TESTS OF FUTURE PAST

Hueston L

Centre for Infectious Diseases and Microbiology Laboratory Services, Pathology West - ICPMR Westmead

“May you live in interesting times” is an English expression allegedly translated from a Chinese curse, it was a common expression in the 1960’s and was famously used in a speech given by Robert Kennedy in Cape Town in 1966. While this expression hasn’t been used to describe laboratory medicine, like it or not laboratory medicine finds itself living in interesting times. Times of upheaval, uncertainty and paradigm shifts but also times of opportunity. In this meeting we will hear a great deal about new technologies and how they have the potential to revolutionise laboratory medicine, but does this mean the demise of older techniques like culture and serology? It is difficult to imagine diagnostic laboratory medicine if serology didn’t exist. It has certainly changed over almost 200 years and in this presentation we will examine if the classic techniques have reached their use by date. Or will we find that scientists in the future will need to return to their past to rediscover techniques they once abandoned, the tests of future past.

ARE THERE SIMILARITIES BETWEEN "STAR WARS" AND AN ANCIENT EPIDEMIC DISEASE?

Crockett, B

St Vincent's Hospital, Melbourne – Staff Clinic

Introduction

Smallpox appeared in Africa around ten thousand years ago but although we can trace it in Egypt, India and China, it did not get to Europe until the Dark Ages. Perhaps it was the Dark Invader aka Darth Vader and Edward Jenner it's Luke Skywalker.

No infective disease has had more impact on the framing of Western civilization than Smallpox.

Polio, Measles, Mumps, Diphtheria, Pertussis, Tetanus, TB and so the list goes on. These are all vaccine preventable diseases that we have made great inroads on toward elimination but where are we really?

What has disappeared? What has been the benefits of mass immunization programs? What of the people who now question the validity of childhood vaccination programs?

Do we still need vaccines if the diseases they are used for have disappeared from our parts of the world?

In 2010 WHO tried a new strategy to finally eradicate polio and as I write we have a case in a Melbourne hospital. Yes right here! We came close to eradication but now some experts are saying enough, give up, as the WHO initiative was to be by 2012.

RUBELLA REPORTING – WHERE DID WE GO WRONG AND WHAT ARE WE DOING ABOUT IT.

Wayne Dimech

NRL, Melbourne, Australia

In 1995, an anti-rubella IgG serum standard, coded RUBI-1-94, was prepared by Statens Serum Institut, Denmark as a replacement for the Second International Reference Preparation for Anti-Rubella Serum. Since this time, most commercially available anti-rubella IgG assays have been calibrated against this standard. However, evidence from peer reviewed journal articles and quality assurance programs indicate that there continues to be a lack of standardisation of results reported by different rubella IgG assays, especially results close to the cutoff, causing difficulty in the clinical interpretation of test results.

There is a lack of information regarding the development of RUBI-1-94. The only publically available documentation on the development of the standard is the instructions for use. This document refers to an international study to assign the potency of the standard but does not provide details of the findings. It also states that "this study has almost been completed". The results were never published. Unpublished data indicate that the testing for potency was performed on first generation enzyme-linked immunoassays, haemagglutination inhibition (HAI) and single radial haemolysis assays. However, metrologic principles require reference materials to be calibrated using a reference method, the highest possible level in the metrologic hierarchy, which is specific for the defined analyte. Given anti-rubella IgG testing targets multiple antigenic sites (E1, E2 and C) with variable avidity and affinity, and that there is no quantitative reference method for anti-rubella IgG available, the approach to establishing potency of the RUBI-1-94 did not meet metrologic requirements.

Initial studies to determine the immune/non-immune cutoff for anti-rubella testing, reported in international units per milliliter (IU/mL), were conducted using HAI and were suggested to be ~24 -48 IU/mL. Subsequently, a NCCLS (now CLSI) Rubella Subcommittee on Rubella Serology suggested cut-off of 15 IU/mL. In 1987 Abbott introduced the IMx rubella IgG assay that had a cutoff of 10 IU/mL. The immune/non-immune cutoff was reviewed by CDC the following year and 10 IU/mL was determined to be appropriate. Since that time, commercial assays have used 10 IU/mL as the cutoff. All publications of these groups that suggested specific cutoffs have indicated that false negative and positive results are to be expected if their cutoff was implemented.

Since 2012, an informal, international committee of interested virologists, in collaboration with the World Health Organization, has conducted meetings discussing mechanisms to address the problems with standardising rubella IgG reporting. Currently, a project is underway to develop a panel of at least 100 plasma samples deemed to be negative for anti-rubella IgG. To date, approximately 50 candidate samples have been identified and a manufacturer enlisted to produce the panels. There is agreement by all major manufacturers of anti-rubella IgG assays to use this panel to establish assay-specific cutoffs.

This presentation will review the development of the international standard, assay cutoff and briefly review evidence of lack of standardisation of rubella IgG reporting. The activities of the informal rubella standardisation committee will be presented.

LABORATORY DIAGNOSIS: THEN AND NOW

Darren Jardine

St Vincents Pathology, Fitzroy, Victoria

Introduction

Demands on diagnostic laboratories are constantly evolving, with laboratories increasingly expected to work under tight operational and fiscal restraints. In order to provide clinicians with accurate timely results it is important to embrace advances in technology. Microbial testing has drastically evolved during the past decade, with new technologies such as mass spectrometry and multiplex molecular testing replacing more traditional methods of microbial identification. This presentation will examine ways in our laboratory is utilising these advances in technology, whilst comparing them to their more traditional counterparts, to meet the demands of a modern microbiology laboratory.

EVERYTHING OLD IS NEW AGAIN

Crockett, B

St Vincent's Hospital, Melbourne – Staff Clinic

Introduction

World Health warned six years ago that infectious diseases were emerging at a never before seen rate. Since the seventies roughly forty diseases have been discovered. I will outline some of these diseases, their global impact and the potential for halting their progress in the future.

We recently have had an Ebola virus outbreak in Guinea that has the potential to spread to other countries. We have seen SARS now MERS and the constant evolution of flu viruses, recently Swine and Avian varieties.

Exposures are greater due to the conquering of the "tyranny of distance". Epidemics can spread rapidly by domestic and international air routes.

