAATs). We evaluated the performance characteristics of the Siemens VERSANT Sample Preparation 1.0 Reagent Kit compared to the Roche MagNA Pure TNAI High Performance Kit. The nucleic acid extracted from both platforms was tested using multiplex real-time PCR. The real-time PCR assay simultaneously detects eight RNA viruses: Influenza virus A and B, human metapneumovirus, parainfluenza virus 1, 2, 3 and respiratory syncytial virus A and B. Both methods were comparable in sensitivity and specificity indicating the suitability for extraction of nucleic acid for respiratory virus detection. An advantage of the VERSANT system is that it offers primary vial loading. Overall, both methods provide a platform capable of efficient extraction, useful for routine diagnosis.

RAPID DIRECT MOLECULAR DETECTION OF PATHOGENS FROM CLINICAL SAMPLES WITHOUT NUCLEIC ACID EXTRACTION.

Dr. Michelle Tabb, Southern Cross Diagnostics

AUTOMATED EXTRACTION OF MICROBIAL DNA FROM WHOLE BLOOD FOR THE UNIVERSAL PCR DETECTION OF PATHOGENS

Van Asten M, Diagnostic Technology

Timely diagnosis of the etiological agents of sepsis is demanded in order to adjust therapy to specific antibiotic treatment. Blood culture is the gold standard of diagnosis taking at least 2 days until identification of the pathogen. Molecular Mass Spectrometry, universal rRNA gene PCR and sequencing are considered a promising ways to the rapid identification of bacteria and fungi in blood samples. Among other parameters contained automation is desirable to avoid contamination. The presentation presents results obtained with a new automated system, *Universal Microbe Detection Arrow*™. It comprises a pretreatment of the blood for the removal of human DNA and the extraction and isolation of pathogen DNA.

NEW TECHNOLOGIES IN MOLECULAR DIAGNOSTICS

Fabrice Magnino
Integrated Sciences Pty Ltd.

Molecular Diagnostics, once considered a relatively scary domain that only experts would touch, is nowadays a fast growing discipline readily accepted into routine laboratory testing

While the current molecular diagnostic techniques used in our laboratories have mostly evolved from technologies developed in research labs years before, the lapse of time between the emergence of new technologies and their implementation in dedicated molecular diagnostic laboratories is even shorter. While PCR and qPCR took about 10 years to reach most of the routine labs, the recently developed technologies such as Next Generation Sequencing (NGS) are likely to take only 3 or 4 years.

This presentation will focus on the latest developments in this field, from the rebirth of PCR after qPCR, the mass spectrometry reorientation to diagnostics and the development of NGS for use in routine labs. I will discuss some of these new approaches and review their promises and challenges.

INCORPORATING HPV GENOTYPING INTO CERVICAL CANCER SCREENING: RESULTS OF THE ATHENA TRIAL

Thomas Wright, Presenting on behalf of Roche Diagnostics

IMPLEMENTATION OF A RAPID COMMERCIAL REAL TIME PCR ASSAY FOR MRSA TESTING USING MOLECULAR AND GENERALIST STAFF IN A CLINICAL MICROBIOLOGY LABORATORY

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Real time PCR technology, where amplification and fluorescent probe detection of target occurs in the same tube, has greatly improved assay turn around times. This has been utilised effectively, for hospital infection control in the detection of Methicillin Resistant *Staphylococcus aureus* (MRSA). Numerous studies have shown that surveillance for and isolation of carriers of MRSA can significantly reduce the incidence of nosocomial infections by these organisms and produce cost-savings. They have also demonstrated comparable sensitivity and specificity to culture methods. To maximise the benefits of these assays, they must be performed seven days a week. This often stretches the molecular resources of a department. The answer to this may be the use of generalists to provide the extra staffing coverage required. This study reports the use of a commercial real time PCR test, the BD GeneOhm™ MRSA assay, in a routine microbiology laboratory. Performance of the test was evaluated and showed good sensitivity and specificity to culture and most importantly was able to detect 57 of 60 epidemic and community MRSA clones, which accounted for 99% of the MRSA isolated in Western Australia. The staff performing the assays were either full time molecular staff or generalists that had no formal molecular training. All staff were trained by an expert user spending three consecutive days with them for continuity. The training was evaluated by assessing failure rates and unresolved rates for both molecular trained and generalist staff. The results between the two groups of staff showed little difference, (run failures 0.20%, 0.24% and unresolved rates 1.41%, 1.52% respectively). With the emergence of real time PCR systems that require minimal molecular expertise, do we need molecular trained staff running these assays? In our experience both molecular trained and generalist staff can adequately perform this testing, but importantly training and monitoring of performance should be undertaken by competent molecular users.

GENERAL SEROLOGY

DIAGNOSIS OF CO-INFECTIONS OF SYPHILIS, HSV AND HIV.
Ginger Weeden, Bio-Rad Laboratories

Co-infections of syphilis, HSV and HIV are not uncommon. The acquisition of one disease actually facilitates the transmission of these diseases by making the body more susceptible to the invasion of these viruses or organisms. So in looking for a primary infection, laboratories would be wise to look for multiple, related disease states as well.

Performing these tests in a timely manner not only increases the efficiency of the laboratory and of the service to the patient, but reduces the risk of mishandling or misplacing the specimen. Learn about methods that make a quicker turn-around-time possible and better patient care achievable.

NOROVIRUS RAPID TESTING PRODUCT
Mark Conte, Alere

GENERAL SEROLOGY

COMPARISON OF THE SIEMENS IMMULITE SYPHILIS SCREEN ASSAY AND THE MUREX ICE SYPHILIS EIA.

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2. Siemens Healthcare Diagnostics

Introduction and Objective: Healthscope Victoria receives approximately 75000 requests for syphilis testing a year. Further automation will improve turn around times for this test. We selected the Immulite 2000 Syphilis Screen as Healthscope Pathology has the Siemens Healthcare ADVIA WorkCell in all its main laboratories. In this study we compared the performance of the ICE Syphilis Murex Diagnostics and the Immulite 2000 Syphilis Screen (Siemens Healthcare Diagnostics).

Materials and Methods: Three hundred (300) samples were tested using the Murex ICE Syphilis EIA (Murex Biotech) and the Siemens Healthcare the Immulite 2000 Syphilis Screen assay. Negative samples were from a low prevalence population while 100 reactive samples were from a high-risk population. Reactive samples from each assay were further tested by RPR (RPR Reditest, Biokit), TPPA (Serodia TP.PA, Fujirebio Inc) and FTA-abs (FTA-ABS Test System MarDx Diagnostics).

Results: For the 200 negative samples a 100% concordance was found between the Murex ICE Syphilis EIA and Immulite 2000 Syphilis Screen.

Seventy-three samples of the 86 reactive samples, were reactive on both the Murex ICE syphilis EIA and Immulite 2000 Syphilis Screen. Thirteen samples were reactive on the ICE Syphilis EIA only. A clinical review as well as previous serological results for these samples found that 5 were from HIV positive patients, 6 from pregnant patients and 2 patients had no relevant clinical history, apart from being in the high-risk population. It was concluded that of these 13 patients the six pregnant patients were most likely false positive results on the Murex ICE syphilis EIA. The other 7 patients had a history of previous reactive Treponemal serology results and or infection.

A review of the negative sample data found that 99% of negative samples returned an S/CO of <0.400. Four of the five HIV patients had a S/CO between 0.4-0.9.