We are seeing the re-activation of old recycled disease due to various causes such as changes to human behavior, migration and travel, world over population, poverty and climate changes.

All is not doom however, we only need to look at the change in HIV infection from a death sentence in the eighties to a rest of life treatable infection now that may well be vaccine preventable in your lifetimes.

We are well on the way to eliminating HPV infection which accounts for most cases of cervical cancer in this country since an effective vaccine has been made available.

MEASLES VIRUS OUTBREAK NORTHERN TERRITORY - 2014

Freeman K, Lines S, Markey P

Northern Territory Government Pathology Services - Serology/Molecular Biology Department, Royal Darwin Hospital, Centre for Disease Control - Northern Territory Government

Introduction

A measles outbreak originated from a returned overseas traveller with local transmission then occurring. A further 5 infected returned travellers from overseas compounded this situation, resulting in a major measles outbreak. Control of the outbreak was obtained by a combination of Measles virus PCR testing of nose/throat swabs and urine specimens of contacts and suspected cases. Contract tracing, advise to Health Care Providers and media releases was provided by the Centre for Disease Control (CDC) – Darwin.

Methods

Measles PCR testing was performed by Nucleic Acid Extraction of specimens using the Roche MagNa Pure System and Amplification/Detection using an In-House Measles virus PCR assay. All positive specimens were referred to the Victorian Infectious Diseases Reference Laboratory (VIDRL) for confirmation and genotyping

A total of 48 patients were diagnosed with Measles virus infection during the outbreak 6 of these cases were recently returned travellers from overseas (Philippines and Singapore).

The CDC was responsible for controlling the outbreak through contact tracing, offering prophylaxis with immunoglobulin or vaccine to contacts, raising awareness amongst clinicians and promoting immunisation in the community.

Results

During the period of 19/01/2014 to 04/04/2014 a total of 1043 specimens were tested with 90 specimens detected for Measles virus (88 confirmed, 2 not confirmed) 4 equivocal specimens (1 confirmed, 3 not confirmed) 51 patients were confirmed as Measles Virus PCR positive, 48 confirmed as Genotype B3, 2 confirmed as Genotype A (Vaccine Strain) and 1 not typeable.

The age distribution was 7 months to 63 years of age, with a bi-modal distribution of <1 year and 17-47 years of age. The majority of cases were in age groups expected to be under-immunised.

12 Patients were hospitalised with 2 Intensive Care Unit admissions with a median stay of 5.5 days.

One household contact of a case, which confirmed as Genotype B3, did not fit the clinical picture of measles. It was thought that this specimen may have been contaminated during home collection in the household of an index case.

The Centre for Disease Control contact traced approximately 2756 people during the outbreak.

Discussion

It is most likely the majority of Measles virus cases have arisen from the first index case, with transmission occurring in households, hospital, shopping centres and child care facilities.

The timely testing of both nose/throat swabs and urines was crucial to the effectiveness of the outbreak response given the potential impact of excluding many contacts from childcare centres and schools.

Conclusion

The control of measles outbreaks is dependent on close collaboration between clinicians, laboratories and disease control staff. As the Northern Territory Government Pathology Services is the only diagnostic Molecular Laboratory in the Northern Territory, we received specimens from all public hospitals and private clinicians, this required exceptional organisation skills from both the Centre of Disease Control and the Serology/Molecular Biology Laboratory. The availability of local molecular diagnostic techniques was fundamental to the success of the outbreak response.

Acknowledgments

Serology/Molecular Biology Team – NTGPS, Royal Darwin Hospital, Centre for Disease Control Darwin, Infectious Disease Team – Royal Darwin Hospital, Victorian Infectious Diseases Reference Laboratory – Molecular Division

MEASLES: THE VIRUS STRIKES BACK, ARE YOUR CHILDREN SAFE?

Lesic, S., Quinn, H. and Hueston, L

The Centre for Infectious Diseases and Microbiology Laboratory Services (C.I.D.M.L.S.), Institute of Clinical Pathology and Medical Research (I.C.P.M.R.) and The National Centre for Immunisation Research and Surveillance (N.C.I.R.S.)

Introduction

Measles is a highly contagious viral disease spread easily through the air via respiratory droplets. This virus effects multiple organ systems leading to severe bacterial infections in the lungs, seizures, inflammation of the brain, hepatitis, blindness, heart muscle damage, kidney failure and even death.

In the past, measles infection was a common occurrence. Thankfully since the introduction of a vaccination program in 1968, measles infection is now uncommon in Australia. This said many areas of the developing world continue to experience outbreaks and due to travel and immigration, spot clusters of infection can still occur across the country. It is therefore vital to determine whether the Australian population is protected against this vaccine preventable disease.

The National Centre for Immunisation Research and Surveillance (N.C.I.R.S.) is funded by the Australian Government Department of Health and Ageing in an effort to promote optimum control of vaccine preventable diseases. One initiative of this organisation is the national serosurveillance program. Previous surveys have been responsible for recommendations of booster injections and the implementation of school-based “catch-up” programs. This year more than 1200 serum samples from diagnostic laboratories across Australia were collected and analysed. Testing was performed using Siemens Enzygnost Anti-Measles Virus/IgG kits. Antibody levels were measured and immunity or susceptibility determined.

Analysis of NSW data collected over 4 serosurveys from 1998 to 2013 showed an increasing rate of equivocal results. This suggests immunity is waning and the state may not be protected from measles infection. Testing and analysis for remaining states and territories has yet to be completed, however preliminary results suggest a similar trend. This presentation explores and evaluates the reasons behind these results.

THE NOVEL PIPELINE OF IMMUNOASSAYS FOR THE QUANTIFICATION OF HEPATITIS B SURFACE ANTIGEN (HBSAG): A NEW LIFE FOR AN OLD MARKER IN CHRONIC HEPATITIS B.

Burdino E [a], Ruggiero T [a], Proietti A [a], Milia MG [a],Olivero A[b], Caviglia GP [b], Marietti M [b], Rizzetto M [b], Smedile A [b], Ghisetti V. [a]

[a] Laboratory of Microbiology and Virology, Amedeo di Savoia Hospital, Torino, Italy
[b] Division of Gastroenterology and Hepatology, Department of Medical Sciences, University of Torino, Torino, Italy

Recent technologic innovations have allowed for the quantitative assessment of the HBsAg levels in serum and HBsAg quantification has been applied for monitoring chronic HBV infection during its natural history as well as for the prediction of response to treatment. The clinical relevance of HBsAg level stems from the apparent correlation with intrahepatic cccDNA levels and influences treatment response.

A new pipeline of immunoassays for the quantification of HBsAg has been recently developed and the LIAISON XL Murex HBsAg Quant assay (DiaSorin, Saluggia, I) is the newest CE approved chemiluminescence immunoassay to detect HBsAg in the setting of blood transfusion as well as to quantify HBsAg level for clinical purposes. The assay is standardized against the World Health Organization Second International Standard with an analytical sensitivity of 0.05 IU/mL and a dynamic range up to 150 IU/mL with onboard dilution.

The aim of the present work is to evaluated LIAISON XL performances against the first licensed quantitative Architect HBsAg immunoassay (Abbott Diagnostics, IL, US), as gold standard, on sequential serum samples (n=152) from 14 patients with chronic HBe-negative hepatitis B (CHB), the majority of them infected with HBV genotype D and undergoing antiviral treatment with different combination of therapies. The 2nd WHO Standard 00/588 for HBsAg was used to assess assay performance.

Evidence of how HBsAg quantification in chronic hepatitis B offers a new tool for a better definition of virological end-points and patients management will be given, as well as that the newest CE approved immunoassay for HBsAg levels, LIAISON XL, is suitable for routine clinical use and can be applied for HBsAg quantification in clinical practice and decision making for patients with CHB.

HBV: A CLINICIAN'S PERSPECTIVE

Batey, R

Alice Springs Hospital

My presentation will address a number of issues that I believe many clinicians struggle with as they deal with HBV in clinical situations across the country. The major areas I will touch on are:

- My clinical history with the HBV
- Some negative experiences - which will cover areas of practice where mistakes have been made and where current responses simply do not answer our need for greater clarity
- Some positive experiences of assisting change in the way we deal with HBV in Australia in 2014.
- Questions I ask as a clinician that are not yet fully answered - and I hope in dealing with these, we can progress our teaching and research agendas in a positive way.
- Current contentment – is it a wise mental state to cling to? Many have relaxed since the arrival of the two drugs, entecavir and tenofovir but it may be that we are living in a fool's paradise. Are we seriously addressing all of the challenges that this virus presents?
- Broadening the clinical base for treatment - an area fraught with fear, concern, territorialism and limited progress.

HEPATITIS B CORE IgM – WORTH DOING ROUTINELY?

Holland J

Laverty Pathology

Introduction

Recent ASHM recommendations for laboratories to consider initiating Hepatitis B core IgM testing (in addition to hepatitis B core total) when initial detection of hepatitis B surface antigen or when detection of isolated hep B core total antibody.

Method

Samples and data collected prospectively from April – September 2013.
Hepatitis B core IgM performed on

a. 100 samples - new diagnosis of hepatitis B – ie hep B surface Ag positive, core total antibody positive no previous positive Hep B surface Ag results in our LIS.
b. 100 samples – isolated hep B core total positive – ie hep B surface Ag and hep B surface Ab negative. No previous isolated hep B core total antibody results in our LIS.

Samples tested using Abbott Architect hepatitis B core IgM assay with supplementary testing of reactive tests on Siemens Centaur assay.

Results

a. Six hep B core IgM positive results detected in the 'new diagnosis' group. Two of these tests were requested by the referrer or by the pathologist when reviewing results prior to release.
b. Three hep B core IgM positive results detected in the 'isolated core' group. All these results were low level reactive on the Architect assay of which 2 samples were negative and 1 sample was equivocal by Centaur.

Discussion

The positivity rate for hep B core IgM in the patient population referred to our laboratory was low. We issue a comment on reports with new diagnosis of hep B surface antigen stating that results are consistent with current (acute or chronic) infection. The hep B core IgM positive results which were detected in the isolated core group are difficult to evaluate but may be false positives.

Conclusion

We will not be instituting routine hepatitis B core IgM testing.

MYCOPLASMA GENITALIUM; NEW DIAGNOSTICS FOR AN EMERGING UROGENITAL PATHOGEN.

Getman, D

Research and Development, Women's Health Diagnostics, Hologic, Inc., San Diego, USA

Background

Mycoplasma genitalium (MG) is an emerging sexually transmitted pathogen implicated as a cause of urethritis in men, and vaginitis, cervicitis, pelvic inflammatory disease, and infertility in women. This study evaluated the analytical performance of an automated transcription-mediated amplification (TMA) assay for MG, and determined the prevalence of MG infection and co-infections with *C. trachomatis* (CT), *N. gonorrhoeae* (NG) and *T. vaginalis* (TV) in urogenital specimens from male and female subjects enrolled in a prospective multi-center clinical study.

Methods

TMA assay analytical sensitivity and specificity was determined using laboratory strains of MG and other non-target microorganisms. Specimens were obtained from male and female subjects consented and enrolled from 7 diverse sites in the United States. Male specimens [physician-collected urethral swab (US) and self-collected male urine (MU)] were obtained from 419 asymptomatic or symptomatic (urethritis) men. Female specimens [physician-collected vaginal swab (VS), endocervical swab (ES), ThinPrep liquid Pap (TP), and self-collected urine (FU)] were obtained from among 800 asymptomatic or symptomatic (urethritis, vaginitis, or cervicitis) women. All samples were tested using the MG TMA assay and FDA-cleared Aptima assays for TV, CT, and NG, on the automated TIGRIS DTS System or Panther System.

Results

High titer (≥10E6 CFU/reaction) samples of non-target organisms tested with the MG TMA assay showed 0% reactivity (S/CO≤ 0.01). Analytical sensitivity (95% LOD) of the assay for MG G37 strain in pooled urine or Aptima sample transport medium was < 0.3 CFU/mL. In male subjects, overall prevalence of MG and CT infections (single and combined infections) was high (17.2% and 18.4%, respectively) while NG and TV prevalence was lower (4.1% and 8.7%, respectively). MG prevalence peaked in ages 21-30 y (23.9%). Slightly more men with only NG infections were symptomatic (67%) compared to men with only MG or only CT infections (52.6%, 51%).