	Specificity	Sensitivity	Inter-assay CV
Immulite 2000 Syphilis Screen	100%	93.5%	10.5%
Murex ICE Syphilis	97.2%	100 %	10.2%

Conclusion: The Immulite 2000 Syphilis Screen proved to have good specificity and sensitivity in this study. However the HIV positive cohort may have weaker immune antibody responses to infection. It was decided that for this group of patients additional tests would be conducted whenever the S/CO values were 0.400 or above to exclude Treponemal infection. These additional tests will be the TPPA and FTA.

Healthscope Pathology decided to implement the Immulite 2000 Syphilis Screen despite the additional testing required for this group of patients. The random access ability of the Immulite 2000 and integration into the Siemens Healthcare ADVIA WorkCell system now allows Syphilis serology to be performed 20 hours per day, six days per week. This has provided significant workflow benefits and has also improved the turn around time for this test.

This presentation will focus on the latest developments in this field, from the rebirth of PCR after qPCR, the mass spectrometry reorientation to diagnostics and the development of NGS for use in routine labs. I will discuss some of these new approaches and review their promises and challenges.

GENERAL SEROLOGY

A UNIQUE ALGORITHM FOR EBV TESTING IN A HIGH VOLUME LABORATORY USING THE THE BIO-RAD BIOPLEX 2200.

Steve Martin, Laverty Pathology

FOR A COPY OF THIS ABSTRACT PLEASE EMAIL: Steven.Martin@laverty.com.au

GENERAL SEROLOGY

LACK OF CORRELATION BETWEEN RESULTS REPORTED BY ABBOTT ARCHITECT AND ROCHE ELECSYS RUBELLA IgG ASSAYS

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NRL, Melbourne, Australia

Introduction: Immunity to rubella virus is determined by the detection of anti-rubella IgG. For many years, commercial rubella IgG assays have been calibrated against an international standard and have reported results in international units per milliliter (IU/mL). However, studies have demonstrated a lack of correlation between the results reported by some assays. In this study rubella IgG results of samples tested in both of the recently released Roche Elecsys and Abbott Architect rubella IgG immunoassays were compared.

Aim:

- To compare quantitative results of samples tested in both the Abbott Architect and Roche Elecsys rubella IgG immunoassays; and
- To compare their qualitative results with a status determined by a haemagglutination inhibition assay (Siemens Health Care Diagnostics, Marburg Germany; HAI) and Western blot (recomBlot, Mikrogen Diagnostik, Neuried, Germany; WB).

Methods: A total of 141 samples were tested in both the Abbott Architect and Roche Elecsys rubella IgG immunoassays and the results were reported in IU/mL. The quantitative results were compared using linear regression analysis. To determine the status, each of the samples was screened using the HAI. Samples with a titre of greater than 1:8 in HAI were confirmed with the WB. The qualitative results of each sample were then compared with the status.

Results: There was poor correlation between the quantitative test results reported by the Abbott Architect and Roche Elecsys rubella IgG immunoassays, with a coefficient of determination of $R^2 = 0.23$. Of the 141 samples, 24 had a negative status and 117 had a positive status. Of the 24 negative samples, the Roche Elecsys immunoassay reported 11 as negative (range: 0 – 6 IU/mL) and 13 as positive (range: 11 – 214 IU/mL). The Abbott Architect immunoassay reported 13 as negative (range: 0 – 5 IU/mL), five equivocal (range: 5 – 10 IU/mL) and six as positive (11 – 22 IU/mL). Of the 117 positive samples, the Roche Elecsys immunoassay reported two as negative (4 and 9 IU/mL) and the Abbott Architect reported one as negative (4 IU/mL) and seven as equivocal (range: 6 – 9 IU/mL).

Conclusion: The findings of the current study showed that the correlation of results reported by the Abbott Architect and Roche Elecsys rubella IgG assays was poor. However approximately the same number of false positive and false negative qualitative results were reported by each assay. Further studies to compare the levels of rubella IgG reported as IU/mL by each assay with the WHO international standard are warranted.

GENERAL SEROLOGY

INVESTIGATION OF LOW-LEVEL RUBELLA IgG RESULTS REPORTED BY FIVE COMMERCIAL IMMUNOASSAYS

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NRL, Melbourne, Australia

Introduction: Interpretation of low-level rubella IgG test results is complicated by a lack of correlation between commercially available rubella IgG immunoassays. Previous comparisons of test results reported by various assays testing the same samples highlight the differences between assays but do not aid in determining which assay has reported the correct result. A haemagglutination inhibition (HAI) and a Western blot (WB) assay were used to assign a positive or negative status to samples having low-level rubella IgG reactivity on five commercial rubella IgG enzyme immunoassays (EIAs). The status for each sample was then compared with the qualitative result reported by each EIA.

Aim: To investigate the level of correlation between the qualitative test results reported by five rubella IgG EIAs with a status derived from HAI and WB testing.

Method: A total of 100 routine clinical samples that had a rubella IgG test result of 40 international units per millilitre (IU/mL) or less on each of four EIAs (Abbott Architect, Abbott AxSYM, bio Merieux VIDAS, and Roche Elecsys) were collected. Eighty-three samples were collected for the Ortho Vitros. To determine a status, each of the 483 samples was screened with HAI (Siemens Heathcare Diagnostics, Marburg, Germany). Samples with a HAI titre of greater than 1:8 were confirmed by WB (recomBlot Rubella IgG, Mikrogen Diagnostik, Neuried, Germany). The qualitative results of the EIAs were compared with the status of each sample.

Results: Of the samples tested in each EIA, 23, 9, 6, 19 and 44 in the Architect, AxSYM, VIDAS, Vitros and Elecsys respectively were found to have a negative status. The qualitative results of the EIAs compared with the status determined by HAI and WB testing are tabulated below. Each EIA reported samples with a positive status as negative and all but the bio Merieux VIDAS reported samples with a negative status as positive. The highest false positive results in IU/mL for each assay were 22, 36, 22 and 35 for the Architect, AxSYM, Vitros and Elecsys respectively.

Rubella IgG status	Negative			Positive		
Assay	Assay test result			Assay test result		
	Negative	Equivocal	Positive	Negative	Equivocal	Positive
Abbott Architect	13	5	5	1	7	69
Abbott AxSYM	6	2	1	2	5	84
bioMerieux VIDAS	6	0	0	6	18	70
Ortho Vitros	17	0	2	11	5	48
Roche Elecsys	29	Not applicable	15	5	Not applicable	51

Conclusion: Although some rubella IgG EIAs specify a range within which an equivocal is reported, the cut-off for determining immune and non-immune status is 10 IU/mL in each EIA. This study indicates that none of the five EIAs included in the study could accurately determine the status of a sample using a single value of 10 IU/mL. It is suggested that laboratories set a “grey zone” when reporting low-level rubella IgG results. Based on the results of this study, the “grey zone” should be from 10 to 20 or 10 to 35 IU/mL, depending upon the assay.

QUALITY ASSURANCE

CAPACITY BUILDING AND PREDICTORS OF SUCCESS FOR HIV DRUG RESISTANCE TESTING IN THE ASIA PACIFIC REGION AND AFRICA

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Objective: TREAT Asia Quality Assessment Scheme (TAQAS) was developed as an external quality assessment programme for laboratories in the Asia Pacific region and Africa that perform HIV drug resistance (HIVDR) genotyping, through expert education and training programmes. We evaluate programme performance and examine factors associated with high quality HIVDR testing.

Methods: Between 12/2005 and 6/2010 two TAQAS test events per annum (five samples per test event per panel) were distributed to participating laboratories for HIVDR testing using their standard protocols (ViroSeq, TruGene or "homebrew"). Panel samples included HIV-positive plasma or diluted culture-amplified virus (wet format; seven panels), or electropherograms (dry format; two panels). Testing, assembly and interpretation methods were documented. Sequences were submitted to NRL, Australia, aligned against panel-specific consensus sequences and analysed in comparison with those of peer participants. Performance was evaluated according to agreement with the consensus sequence, detection of drug resistance mutations (DRMs) and detection of mixtures of wildtype and resistant virus (mixtures). Participants were encouraged to review and compare results, and amend and improve their testing methods, as appropriate. High quality performance was defined as detection of ≥95% of DRMs in the consensus sequence. Factors associated with a laboratory performance were examined using mixed-effect logistic regression models (using SAS and Stata).

Results: Participants (N=22) in 13 countries received 45 samples (30 subtype B; 15 non-B subtypes) in nine panels. Eight laboratories reported results for all 9 panels, 2 for 8 panels, 4 for 6 panels, 3 for 5 panels, 3 for 4 panels, 1 for 3 panels, 1 for 2 panels and 1 for 1 panel. Detection of DRMs averaged 93-98% in wet panels and up to 100% in dry panels. Factors associated with performance quality included sample complexity factors [number of DRMs ($p<0.001$) and number of DRMs as mixtures ($p<0.001$)] and laboratory performance factors [detection of mixtures ($p<0.001$) and sequence agreement with the consensus sequence ($p<0.001$)]. The sample format (wet or dry), subtype [B or non-B], type of assay used [commercial or "homebrew"], sequencing location, assembly and interpretation methods were not associated with performance. Six participants detected <80% of DRMs in early panels, with subsequent improvement in detection of DRMs.

Conclusion: TAQAS achieved high-quality HIVDR genotyping in Asian, Pacific and African laboratories participating in this collaborative network. Sample complexity and ability to detect mixtures were found to influence performance quality. Laboratories conducting drug resistance genotyping are encouraged to participate in external quality assessment programmes.

QUALITY ASSURANCE

SIX MONTH REVIEW OF THE NEW AND IMPROVED RCPA SEROLOGY QAP SCORING SYSTEM.

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Introduction
The RCPA Serology Quality Assurance Program (SQAP) provides Quality Assurance to over 800 laboratories in 34 countries worldwide for Infectious Diseases using both Serological and Molecular Techniques. Accredited as a Provider of Proficiency Programs with international recognition, the SQAP uses the consensus as the assigned value to which participant performance is evaluated. Since the introduction of scoring for qualitative results, the SQAP has become increasingly concerned that some participants with aberrant results are being overlooked by the 80% consensus with a 95% confidence limit scoring method. As of the 2011 survey year, the SQAP has trialled a new and improved scoring system eliminating the 95% confidence limit. The SQAP assessed this new scoring method against the old, to measure its efficacy in alerting participants to potential performance issues.
Methods
Data obtained for the first six months of surveys for 2011 using the new scoring method, was rescored using the old scoring method with the 95% confidence limits.
Results
The first half of the 2011 survey year results were evaluated using both the new and old scoring systems. Differences in Participant Scores were noted, highlighting potential performance issues to participants in a timely manner.
Conclusions
Up until 2011, it has been noted on occasions that participants have returned an aberrant result, but due to a lack of consensus (80%, CI 95%) the result was not scored on the Participant Assessment Report. With the new and improved scoring system, these participants are being alerted to potential performance issues early.

QUALITY ASSURANCE

OCCUPATIONAL HEALTH AND SAFETY – CASE STUDY REVIEW

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Background: The RCPA Serology Quality Assurance Program (SQAP) 'Specimen Free' Module comprises various scenarios and case studies, with the objective of encouraging discussion amongst scientific and technical staff without testing actual specimens. The 'Specimen Free' module may be used as a tool to assist with training and continuing education. Responses from participating laboratories are collated into a report together with a discussion, so that participants are able to review and compare their responses against other participating laboratories. The topics are varied from diagnostic related case study to other issues which are often encountered in a routine diagnostic pathology laboratory, such as Occupational Health and Safety.
Method: The Occupational Health and Safety case study used a scenario where an employee cut her finger on broken glassware. The glassware was soaking in bleach and the employee had seen that it was broken prior touching it. It was late in the afternoon; she bandaged her finger and went home for the day.
Participants were asked to provide responses to three questions. Firstly, what the employee should have done in this situation. Secondly, what the laboratory manager should do next on the next day after noticing and querying the injury. Finally, what the manager and employee should have done to reduce the risk of re-occurrence.
Results: Twenty-four participants returned responses.
For the first question, participants identified six different actions which the employee should have taken, including 'Apply first aid',' Report incident to Supervisor/Manager', 'Safe disposal of the broken glassware', 'Complete accident/incident form', 'Seek medical attention' and 'Report the hazard'.
For the second question, participants suggested actions which the manager should have taken after noticing the injury on the next day, the responses include 'Fill incident form', 'Medical assessment', 'Investigate Incident/Risk assessment', 'Educate OH&S issues' and 'Ensure appropriate disposal of broken flask' .
For the third question, participants provided answers from both employee's and manager's point of view, employee's responsibilities including, 'Use appropriate PPE', 'Inform supervisor immediately', 'Read/understand safety policies' and 'Dispose broken glassware/other'. Participants suggested that the manager's responsibilities as an employer include, 'Educate staff regarding OH&S Issues', 'Establish vaccination/immunization status', 'Ensure workplace safety regularly and training', 'Ensure protocol for disposal of broken glassware' and 'Check and eliminate hazards'.
Discussion/Conclusion: From the responses submitted, it is clear that participants understand the procedure of incident reporting, actions to be taken and responsibilities of employees and employer to prevent incidents and accidents occurring in the laboratory.
Most participants identified the appropriate actions to be taken for each question posed. Furthermore, most also agreed on what actions should be prioritised. The responses were satisfactory and demonstrated a comprehensive understanding of responsibilities in Occupational Health and Safety.

QUALITY ASSURANCE

UTILITY OF SWAB SAMPLES IN EXTERNAL QUALITY ASSESSMENT SCHEMES FOR *C. TRACHOMATIS/N. GONORRHOEAE* AND HERPES SIMPLEX VIRUS TYPES 1&2 TESTING

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Introduction: NRL provides Quality Assurance (QA) programmes to laboratories that test for infectious diseases by serological and/or nucleic acid testing (NAT) techniques. One of the components of QA is External Quality Assessment Schemes (EQAS). NRL EQAS are designed to be treated in the same manner as clinical samples. However, for the herpes simplex virus types 1 and 2 (HSV-1/2) and the *Chlamydia trachomatis* and *Neisseria gonorrhoeae* (CTNG) programmes, the current EQAS format does not reflect routinely encountered swab samples.

Objective: To examine the feasibility of design and transport of dry swab panels for CT/NG and HSV-1/2 NAT EQAS.

Methods: Log_{10}-serial dilutions of *C. trachomatis* elementary bodies (CT), *N. gonorrhoeae* bacteria (NG) and cell culture supernatants of herpes simplex virus types 1 & 2 (HSV-1 and HSV-2) were seeded onto sterile polyester-tipped plastic swabs and allowed to air dry inside a class two biological safety cabinet. Twenty replicates at each concentration for each analyte were then stored at room temperature (RT) and at -20°C. Five replicates of each concentration, for each analyte, from each storage temperature were tested at 2, 7, 13 and 21 weeks post manufacture. Swabs were extracted using a semi-automated extraction method and amplified by established qPCR assays. The results were analysed to examine the stability of samples at different storage temperatures and determine the lowest detectable concentration of input DNA for each analyte.