Among female subjects, prevalence of MG and TV infections was high (21.6%, 23.3%) in endocervical swab samples and in vaginal swab samples (21.7%, 21.7%), with highest MG infection rate in VS and ES specimens from young women ages 14-20 (24.4%, 20.0%, respectively). CT and NG prevalence rates ranged from 3% to 9.4% in these sample types. FU sample prevalence rates for TV, CT, MG and NG were 11.5%, 8.1%, 7.2%, and 1.5%, respectively.

Conclusions

The MG TMA assay is sensitive and specific for detection of MG 16s ribosomal RNA. Testing of urogenital samples showed higher MG prevalence in younger vs older males and females. Vaginal swabs and male urethral swabs had the highest MG positivity rate among sample types tested. Males and females infected with MG alone had symptomatic rates that were similar to subjects infected with only NG. These results show MG infection is common in young men and women with symptoms of urogenital tract inflammation.

THE IMPACT OF CHANGES IN TESTING ON NOTIFIABLE CONDITIONS IN NSW

Adamson S, Spokes P, Sheppeard V

Health Protection NSW, NSW Health, Australia

Introduction

Methods for infectious disease diagnostic testing are changing rapidly, which has potential to affect detection and reporting of notifiable conditions. Timely and accurate notification of infectious diseases to health authorities allows implementation of public health measures to control further spread of these infections.

Objectives

To consider the impact of recent changes in infectious disease diagnostic testing on notfications, and implications for public health response.

Methods

Recent changes in infectious disease diagnostic testing, and the impact of these changes on detection of notifiable conditions and measures taken by NSW public health to adapt to these changes in 2013 - 2014 were reviewed.

Results

Recent introduction of rapid testing methods for some notifiable conditions (eg screening using multiplex nucleic acid tests for enteric pathogens, and for sexually transmitted infections) has resulted in earlier detection and notification for public health response. For some notfiable infectious diseases, rapid pathogen detection (eg antigen tests for dengue, legionella, HIV, HCV, and molecular tests for respiratory pathogens, measles and syphilis) rather than awaiting seroconversion has also reduced the time to case confirmation.

However, culture independent diagnostic testing using molecular methods may also lead to loss of information for public health response due to lack of pathogen isolation and characterisation, including anti-microbial susceptibility. Examples include lack of *Neisseria gonorrhoeae* isolates for detection of antimicrobial resistance, and loss of influenza virus isolation in local laboratories for typing and vaccine development. Recent introduction of molecular detection of *Shigella spp* using a target which also detects Enteroinvasive *Escherichia coli* (EIEC) has also led to potentially unnecessary early treatment and public health response for shigellosis prior to confirmation by reflexive culture and isolation. NSW notification data from four large laboratories for January–June 2014 showed that of 137 *Shigella spp*,/ EIEC detections by nucleic acid testing, only 40 were confirmed as *Shigella spp* by reflexive culture.

Point of care testing (PoCT) for infectious diseases is becoming increasingly available (eg HIV, influenza). While most PoCT currently requires confirmation by standard laboratory testing, if this is not done there is potential to miss cases that would have been notified by a laboratory. Provision of HIV PoCT at community sites in NSW for high risk groups has been successful in demonstrating increased testing rates through accessing people not previously tested (15-20% of those tested), or not regularly tested (25-35% of those tested).

Conclusions

Increasing use of rapid testing methods, particularly for direct pathogen detection, has been beneficial for public health response due to faster confirmation of cases, However, this has also led to loss of pathogen characterisation. Public health protocols for management of notifiable infectious diseases will need to be reviewed to account for loss of information and may require development of additional methods to identify outbreaks.

Increasing availability of point of care testing for infectious diseases, and potential self-testing, may further impact on detection and reporting of notifiable conditions, and public health management in the future.

EVALUATION OF MULTIPLO RAPID TP/HIV ANTIBODY TEST

Nilukshi Arachchi and Wayne Dimech

NRL, Melbourne, Australia

Introduction

The Multiplo Rapid TP/HIV antibody test ([Multiplo] - MedMira, Halifax, Canada) is a multiplex device designed to detect, simultaneously, the presence of antibodies to *Treponema pallidum* (TP) and/or human immunodeficiency virus types 1 and 2 (HIV). The device employs a proprietary Rapid Vertical Flow Technology™ test membrane that allows the detection of specific antibodies for each analyte in human whole blood, plasma or serum.

Objective

The aim of this study was to evaluate the sensitivity and specificity of the individual analytes of the Multiplo test.

Method

A total of 400 specimens were included in the study. Of these, 100 were selected as confirmed HIV positive (with unknown TP reactivity), 80 as confirmed TP positive (with unknown HIV reactivity) and 220 as confirmed negative for TP and HIV. Positive specimens were human plasma specimens, retrieved from the NRL sample bank, each having previously been confirmed positive using NRL's testing strategies. Negative specimens were obtained from the Australian Red Cross Blood Service each having been screened negative for HIV and TP. The vials of positive and negative specimens were prepared into a panel in random order, blinded to the study operator, and labelled 1 to 400.

All Multiplo testing was performed as per the manufacturer's instructions for use. The Multiplo test results were compared with the reference results. The sensitivity and specificity, including the 95% confidence intervals (95% CI) of the Multiplo results were estimated as per the following categories:

- Multiplo specificity based on specimens that were negative for both TP and HIV;
- Multiplo sensitivity based on specimens that were dually positive for TP and HIV;
- Multiplo TP sensitivity based on specimens that were true positive for TP, and the HIV reactivity was either negative or unknown; and
- Multiplo HIV sensitivity based on specimens that were true positive for HIV, and the TP reactivity was either negative or unknown.

Results

A total of 6 of 400 specimens were repeatedly invalid and the results removed from the study. All six specimens had been initially included into the study as HIV positive specimens. The results of all test controls were as expected. Multiplo specificity (95% CI) was 98.6% (95.7-99.6%) for each of TP and HIV in dually negative specimens. Multiplo sensitivity (95% CI) was 100% (74.7-99.4%) for each of TP and HIV in co-infected specimens. Multiplo sensitivity (95% CI) for specimens included in the study with known positive reactivity for TP (HIV unknown or negative) was 87.0% (76.2-93.5%) and 98.8% (92.8-99.9%) for HIV positive specimens (TP unknown or negative).