Results: The HSV-1/2 swabs were stable, with no significant difference in quantification cycle (Cq) values between samples stored at -20°C and samples stored at RT. For HSV-1 samples with the highest input concentration, the mean difference in Cq between the two storage temperatures was 0.3 cycles. For the swabs containing the highest HSV-2 input concentration, the mean difference in Cq values between the two storage temperatures was 0.5 cycles.

For the CT swabs, the mean differences in Cq values between the two storage temperatures for each of the three different input concentrations were approximately one cycle (approximately 0.5 log_{10} DNA copies/mL), with samples that were stored at -20°C having a lower Cq than those stored at RT. While results were reportable for all swabs seeded with the highest input concentration, 32/40 samples with the lowest input concentration were not detectable using the single copy target assay. These observed differences would have negligible effects in samples containing higher DNA concentrations and assays that utilise multicopy targets.

Swabs seeded with NG showed similar results to those containing CT, where the mean difference in Cq values between the two storage temperatures was also approximately one cycle. Swabs stored at -20°C had a lower Cq than those stored at RT. NG DNA was amplified from all input concentrations, most likely due to the multicopy target used for NG detection.

Conclusion: It is feasible to supply EQAS samples for CTNG and HSV-1/2 on dry swabs. One advantage of the dry swab is that samples do not require specialised storage conditions as the DNA are stable at RT and at -20°C. Swab panels must be designed with care. When choosing the concentration of input DNA, the ability to detect DNA will be compounded by assay design in addition to the swabs' storage conditions.

QUALITY ASSURANCE

USING CYTO-CHEX TUBES TO PREPARE CD4 IQC SAMPLES IN VIETNAM

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Background

Using internal quality control (IQC) in CD4 count test is an essential step to assure accuracy and precision of the test. Up to now, IQC samples for CD4 count testing are not widely used in Vietnam due to its expensive cost. Thus, we proposed to prepare CD4 IQC samples by using stabilized blood from commercial reagents with low cost.

Cyto-Chex BCT tubes from Streck, Inc can be used for the preservation of whole blood samples for immunophenotyping in flow cytometry for 14 days under the conditions established by the manufacturer. However, that period is too short to use the stabilized blood as IQC samples. We attempted to increase the stability time from the blood cells by using Cyto-chex tubes and changing the temperature condition of storage.

Methods

Blood was drawn from donors in Cyto-chex tube and stored at room temperature and 4^0C. The samples were be tested for Lympho T CD4 percentages and absolute count using flow cytometry technique on FacsCount and FacsCalibur instruments.

Mean, coefficient of variation (CV), standard deviation (SD) were calculated and presented by using standard deviation index (SDI) in order to monitor the integrity of the samples during storage time.

Results:

The stabilized blood in Cyto-Chex tubes were tested for CD4 expression on lymphocytes everyday by FacsCalibur machine. After 18 days (14 runs), data results showed a mean of 35% (34-38%), CV of 2.7 and SD of 0.9. However, the cell morphology and CD4 expression showed some degradation and or samples can't be analyzed for the next run.

In order to increase the life-span for the sample, we kept them in 4^oC and monitor CD4 percentages, absolute count (CD4 AC) and cell morphology. The results have shown that the stabilized blood from Cyto-chex tubes can maintain integrity of CD4 expression and cell morphology after 40 days (10 runs). No result is out of ± 2 SDI.

Value	Pasteur Institute	
	CD4 AC	CD4%
N	10	
Mean	728	35
CV	30.8	0.3
SD	4.2	0.9

To confirm data and continue surveying the variation of CD4 absolute count test (CD4 AC), these samples were transferred to 2 other centers, Pham Ngoc Thach Hospital ('PNT') (1) and Tropical disease Hospital ('TD Hospital') (2), which use single platform – FacsCalibur and Facscount for CD4 count test. During 40 days, these two centers performed CD4 count tests and the results are presented as follows:

For the first lot:

Value	PNT Hospital		TD Hospital	
	CD4 AC	CD4%	CD4 AC	CD4%
N	12		13	
Mean	719	36.0	798	36.0
CV	61.4	1.0	17.1	1.3
SD	8.5	2.9	2.1	3.4

For the second lot:

Value	PNT Hospital		TD Hospital	
	CD4 AC	CD4%	CD4 AC	CD4%
N	16		22	
Mean	742	35	780	36
CV	59.5	1.0	20.2	1.2
SD	8.0	3.0	2.6	3.5

Both of lots show good results in the stabilization of whole blood during 40 days.

Discussion

From the data, we conclude that whole blood drawn in Cyto-chex tube kept at 4^0C can be preserved up to 40 days for CD4 count test.

In this research, because of limitation on time and donors, we have not compared mean values of two conditions of storage. Therefore, we suggest that the future research should focus on whether storage condition at 4^0C affects the percentage of CD4 expression.

After storing at 4^0C, stabilized blood did not show many alterations on CD4 expression or cell morphology and the life-span of the samples was increased to 40 days, which can serve the purpose as CD4 IQC samples..

For the next step, we plan to increase the number of donors, include HIV-infected individuals with low CD4 absolute count and also to evaluate the stabilization of the samples during transportation conditions.

QUALITY ASSURANCE

EXPLORING QUALITY ASSURANCE QUESTIONS WITH PROFICIENCY TESTING DATA FOR THE LEPTOSPIROSIS MICROSCOPIC AGGLUTINATION TEST

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Introduction

The microscopic agglutination test (MAT), used globally for diagnosis of leptospirosis, measures serum antibodies in human beings and animals. The MAT uses live cultures of leptospiral bacteria as test antigens. Sera to be tested are serially diluted, and antigen culture is added to each dilution. After incubation, agglutination is observed, and the result is expressed as a titre. A testing laboratory typically maintains a panel of antigen cultures of several serovars, because leptospiral bacteria show great antigenic diversity. Titres reported by participants in the International Proficiency Testing Scheme for the Leptospirosis MAT, testing the same sample with cultures of the same serovar, can vary widely. The causes of this variation are not well understood, but probably include the sources of antigen cultures, how and for how long the cultures have been maintained, and minor variations in how the test is performed. It is of interest to know whether some laboratories systematically report higher titres than other laboratories, irrespective of the serovars with which they test. Results from two rounds of the International Proficiency Testing Scheme have been used to explore this question.

Methods

(a) Homologous titres to each of four serovars were analysed for each of two proficiency testing rounds. The serovars were Australis, Canicola, Icterohaemorrhagiae and Tarassovi in Round 7 (2008), and Canicola, Grippotyphosa, Icterohaemorrhagiae and Sejroe in Round 8 (2009). Available titres, transformed as logs to the base 10, were compared for each of six possible pairs of serovars for each round, e.g. titres to serovar Australis were compared with titres to serovar Canicola from Round 7. Between 26 and 63 pairs of titres where available for each of the twelve comparisons. Within each comparison, titres were classed as High or Low, according to whether they were higher or lower than the mean log of the titres for the serovar concerned. It was postulated that, if there were a general laboratory effect on titres, there would be more agreement between titres in each comparison (High-High and Low-Low) than disagreement (High-Low and Low-High).
(b) Forty-one laboratories were identified that showed at least one High-High agreement in a Round 7 comparison, and that also participated in Round 8. The other results from these laboratories were analysed, to determine whether a tendency to report high titres in Round 7 was reflected in a similar trend in Round 8.

Results

(a) The ratio of agreement to disagreement ranged from 1.00 to 3.15 across the twelve individual comparisons. The overall ratios were 1.47 for Round 7, 1.88 for Round 8, and 1.65 combining both. In all there were 326 agreements and 197 disagreements. This was significantly different from a 1:1 relationship, expected if there were no general laboratory influence on titres (Chi-square = 31.8, $p < 0.001$).
(b) Of 41 laboratories that showed at least one High-High agreement in Round 7, 25 (61%) also showed at least one High-High agreement in Round 8. In contrast, this group showed only 8 Low-Low agreements in Round 7, and 6 Low-Low agreements in Round 8 (14/82 in combination, 17%).

Discussion

These results demonstrate that, overall, some laboratories report higher MAT titres than others, and that a tendency to report higher titres in one proficiency testing round predicts a similar tendency in a subsequent round. This illustrates the desirability of exploring the influence of a range of different factors on MAT titres. More controlled experiments to investigate such factors are needed, but proficiency testing results may be a significant supplementary source of required information.

QUALITY ASSURANCE

THE IMPLEMENTATION OF QUALITY MANAGEMENT SYSTEM IN CLINICAL LABORATORIES IN VIET NAM: ACHIEVEMENTS AND CHALLENGES

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Laboratory quality management system was recently introduced into clinical laboratories in Viet Nam. Several trainings have been conducted for both technicians and managers of the local facilities. The training packages were developed by in-country CDC laboratory team in collaboration with local experts from different governmental and non-governmental organization such as National Institute of Hygiene and Epidemic, Pasteur Institute Ho Chi Minh City, Viet Nam Administration of Medical Service, LIFE-GAP, Family Health International, SCMS and Clinton Health Access Initiative. The trainings were based on the “12 Quality Systems Essentials” model developed by the Clinical and Laboratory Standards Institute (CLSI) and some models from the Strengthening Laboratory Management towards Accreditation (SLMTA) package. Trainings were divided into two parts of which six quality essentials are included in each part. During the interval of the trainings, quality assurance and technical assistance were provided to the local laboratories in order to support the improvement projects that trainees proposed to apply into their facilities.

After the first trainings, it is showed that all trainees were aware the important of the laboratory quality management system. Many of them have passed their knowledge obtained through the trainings to their colleagues and higher managers. As a result, quality management system has been applied into the facilities base on the resources and innovation of each laboratory. Most of the laboratory started with the non-expenditure activities including the work space reorganization, arrangement of essential equipments, and establishment of organizational charts, duty roster and standard operation procedure (SOP) development. Several laboratories went further in implementing budget-required activities such as equipment maintenance and calibration, facility renovation for better work flow, participating in external quality assessment (EQA) programs, sending personnel to the training courses and so forth. With national and international resources, some laboratories have applied the international standard quality management system (e.g., ISO 15189) into their facilities. However, despite of extensive support and technical assistance from governmental and non-governmental organizations, some laboratories are still facing with many challenges. These laboratories are normally located at provincial/district level or smaller clinics. Their current systems are conservative and difficult to change without innovation and enthusiasm.

In summary, the implementation of quality management system in clinical laboratories in Viet Nam showed positive results as well as a huge demand of training and support for applying quality management processes. It is necessary to expand the training program with providing more frequent technical assistance for the local laboratory.

QUALITY ASSURANCE

HEMATOLOGY AND BIO-CHEMISTRY EQAs WITH THE IMPROVEMENT CAPACITY OF MEDICAL LABORATORIES IN MONITORING AND TREATMENT OF HIV PATIENTS IN HOCHIMINH CITY - VIETNAM

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Center for Standardization and QC in Medical Labs of HCMC (CSQL of HCMC) (1)

Hochiminh City AIDS Committee (2)

Abstract:

External Quality Assessment Scheme (EQAs) is one of methods for evaluating the reliability of the medical diagnosis, it helps the laboratories find out the errors and have the corrective actions, so that they will give the better results to the patients.

Center for Standardization and QC in Medical Labs of HCMC (CSQL of HCMC) co-ordinated with Hochiminh City AIDS Committee (HCMC PAC) and US Center for Disease Control and Prevention (CDC VietNam) has expanded the EQAs for hematology and bio-chemistry to 22 hospitals /laboratories (participants) to improve their capacity in medical diagnosis, in order to strengthen their monitoring and treatment to HIV patients.

We detected strong evidence of improvement in participants' diagnostic capacity (hematology and bio-chemistry) after 6 months from the expansion of the EQAs program. The rate of errors (the results z-score which are out of ± 3) reduced when comparing the errors from sample 1 to sample 6:

- Hematology: 8.17% in sample 6 compare to 25.69% in sample 1
- Bio-chemistry: 21.18.% in sample 6 compare to 27.17% in sample 1

These results reflect a good improvement on the hospital's capacity to care for their patients, especially, HIV patients.

Key words: EQAs, CDC, CSQL of HCMC, z-scores, hematology, bio-chemistry

GENETIC PREDICTORS OF HCV TREATMENT OUTCOME.
Alex Thompson, St Vincent's Hospital

HEPATITIS C

A PERFORMANCE EVALUATION OF THE ABBOTT ARCHITECT HCV AG ASSAY. NEW POSSIBILITIES. NEW ALGORTIHMS.

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With the recent release if the Abbott Architect HCV Ag CMIA assay a performance evaluation was performed. Results indicated a clinical utility for this test not previously possible with current serological testing algorithms.
This evaluation demonstrated the performance characteristics of this assay and proposes new testing algorithms for targeted populations.

HEPATITIS C

A PERFORMANCE EVALUATION OF COMMERCIALLY AVAILABLE HCV SEROLOGY ASSAYS INCLUDING POC RAPID TEST.

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With the proposed introduction of serological testing for HCV Ag in targeted populations an evaluation of established HCV assays was performed in order to design an algorithm that would satisfy testing requirements.
A Rapid POC assay was also evaluated alongside current methodologies to give an indication of the clinical utility of these assays for anti-HCV screening outside a laboratory setting.

HEPATITIS C

Routine Surveillance of HCV Genotype in Sydney: easy as 1a, 2b, 3c :
Differences in HCV genotype reporting patterns with Versant HCV genotype 1.0 and 2.0 (LiPa) assays
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Introduction/Objectives: To determine if HCV genotype patterns have changed over the period of January 2008 to May 2011, particularly with the transition from the Versant Lineprobe HCV Genotype 1.0 Assay (LiPA) to the Versant HCV Genotype 2.0 Assay (LiPA) in May 2009.

Methods: Samples received for HCV genotype testing during the period 1 January 2008 to 20 May 2009 were tested on the Versant HCV Genotype 1.0 Assay (LiPA) (n=434). Samples received after 21 May 2009 to present were tested on the Versant HCV Amplification 2.0 Kit (LiPA) and the Versant HCV Genotype 2.0 Assay (n= 521). Versant HCV Amplification 2.0 Kit (LiPA) provides amplification of both 5`UTR and CORE region of HCV genome. The produce is biotinylated DNA whish is then used on the Versant HCV Genotype 2.0 Assay (LiPA).

Results: HCV Genotype results obtained from the Versant Lineprobe HCV Genotype (LiPA) 1.0 Assay and the Versant Lineprobe HCV Genotype (LiPA) 2.0 Assay are outlined in Table 1.

Table 1. HCV Genotype patterns obtained with the Versant HCV Genotype (LiPA) 1.0 Assay and the Versant Lineprobe HCV Genotype (LiPA) 2.0 Assay.