Conclusion

The Multiplo assay was simple to use, rapid and generally produced results that were easily read. Multiplo assay could be considered as an assay to screen for HIV and TP in the same test. The format is suited to antenatal and sexual health clinic screening for sexually transmitted infections.

EVOLUTION IN REAL-TIME: GLOBAL DIVERSIFICATION OF HIV-1 AND IMPLICATIONS FOR DIAGNOSTIC ASSAYS

Hackett J Jr., Berg M, Yamaguchi J, Vallari A, Holzmayer V, Swanson P, Harris B, Luk K-C and Brennan C

Abbott Diagnostics, Abbott Park, IL USA

Introduction

HIV-1 is characterized by an exceptional level of genetic diversity and a rapid rate of evolution. There are three primary sources of genetic diversity: (1) transpeciation of related lentiviruses (simian immunodeficiency viruses) from non-human primate hosts, (2) an error-prone reverse transcriptase enzyme that lacks proofreading capability and (3) recombination. Analysis of global HIV-1 strains reveals that there are four primary lineages of HIV-1 designated as groups M, O, N and P. Group M strains, which represent the vast majority of HIV-1, are further subdivided into subtypes, designated A-K, and intersubtype recombinants. Currently, there are 61 recognized circulating recombinant forms (CRF). Based on recent estimates, recombinant forms of HIV-1 represent 20% of global infections. Sequence diversity between HIV-1 groups and subtypes can be substantial; envelope sequences differ by 20-30% between subtypes and up to 50% between groups. This high level of sequence diversity has the potential to influence assay performance thus has important implications for screening, diagnostic and patient monitoring assays. Recognizing the importance of this issue, Abbott pro-actively initiated a comprehensive program designed to monitor global diversification of HIV, search for newly emerging strains, and assemble a well-characterized panel of genetically and geographically diverse HIV-infected specimens to provide a foundation for assay development and enable rigorous performance evaluations.

Methods

Specimens from HIV-1 infected individuals were collected from 14 countries in Africa, Asia, Europe and South America. Identification of rare variants was focused on Cameroon and involved collections of thousands of small volume specimens from clinics and hospitals in both rural and urban settings. Rare variants were identified using an algorithm of sequential env gp120 V3 and env gp41 IDR peptide EIA's followed by molecular analysis. Large volume panels of HIV-1 seropositive blood donor specimens were routinely characterized using multiple serological assays and by sequencing of gag p24, pol integrase (IN) and env gp41 immunodominant region. Next-generation sequencing (NGS) using pan HIV-specific protocols for library generation from virus isolates and human plasma specimens was performed on an Illumina MiSeq instrument.

Results

Screening for non-group M variants yielded >200 group O, 9 group N and 1 group P infection. Based on our data, the prevalence of group O, N and P is 1-2%, 0.1% and <0.01%, respectively, among HIV-1 infected individuals in Cameroon. Characterization of large volume specimens (n>1600) yielded a genetically and geographically diverse panel representing the major subtypes and numerous CRFs and unique recombinants. Recombinants accounted for more than 60% of the panel with 78% of these comprising recognized CRFs. Application of NGS using pan-HIV specific amplification yielded full genome sequences of group M, N, O and HIV-2 virus isolates. Complete genome coverage was also observed for selected HIV-1 infected plasma samples.

Conclusions

The rapid rate of evolution, ongoing diversification and emergence of new strains will continue to pose a challenge to HIV-1 diagnostic and screening tests. Development of well characterized panels of genetically and geographically divergent specimens coupled with sequence information from diagnostically-relevant regions of the genome provides an invaluable resource for assay development and evaluation. Studies utilizing diverse HIV-1 specimens have revealed differences in p24 Ag detection sensitivity between HIV Ag/Ab combo assays. Application of NGS shows promise for increasing overall genome coverage and has the potential to revolutionize surveillance strategies. The continued diversification and global redistribution of HIV groups, subtypes and recombinants make it imperative that serological and molecular assays be designed to provide reliable performance on all HIV infections.

ASSURING THE QUALITY OF IN VITRO DIAGNOSTICS: WHO PREQUALIFICATION AND POST-MARKET SURVEILLANCE

Sands A

Department of Essential Medicines and Health Products, World Health Organization

Introduction

In settings without adequate regulatory capacity to assess the suitability of diagnostics for their intended use (pre-market assessment) and to monitor the on-going quality of diagnostics (post-market surveillance), WHO fills a gap through its WHO Prequalification of In Vitro Diagnostics Programme. The newly streamlined WHO prequalification assessment procedure allows for global stakeholders and national authorities to have a source of independent technical review of safety, performance and quality ahead of national registration. However, once products are placed on the market, their quality needs to be continually monitored, both proactively and reactively through post-market surveillance activities.

Proactive elements of post-market surveillance include independent lot verification testing and review of data generated through external quality assessment schemes and quality control programmes at user level. Reactive elements of post-market surveillance such as vigilance through reporting of complaints and quality issues are another important source of post-market information.

Materials and Methods

The author will present on the streamlined approach to WHO prequalification assessment of in vitro diagnostics with a focus on products used in resource-limited settings. The author will present on proposed WHO normative guidance and simplified systems for post-market surveillance of in vitro diagnostics, including complaint reporting and lot verification testing.

VALIDATION OF NON-WESTERN BLOT ALGORITHMS FOR HIV TESTING

Best, Susan

NRL, Melbourne, Australia

Internationally, HIV testing is often conducted using algorithms that do not include Western blot (WB) but instead comprise a combination of two or three immunoassays (IA). Using these algorithms, specimen testing is conducted on each of the assays in a particular sequence and may include a combination of rapid, semi-automated and / or automated IAs. This approach is taken to reduce cost, complexity and turnaround time. When an algorithm that comprises only a combination of IAs is rigorously validated, its performance can approach that of the gold standard, namely an algorithm that includes WB. However, an un-validated algorithm carries with it the risk that that some specimens may show common false-reactivity amongst the chosen IAs. This can lead to individuals being incorrectly identified as HIV positive.