HCV Genotype	Versant HCV Genotype (LiPA) 1.0 Assay	Versant HCV Genotype (LiPA) 2.0 Assay
1a	15.2%	45.1%
1b	10.6%	11.7%
1a or 1b	5.3%	0%
1	9.7%	0.8%
2a or 2c	1.9%	1.2%
2b	4.6%	3.8%
2	2.3%	0.4%
3a	25.1%	30.3%
3b, 3c or 3k	0%	0.6%
3	1.6%	1.5%
4	0.2%	1.0%
5	0%	0.2%
6	Not available	0.8%

Discussion: The Versant HCV Genotype 2.0 Assay (LiPA) has allowed samples of genotype 1 to be further differentiated into specific subtypes. There has been reduction in the reporting of genotype 1 only samples with the majority of these samples classified as subtype 1a. The ability of Versant HCV Genotype 2.0 Assay (LiPA) to detect genotype 6 samples is an improvement on Versant Lineprobe HCV Genotype1.0 Assay (LiPA) which misclassified genotype 6 samples as genotype 1.

HEPATITIS C

INTERLEUKIN 28B POLYMORPHISM IDENTIFICATION: ASSAY VALIDATION

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Introduction: Recently, genome-wide association studies identified a single nucleotide polymorphism (SNP) upstream from the interleukin (IL) 28B gene on chromosome 19 as a major predictor of treatment response in patients infected with HCV genotype 1. Patients with a C/C genotype responded better to pegylated interferon and ribavirin treatment than patients with a T/T genotype or the heterozygous C/T genotype. A SNP assay has been developed using genomic DNA extracted from liver tissue or peripheral blood mononuclear cells, however, these samples are not easy to obtain. Our aim was to assess the utility of using plasma as a source of genomic material for the IL28B SNP assay.

Methods: DNA was extracted from buffy coat and plasma samples using a number of different extraction kits. Plasma samples were extracted using the QIAGEN QIAamp DNA Mini Kit, the QIAGEN DX reagent kit on the Corbett X-tractor and the Abbott DNA extraction kit on the m2000sp platform. Buffy coat samples were extracted using the DNA Mini Kit and ArchivePure DNA Blood kit (Quantum Scientific). The samples were tested in an allelic discrimination assay on the ABI 7500 system, using selective TaqMan probes. Upon completion of the assay, the results and threshold cycle (Ct) values of the extracted samples for each extraction method were analysed.

Results: Both buffy coat and plasma samples provided sufficient genomic DNA to identify the IL28B SNP genotype. Both kits used to extract genomic DNA from buffy coat samples provided similar results. The three plasma extraction procedures differed in the amount of genomic DNA produced as demonstrated by the variation in the Ct values. The DNA Mini Kit was consistently the most efficient, closely followed by the DNA extraction on the Abbott instrument with those extracted on the Corbett X-tractor having the lowest yields. The Corbett extracted material gave indeterminate results more frequently than either of the other methods. IL28B polymorphism results were identical in matched plasma and buffy coat samples, as expected.

HEPATITIS C

EVALUATION OF A NOVEL TESTING STRATEGY FOR HEPATITIS C RNA DETECTION USING DRIED BLOOD SPOTS

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Background: Currently, nucleic acid testing for blood-borne viruses, including Hepatitis C virus (HCV), often involves collecting large volumes of plasma or serum and requires stringent handling procedures to preserve specimen integrity . In many situations, particularly in field or resource limited settings venepuncture and subsequent processing may not be possible. Large epidemiological surveillance of HCV and HIV has been undertaken by adapting serological tests for use with DBS samples and has successfully informed public health and prevention policy. The application of DBS in single and pooled sample testing is viewed as enhanced surveillance of HCV in such cohorts.

Aims: This study examines a testing strategy using dried blood spots (DBS) on a Whatman 903 Protein Saver Card ® as an alternate specimen type for nucleic acid detection using the Roche Diagnostic COBAS ® Amplicor ® platform for Hepatitis C RNA (Roche Molecular Systems CA USA) . Dried blood spotted onto #903 specimen collection card each containing 50 μL of whole blood were dried for over three hours to overnight and were lysed in the a commercially available NucliSENS ® Lysis buffer. Two complete spots were eluted in 2 mL screw capped tubes tube and incubated for thirty minutes on a roller mixer (up/down movement) at room temperature. The RNA was then extracted using the NucliSENS ® EasyMAG ® extraction system (Biomerieux France).

Results: Single eluate testing confirmed specimens that had been previously detected by conventional HCV RNA PCR returned positive results in the DBS sample with a lower limit of detection estimated to be as low as 24 IU/mL. A pooling strategy was also evaluated using DBS prepared with a 4:1 ratio of negative and positive specimens. The viral load values of the positive specimen in the pool ranges from 10^2 to 10^4 IU/mL and all pools returned detectable RNA results in every pool tested.

Conclusions: We conclude that these results show a comparable level of sensitivity for testing when compared to conventional plasma or serum specimens. Utilisation of DBS specimen collection methods could overcome access issues with specimen collection in remote or resource constrained settings. These preliminary results suggest that DBS specimen can be successfully applied to large sero-epidemiological surveillance surveys of HCV in affected populations. The novel application of pooling samples will further reduce improve efficiencies in reagent and labour costs. Data of the validation experiments will be presented.

HEPATITIS B

AN IMPROVED ABBOTT ARCHITECT ASSAY FOR THE DETECTION OF HEPATITIS B VIRUS SURFACE ANTIGEN

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Introduction: The sensitive and accurate detection of Hepatitis B virus (HBV) surface antigen (HBsAg) is critical to the identification of infection and the prevention of transfusion transmitted disease. To reduce the early window period during the acute phase of infection, improvement in HBsAg assay sensitivity is essential. Additionally, the sensitive detection of HBsAg mutants that are continually evolving is a necessity.
Methods: We developed a fully-automated HBsAg assay compatible with the Abbott ARCHITECT®. This magnetic microparticle-based assay utilizes anti-HBsAg monoclonal antibody to capture antigen present in serum or plasma. Captured antigen is then detected using anti-HBsAg antibody conjugated with the chemiluminescent compound.
Results: The sensitivity of the ARCHITECT HBsAg Qualitative II assay was improved as compared to the current ARCHITECT and PRISM HBsAg assays, as well as competitor HBsAg assays. The enhancement in assay sensitivity resulted in the earlier detection of acute phase samples as demonstrated by detection of additional seroconversion panel members, as well as improved analytical sensitivity using the WHO 2nd International Standard (0.020 IU/ml). The ARCHITECT HBsAg Qualitative II assay also efficiently detected all viral genotypes and mutants evaluated, and had better overall mutant sensitivity as compared to other HBsAg assays. Additionally, the sensitivity improvement of the assay did not compromise assay specificity, being greater than 99.9% in multiple studies evaluating various blood donor and diagnostic populations. No adverse effects on assay performance were observed when testing potentially interfering specimens from other viral or bacterial infections, autoimmune diseases, or conditions such as pregnant or multiparous females, elevated IgG and/or IgM levels, human anti-mouse antibodies (HAMA), hemodialysis, or multiple transfusion recipients.
Conclusion: Data from these evaluations demonstrate that the ARCHITECT HBsAg Qualitative II assay has better sensitivity, both seroconversion and analytical, than other HBsAg assays. Additionally, despite the increased sensitivity, assay specificity is statistically identical to the current ARCHITECT HBsAg Qualitative assay.

HEPATITIS B

A NEW "MUTANTS SENSITIVE" LIAISON XL ® ASSAY FOR QUANTIZATION OF HEPATITIS B VIRUS SURFACE ANTIGEN (HBSAG).

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Objective: Improvement in HBsAg assay sensitivity is essential to reduce the window to detect an acute HBV infection and its quantization support the role of HBsAg as a predictive marker for the anti-HBV treatment response. Additionally, it is a necessity to improve both specificity features and sensitive detection of HBsAg mutants, that continue to be a risk due to vaccine escape, immune selection and reverse transcriptase errors. The aim of the work was to develop and assess a highly specific quantitative immunoassay for HBsAg detection in human serum/plasma, capable of recognition of all mutants, to be used on the new LIAISON® XL analyzer.

Methods: The quantitative detection of Hepatitis B virus surface antigen is a two-step sandwich chemiluminescence immunoassay (CLIA). Comparable sensitivity for detection of different mutants and genotypes is assured by mouse monoclonal antibodies directed to highly conserved epitopes of HBsAg inner region that can detect HBsAg when used in combination with a complex detergent mixture. A mixture of mouse monoclonal antibodies is used for coating magnetic particles (solid phase) and a different mixture of mouse monoclonal antibodies directed to different epitopes is linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, HBsAg present in calibrators, samples or controls binds to the solid phase. During the second incubation, the antibody conjugate reacts with HBsAg already bound to the solid phase. After each incubation, the unbound material is removed with a wash cycle.
Subsequently a flash chemiluminescence reaction is induced. The light signal, and hence the amount of isoluminol-antibody conjugate, is measured by a photomultiplier as relative light units (RLU) and is directly proportional to HBsAg concentration present in calibrators, samples or controls. The analyzer automatically calculates the HBsAg concentration (as IU/mL) and grades the results.

Results: 5,201 serum and plasma specimens collected in two blood banks were run. The assay showed 99.88% diagnostic specificity at screening and 99.98% after retest of initially reactive samples. 911 specimens from hospitalized patients, dialysis patients, pregnant women, high-risk subjects, and another population of 2,000 specimens from a laboratory routine, were also tested obtaining a diagnostic specificity of 99.78% and 100% respectively. The assay showed 100% diagnostic sensitivity by testing a panel of 10 recombinant mutants and 424 specimens from preselected HBsAg positive patients (86 of whom with defined HBsAg subtypes). 32 seroconversion panels were also evaluated in comparison CE-marked HBsAg assays. The results show that the LIAISON® XL Murex assay detected HBsAg one bleed earlier in five out of 32 panels and one bleed later in two out of 32 panels. Both assays exhibited equivalent HBsAg detection in 25 out of 32 panels.

Conclusions: The LIAISON® XL Murex HBsAg Quant direct two-step sandwich CLIA assay ensures reliable data showing high analytical performance in the quantitative determination of HBsAg in serum/plasma specimens. A unique capability in detecting both the described HBsAg mutants, subtypes and genotypes is then highlighted like an excellent specificity to meet laboratory needs.
Nevertheless additional investigations with clinical samples should support algorithm definition with this quantitative application.

HEPATITIS B

VERIFICATION OF THE COBAS AMPLISCREEN HBV TEST

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Introduction

NRL conducted a verification of the COBAS AmpliScreen HBV assay to determine the sensitivity, specificity and robustness of the assay in use at the NRL. The AmpliScreen HBV Test is a qualitative *in vitro* test for the direct detection of HBV DNA in human plasma. The test is intended for donor screening of individual donors of whole blood, blood components, source plasma and other living donors. It is also intended for use to screen organ donors when specimens are obtained while the donor's heart is still beating or from cadaveric (non-heart-beating) donors. The AmpliScreen HBV assay can detect blood and tissue donors who are in an early acute phase of HBV infection, before HBsAg can be detected by EIA.

Methods

The COBAS AmpliScreen HBV Test uses a generic sample DNA extraction technique (MultiPrep Specimen Processing Procedure), along with automated amplification and detection on the COBAS AMPLICOR™ Analyser. The assay incorporates an Internal Control for monitoring assay performance in each individual test, as well as the AmpErase® enzyme (uracil-N-glycosylase) to reduce potential false reactivity due to contamination by previously amplified material (amplicon). The test involves:-

1. Specimen Processing and DNA extraction.
2. PCR amplification of target DNA using HBV-specific biotinylated primers to amplify both HBV and IC DNA.
3. Hybridisation of the amplified products to magnetic particle bound oligonucleotide probes, specific to the targets (HBV and IC DNA).
4. Detection of the probe-bound amplified products by colorimetric determination .

All samples used for this verification were characterised by multiple HBV DNA nucleic acid assays, both qualitative and quantitative.

- 45 HVB DNA positive samples collected from living donors
- 40 HBV DNA positive samples collected from cadavers
- 36 HBsAg negative samples collected from living donors
- 36 HBsAg negative samples collected from cadavers

Results

HBV AmpliScreen was positive for all 45 HBV DNA positive samples (various genotypes and antibody escape mutants) collected from living donors. All 40 samples collected from cadavers were also all positive.

The HBV AmpliScreen assay returned negative results for 70 of the 72 HBsAg negative samples tested. Of the two exceptions one was a sample from a living individual from which the internal control could not be amplified during PCR. The second exception was a sample from a living individual which initially gave a positive result. Upon retesting this sample returned a negative result.

As part of the verification 39 replicates of a 10 IU/ml sample which had been calibrated against the WHO international standard was tested. All replicates except one returned a positive result.

Conclusions

- The assay successfully detected HBV DNA from several common genotypes and escape mutants.
- The matrix of cadaver plasma did not adversely affect the detection of HBV DNA.
- An estimated sensitivity of 100% (95% CI 94% - 100%) was obtained.
- The rate of false positive reactivity in negative samples met the acceptance criteria indicating that the robustness of the test was adequate. Retesting samples that give a positive result has been included in the testing strategy.
- An estimated specificity of 98.6% (95% CI: 915 – 100%) was obtained.
- At 10 IU HBV DNA /ml the detection rate was 97%.
- The results of this study verify the performance of the HBV AmpliScreen test in NRL’s hands.

HIV AND T CELL IMMUNITY

PREVALENCE OF TRANSMITTED DRUG RESISTANCE IN PRIMARY INFECTED HIV PATIENTS IN SYDNEY - 2005 – 2010

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Background: The implementation of antiretroviral therapy has led to a marked drop in mortality rates of patients with HIV infection. With the wide spread use of anti-retroviral drugs (ARV) on a global scale the emergence of treatment failure attributed to drug resistance has been a major public health concern. Primary or transmitted resistance has emerged as a potential threat to the success of antiretroviral therapy. Transmission of HIV drug resistance to uninfected individuals raises serious clinical consequences as it may compromise the response to initial therapy.

Aim: This study was to characterize rates associated with transmitted drug-resistant HIV-1 infection. Prospectively collected genotypic resistance data from antiretroviral therapy (ART)-naive individuals was collated from newly diagnosed cases of HIV-1 infection from January 2005 through December 2010. Genotypic resistance was determined in plasma RNA using the TruGene HIV GART assay (Siemens, USA). The specific drug resistance mutations collected were based on the recommended World Health Organisation form, as published by Bennett *et al* 2009. Genotypes were analysed using the Stanford software (http://hiv-4.stanford.edu).

Results: Data will be presented on the trends in transmitted HIV drugs resistance of the five year observational period.

Conclusions: Surveillance data of ARV drug resistance in cases of recent HIV infection is an important source of sentinel surveillance data to observe trends in transmitted HIV drug resistance. Monitoring of patterns of transmitted HIV drug resistance provides an opportunity to optimise treatment guidelines at the population level but also to individualise effective treatment strategies in newly acquired cases of HIV infection.