When choosing IAs for an algorithm that will not contain WB, thought should be given to minimising the possibility of common false reactivity between the selected assays. This presentation will discuss a range of approaches to choosing IAs so that the likelihood of a false positive final interpretation from the algorithm is minimised.

HEPATITIS E VIRUS SERO-PREVALENCE: DOES LOCALLY ACQUIRED INFECTION OCCUR MORE FREQUENTLY THAN WE THINK?

Faddy HM[1, 2], Shrestha AC[1, 2], Flower RLP[1]

[1] Research and Development, Australian Red Cross Blood Service, Australia
[2] School of Medicine, the University of Queensland, Australia

Introduction/Objectives

Hepatitis E virus (HEV) is emerging as a public health concern in many industrialised countries. Traditionally HEV is often associated with travel to developing countries, where large water-borne outbreaks occur. However, in many industrialised countries autochthonous HEV is increasingly being identified, mainly associated with ingestion of contaminated undercooked food (primarily pork) or contact with infected animals. Infection is usually asymptomatic in immunocompetent individuals, however, in a small proportion of infected individuals more severe infection has been reported and chronic infection has occurred in solid-organ transplant recipients and in patients with immune suppressive conditions. HEV is nationally notifiable in Australia, with the majority of cases reported in travellers and only one published locally acquired case to date. In Australian commercial piggeries, HEV IgG seroprevalence of up to 95% has been documented. In this study we measured HEV seroprevalence in a cohort of blood donors from around Australia.

Methods

Plasma samples (n=3,237) from individual blood donors were tested for HEV IgG (Wantai HEV-IgG ELISA, Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.; ELISA 1). Donor demographics, including age, gender, state of residence and overseas travel disclosure, were obtained. All samples testing positive for HEV IgG, as well as age matched controls (testing negative for HEV IgG on the Wantai assay), were tested for HEV IgG with a second ELISA (MP Biomedicals HEV ELISA IgG, MP Biomedicals Australia Pty Ltd.; ELISA 2).

Results

Using ELISA 1, HEV IgG was detected in 194 donors (5.99%, 95% CI 5.18-6.81). As expected, seropositivity increased with increasing donor age ($p < 0.05$). There was no difference in HEV IgG seropositivity between the sexes (females: 5.37% (95% CI 4.21- 6.53); males: 6.50% (95% CI 5.36-7.65); $p > 0.05$), nor between donors residing in the different states and territories ($p > 0.05$). Importantly, HEV IgG was detected in 3.37% (95% CI 1.63-5.10%) of donors not reporting overseas travel. Of the 194 samples that tested positive for HEV IgG on ELISA 1, 92 (47%) were also positive for HEV IgG on ELISA 2, while 1 of the 200 (0.5%) 'age matched' controls was weakly positive on this assay.

Discussion/Conclusion

This study showed that for this sample population, 6% of Australian donors had serological evidence of a prior infection with HEV (based on testing with the Wantai assay), which is similar to other non-endemic countries that also used this assay. We also demonstrated HEV seropositivity in donors who did not report international travel, suggestive of locally acquired infection. The ELISA results were concordant for 47% of HEV IgG positive samples, an observation consistent with the known variability between different HEV ELISAs, indicating that care should be taken when interpreting the results of HEV serology.

FOCUS ON EBV

Anna-Maria Costa[a], Jeff Chan[b], and Sepehr N. Tabrizi[a, c, d]

[a]Department of Microbiology and Infectious Diseases, Royal Children's Hospital, Victoria, Australia
[b]Southern Cross Diagnostics, Sydney, Australia
[c]Murdoch Children's research Institute, Parkville, VIC, Australia
[d]Department of Obstetrics and Gynaecology, University of Melbourne, VIC, Australia

Introduction

Molecular detection of EBV DNA is a useful diagnostic tool for evaluation of EBV-related diseases. PCR-based EBV diagnostic techniques may help with the identification and monitoring of EBV-related diseases in patients.
We evaluated quantification of EBV by the Simplexa EBV PCR assay (Focus Diagnostics, USA) with existing extraction and post-elution liquid handling instruments in our laboratory.

Methods

Ten-fold serial dilutions from 5.70 $\log_{10}$ IU/mL to 0.70 $\log_{10}$ IU/mL of EBV WHO International Standard were prepared in triplicate in a matrix of both pooled EBV-negative plasma and pooled EDTA whole blood samples. Nucleic acid extraction was performed on the MagNA Pure 96 instrument using the MagNA Pure 96 DNA and Viral RNA Small Volume Kit. Extraction was performed with 200uL of seeded EDTA whole blood or plasma sample eluted into 50uL. Post elution liquid handling for PCR set-up into the 3M Universal disc was formatted on the Tecan EVO 100. EBV quantitative PCR was performed with 5uL NA extract and 5uL master mix utilising the 3M Integrated Cycler.

Results

The serial dilutions of EBV WHO International Standard in pooled plasma and EDTA whole blood showed good correlation between sample types with the lowest limit of detection for plasma at 1.7 $\log_{10}$ IU/mL, and EDTA whole blood at 2.7 $\log_{10}$ IU/mL.

Discussion

Preliminary testing of triplicate dilutions suggests that the assay may have a higher sensitivity in pooled plasma samples compared to pooled EDTA whole blood samples, however, this assay shows good dynamic range in both pooled plasma and pooled EDTA whole blood samples.

The Simplexa EBV PCR assay can be incorporated into a diagnostic laboratory using existing extraction and post-elution equipment, and only requires the Integrated Cycler as additional instrumentation.

OUR EXPERIENCE: *CLOSTRIDIUM DIFFICILE* REAL-TIME PCR TESTING MADE SIMPLE

Pyretzis E, McIlvride K, Sivertsen T, Chambers I

Douglass Hanly Moir Pahology, Sydney, Australia

Introduction

Clostridium difficile is an anaerobic Gram positive, spore forming bacillus that causes antibiotic-associated colitis and diarrhoea. Symptoms range from mild nuisance diarrhoea, fever, abdominal pain to pseudomembranous colitis and if untreated can become life-threatening. The disease is related to the production of two potent exotoxins, toxin A (enterotoxin) and toxin B (cytotoxin) where toxin B is the essential virulence factor of *C. difficile*. The urgency of diagnosing the virulence factors of *C. difficile* becomes apparent when severity and spread of infection is considered. *C. difficile* is transmitted from person to person by the oral-faecal route. Once introduced into an environment such as hospitals or nursing homes, *C. difficile* forms spores which are highly resistant to decontamination agents. While *Clostridium difficile* associated disease (CDAD) affects all age groups, most severe infections are related to hospitalised elderly, which could rapidly result in death if untreated.

Therefore a prompt and accurate diagnostic test is required to detect the pathogen and administer appropriate treatment accordingly, limiting spread of infection. In the past, enzyme immunoassay (EIA) was employed to test for the presence of either toxin A and B combined or toxin B alone. EIA is a rapid test however it has been shown that further confirmation testing such as glutamate dehydrogenase (GDH) was required due to low sensitivity rates. While specificity is generally good and false-positives uncommon, the diagnostic sensitivity of these assays is only moderate and the false-negative rate is unacceptably high. In recent years, application of real time PCR-based assays for infectious disease diagnosis has been proven advantageous in terms of sensitivity, specificity and turn around time. The Focus Diagnostic Simplexa *C. difficile* Universal Direct assay (distributed by Southern Cross Diagnostics) is one such real time PCR-based assay currently being used in Molecular Biology at Douglass Hanly Moir Pathology.

The assay which is run on the 3M Integrated Cycler, detects toxogenic *C. difficile* in liquid or unformed stool samples and DNA extraction and amplification is performed in one step. The gene sequence targeted for detection of *C. difficile* is within a well conserved region of the toxin B gene (tcdB). The tcdB gene of *C. difficile* (which encodes toxin B) is considered to be its primary virulence factor and is present in virtually all diarrhoeagenic strains, including the hypervirulent O27 ribotype. Detection of the tcdB gene by PCR provides rapid and accurate diagnosis, which in turn allows prompt implementation of treatment and infection control measures. My presentation will focus on our implementation of this assay as a routine diagnostic test in Molecular Biology at Douglass Hanly Moir Pathology

Title:

VELA DIAGNOSTIC's *SENTOSA*® SQ HCV GENOTYPING ASSAY – A NEW NGS HCV DIAGNOSTIC KIT

Authors:

Rui Zhang, Lee Charlie, Siow Rouh San, Lou Chao Ping, Choi Dawn, Leong See Ting, Kung Terrence, Huang Wen, Rakhmanaliev Elian

Affiliation(s):

Vela Research Singapore Pte. Ltd.

Abstract:

Introduction:

Sanger sequencing has been the standard method in clinical DNA sequencing for several decades. But next-generation sequencing (NGS) technology is now revolutionizing the field of clinical diagnostics. NGS is highly efficient, producing an enormous amount of information at low cost in a relatively short period of time. Genotyping of hepatitis C virus (HCV) is extremely important for monitoring of antiviral therapy. Vela Diagnostics has developed a novel from-sample-to-result NGS-based automated workflow for HCV genotyping.

Methods:

The *Sentosa*® HCV Genotyping Assay is designed for genotyping of HCV in human plasma and serum based on sequencing of several HCV regions using the *Sentosa*® SX101 nucleic acid extraction platform in conjunction with the *Sentosa*® NGS system. The *Sentosa*® data reporting software automatically generates information that is reported to the laboratory information system via *Sentosa*® Link.

Results:

Sentosa® HCV Genotyping Assay is a part of a two day automated NGS workflow requiring minimal hands-on time It is able to run up to 15 clinical samples simultaneously. A unique feature of the system allows the monitoring of contamination by PCR products, RNA extraction and workflow failures as well as the presence of PCR inhibitors. The limit of detection for HCV genotypes 1-4 and 5-6 is 1,000 IU/mL and 2,000 IU/mL, respectively. Assay reproducibility based on different performance parameters was determined to be 98.9%. No occurrence of cross-contamination using highly concentrated positive samples and no cross-reaction with clinically relevant viruses and human genomic DNA has been observed.

Discussion/Conclusion:

The *Sentosa*® SQ HCV Genotyping Assay provides a ready-to-use efficient, sensitive and reliable solution for the genotyping of Hepatitis C Virus. The *Sentosa*® SQ HCV Genotyping Assay is integrated into Vela's *Sentosa*® system. The system consists of a multi-purpose *Sentosa*® SX101 robotic platform which performs automated lysis, RNA extraction, RT-PCR set-up and library preparation and is integrated into the *Sentosa*® NGS system. This approach provides standardized versatility from sample input to result.

HEPATITIS B VIRUS DNA SCREENING OF AUSTRALIAN BLOOD DONORS REVEALS THE PRESENCE OF OCCULT HEPATITIS B VIRUS INFECTION

Kiely P[1], Seed CR[2], Margaritis AR[2] and Yang H[3].

[1] Australian Red Cross Blood Service, Melbourne

[2] Australian Red Cross Blood Service, Perth

[3] Australian Red Cross Blood Service, Sydney

Introduction

Until recently, the detection of hepatitis B virus (HBV) infection in Australian blood donors has been primarily based on screening for HBV surface antigen (HBsAg). However, in July 2010 the Australian Red Cross Blood service implemented HBV nucleic acid testing (NAT). Subsequently, the screening of blood donors by HBV NAT has revealed the presence of occult HBV infection (OBI), a form of chronic infection characterised by detectable serum HBV DNA and antibodies to HBV core antigen (anti-HBc) but without detectable HBsAg.

Objectives

To describe the detection of OBI in Australian blood donors during the first three years of HBV NAT.

Methods

During the first three years of HBV NAT (5 July 2010 to 30 June 2013) all donors were screened for HBV DNA using the PROCLEIX ULTRIO (HIV-1/HCV/HBV multiplex) assay (Ultrio) on the PROCLEIX TIGRIS automated system (Gen-Probe/Novartis Diagnostics, San Diego/Emeryville, CA). Samples initially reactive on the Ultrio assay were 'discriminated' to identify the specific virus using the PROCLEIX HIV-1, HCV and HBV discriminatory assays. All donors were also screened for HBsAg using the Abbott PRISM HBsAg chemiluminescent immunoassay (Abbott Diagnostics, Delkenheim, Germany). Samples reactive on the NAT and/or HBsAg assays were also tested for anti-HBc by either the AxSYM CORE or Architect anti-HBc II assays, and for anti-HBs by either the AxSYM AUSAB or Architect AUSAB assays (Abbott Diagnostics, Delkenheim, Germany).