HIV AND T CELL IMMUNITY

PROSPECTIVE ESTIMATION OF INCIDENCE IN NEWLY DIAGNOSED CASES OF HIV INFECTION AT ST VINCENT'S HOSPITAL, SYDNEY, 2005 – 2011 – AN UPDATE

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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 characterizing the epidemic and to monitor the effectiveness of prevention programmes.

A number of immunoassays have been developed which distinguish between individuals with established HIV infection and those which have been infected within the previous six months. There has been growing interest in the application of laboratory methods to measure HIV-1 incidence to monitor the effectiveness of prevention programs, targeting resources and second generation surveillance. In 1997, Janssen et al (CDC USA) developed the 'detuned' or 'Less sensitive' ELISA by modifying a first generation lysate EIA for HIV-1 infection (infection within <176 days). This has been out of production since 2008. More recently, the BED Capture HIV-1 IgG incidence EIA (Calypte Biomedical, USA) estimates incidence by measuring HIV-1 specific IgG with total IgG (infection within <153 days).

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

The NSW State Reference Laboratory (SRL) for HIV at St Vincent's Hospital identifies more than 60% of newly identified cases of HIV infection for NSW. In 1995, the SRL commenced incidence testing of all newly identified cases of HIV infection.

Year of Diagnosis	2005	2006	2007	2008	2009	2010	2011
	(n=286)	(n=230)	(n=255)	(n=220)	(n=253)	(n=245)	(n=135)
Incident HIV infection	37.9%	38.6%	37.5%	44.5%	42.3%	39.6%	48.10%
Established HIV infection	62.1%	61.4%	62.5%	55.5%	57.7%	60.4%	51.90%

This study shows that approximately 40% of newly identified cases of HIV are presenting with incident HIV infection. On analysis of recent statistics, it has been observed that the number of incident cases presenting to the SRL are increasing, perhaps indicating increased patient awareness of the importance of regularly being tested for HIV. These results suggest these tests may be useful for population estimates of recent infection however, use for individual patient management is not recommended.

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.

HIV AND T CELL IMMUNITY

ASSESSMENT OF A GENOTYPIC ASSAY FOR DETERMINATION OF CHEMOKINE RECEPTOR TROPISM HIV-1

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Introduction/Objectives:
In order to achieve therapeutic efficacy it is necessary to determine the co-receptor tropism of HIV-1 (CCR5-tropic or CXCR4-tropic virus), prior to the prescription of CCR5 inhibitors (e.g. Maraviroc, Selzentry). The current standard assay (phenotypic) for determining HIV-1 tropism (*Trofile,* Monogram) is expensive and has a slow turnaround time. Therefore we have assessed an alternative assay to detect co-receptor tropism based on a genotyping assay by sequence analysis of the HIV-1 *env* gene (V3 loop) using 59 clinical samples and 30 Quality Assurance Program (QAP) samples. Viral tropism was determined using an online algorithm, *geno2pheno* (Max-Planck Institute, Germany). This algorithm predicts a False Positive Rate (FPR%), determining whether a sequence is likely or not to be CXCR4 tropic (FPR% <20% = CXCR4; FPR% >20% = CCR5).

Methods:
We employed a genotyping assay (Swenson, LC *et al*.) utilizing a nested PCR with secondary back-up primers in conjunction with *geno2pheno* (using "German recommendation" settings) in order to determine HIV-1 tropism based on sequencing of the V3 loop envelope gene from plasma viral RNA. We initially analysed the assay's ability to detect mixtures of virus by mixing two laboratory HIV -1 strains: CCR5-tropic virus (Bal-1) and CXCR4-tropic virus (NL4-3). We further assessed the ability to detect co-receptor tropism and determine the sensitivity and reproducibility of the assay in 59 clinical samples and 30 QAP samples.

Results:
We were able to detect CXCR4-tropic virus in a mimic mixture of laboratory HIV-1 strains in negative plasma with mixtures containing a minimum of 20% CXCR4 tropic virus. We found the assay variability increased and sensitivity decreased as the patient's viral load (VL) decreased. We therefore set a limit of detection (LOD) for clinical samples with HIV-1 VLs >2000 copies/mL. We amplified, sequenced and designated a viral tropism for 96.6% (57/59) of patient samples and 96.7% (29/30) for QAP samples (from two separate QAPs, Vancouver and Germany). Each of these samples were amplified and sequenced in triplicate from either first round PCR, nested PCR or by nested PCR using secondary back-up primers. The QAP sample results showed a high degree of concordance between the FPR% from our laboratory and the reference laboratories (R^2 = 0.99). From the 59 clinical samples, 57 samples were amplified and sequenced in triplicate and showed minimal variation between triplicates (SD $\pm$ 0.7). However, there was variation between concordance of attributed viral tropism in 3.5% (2/57) of samples.

Discussion/Conclusions:
We evaluated the V3 loop genotypic assay via several methods, the first of which we showed the assay can detect tropism of HIV-1 virus laboratory strains to a level of 20% mixed population of laboratory-strain mimicking CXCR4-tropic virus, thus showing we can identify CCR5- -tropic and CXCR4-tropic viruses. We found that patient samples with a VL of >2000copies/mL could be amplified and sequenced with a higher degree of sensitivity and less variability and therefore set this VL for the assay LOD. We reported results on 96.6% from 59 clinical samples and 96.7% from 30 QAP samples, showing a high degree concordance and specificity at the level of FPR%, as well as good reliability and reproducibility of the assay. The 3.5% of clinical samples that were not concordant were most likely due to the primer mismatches due to virus mutations. We evaluated the necessity for determining viral tropism in 59 clinical samples by assaying them in triplicate, showing that in 3.5% of samples the FPR% will vary and result in discordant assigned tropisms, indicating the necessity for the use of triplicates in a clinical setting. This assay provides good concordance, sensitivity and reliability and in conjunction with a relatively quick turn-around time and cost efficiency, offers an excellent alternative to current clinical assays detecting HIV-1 viral tropism.

HIV AND T CELL IMMUNITY

T CELL IMMUNITY

Bill Sewell, Garvan Institute

Garvan Institute for Medical Research, St Vincent's Pathology, and UNSW St Vincent's Clinical School, Darlinghurst NSW 2010

This lecture provides an overview of essential aspects of the T cell response. Key similarities between T and B cells include a very diverse range of antigen receptors, and clonal responses of specifically activated cells, giving rise to both effector cells and memory cells. A key difference is that while B cells can be activated by many different types of free extracellular antigen, T cells only respond to protein antigens, and only after they have been processed into peptides and presented by another cell, bound to major histocompatibility (MHC) molecules. Dendritic cells are the most effective cell type that presents antigen to both CD4 (helper) and CD8 (cytotoxic) T cells. Another key difference is that while a B cell clone gives rise to plasma cells that secrete their antigen receptor molecules (secreted immunoglobulin), T cells do not secrete their T cell receptors (TcR). Instead, T cells mediate immune responses by secreting cytokines or cytotoxic molecules. CD4 T cells "help" a range of other cells by differentiating into various subsets of T helper cells that secrete different cytokines, of which Th1, Th2 and Th17 are the best described. In the diagnostic laboratory, it is much more difficult to measure specific T cell responses than specific antibody responses. The best-established assays for specific T cell responses are interferon-gamma release assays, that measure Th1 responses. There is potential for a variety of other T cell assays to be introduced into the diagnostic laboratory.