Results

(i) During the three years of HBV NAT a relatively small number of donors with HBV acute serological window period infection (HBV DNA+/HBsAg-/anti-HBc-) were detected annually (2, 6 and 0 cases, respectively) and the annual differences in detection rates were not significant. (ii) In contrast, a substantially higher number of donors with OBI were detected annually (27, 15 and 9 cases, respectively) and the annual OBI detection rate significantly decreased over the three year study period (4.87, 2.72 and 1.72 per 100,000 donors respectively). (iii) The number of HBV inconclusive donors (non-discriminated NAT reactivity/HBsAg-/anti-HBc+) detected annually was 28, 24 and 16, respectively, but the annual detection rate (5.05, 4.98 and 3.06 per 100,000 donors, respectively) did not vary significantly over the 3-year study period. Of the HBV inconclusive donors who were available for followup testing, the annual percentage who were confirmed as OBI at followup was 11.4%, 17.1% and 14.3%, respectively. (iv) Both the OBI and HBV inconclusive donors were predominately repeat donors (82.4% and 91.2%, respectively) while, in contrast, only a minority of the acute serological window period donors were repeat donors (25.0%). (v)The annual percentage of OBI and HBV inconclusive donors with one or more NAT NR donations prior to the index NAT reactive donation showed an overall increase over the 3-year study period and reached statistically significance levels for the OBI donors (29.6%, 73.3% and 55.6%, respectively) but not for the HBV inconclusive donors (60.7%, 75.0% and 87.54%, respectively). (vi) In addition, the average period of time from the first NAT non-reactive donation to the index NAT reactive donation increased annually for both OBI donors (6.6, 13.9 and 18.4 months, respectively) and HBV inconclusive donors (5.5, 13.2 and 19.7 months, respectively).

Conclusions

The implementation of HBV NAT by the Blood Service in July 2010 has provided further insight into the epidemiology and complexity of HBV infection in Australian blood donors. Specifically, it has revealed the presence of HBV infection without detectable HBsAg, both acute serological window period infection and chronic OBI. The significant overall decline in the detection rate of OBI in repeat donors may in part reflect that during the initial period of HBV NAT screening, those OBI donors with relatively higher levels of HBV DNA will be detected the first time they are screened by HBV NAT while those with lower, intermittently detectable levels of HBV DNA may only be detected after several NAT non-reactive donations. Our experience of 3 years of HBV NAT has also demonstrated the need for careful follow up of donors with non-discriminated NAT reactivity with concomitant anti-HBc reactivity as a number of donors with this result profile were confirmed as OBI at followup.

SCREENING FOR BLOOD-BORNE VIRUSES IN ORGAN AND TISSUE TRANSPLANTATION

Cristina Baleriola, Chee Choy Kok, Sanghamitra Ray, Peter Robertson, William D Rawlinson

SEALS NAT Laboratory, Prince of Wales Hospital, Randwick, NSW 2031, Australia

Serology and nucleic acid test (NAT) screening for HBV, HCV and HIV are key steps to prevent blood-borne virus (BBV) transmission from organ donors. We assessed optimal use of NAT in the solid organ transplant setting for 4 years through evaluation of requests and results for NAT and serology testing in routine and increased risk donors (IRD).

Review of NAT and serology results for donor screening from October 2009 to December 2013 showed NAT was performed on a total of 458 donors; 109 performed prospectively while 349 performed retrospectively. There were 315 organs (2.9 organs/donor) retrieved from IRD and 1,211 organs retrieved from 349 average-risk donors (3.5 organs/donor).

NAT screening of 44 serology positive (1 HIVAb+, 11 HCVAb+, 32 HBV HBcAb or HBsAg+) donors resulted in transplantation of 79 additional organs that without NAT screening would either have not been used or used with restrictions. The NAT assays demonstrated the HIV donor was NAT positive, the HCV donors were 9/11 NAT positive, and the HBV donors were 3/32 NAT positive. Of the HBV donors, 29/32 were HBcAb positive and 3/32 NAT positive. Donors accepted for transplantation were HIV 0/1, HCV 4/11 (all of whom were NAT positive and transplanted into HCV RNA positive recipients) and 21/32 HBcAb positive donors transplanted. Most transplanted organs with positive serology and negative NAT were from HBcAb positive donors (n=21 donors with 75 organs transplanted) and 4 organs retrieved from 4 HCV positive donors.

The availability of a 24/7 NAT screening service for organ donors provides diagnosis within 8 hours of blood delivery. This enabled the use of organs from donors with positive serology but negative NAT and donors with false-positive serology results. This algorithm allowed use of organs from IRD with safer expansion of the donor pool.

A RAPID HCV GENOTYPING ASSAY TO EXPAND THE DONOR POOL IN ORGAN AND TISSUE DONATION

Ray S[1], Baleriola C[1], Kok CC[1], Rawlinson W [1,2]

[1] South Eastern Area Laboratory Services, Prince of Wales Hospital, Randwick, NSW 2031
[2] School of Medicine, University of New South Wales, Kensington, NSW 2052

Introduction:

Transplantation of organs from HCV antibody positive donors to HCV PCR positive recipients is a standard practice in many countries, including Australia, to increase the number of transplanted organs. Donor HCV genotype can potentially alter the long-term outcome of the transplanted graft as HCV genotype 1 shows lower response to interferon-ribavirin based therapies and may transmit the infection in a more aggressive manner to recipients. Currently there is no commercially available genotyping assay capable of distinguishing HCV genotypes within the time-span established for organ transplantation (approximately 8 hrs). SEALS has developed a rapid HCV genotyping assay to distinguish genotype 1 and non-1, by use of melting curve analysis (MCA) using a single set of fluorescent resonance energy transfer (FRET) probe. The performance characteristics of the assay were evaluated using the INNO-LiPA™ as reference method (gold standard).

Objectives:

- To develop a rapid HCV genotyping assay to distinguish genotype 1 and non-1
- To assess the performance characteristics of the assay using the INNO-LiPA™ as reference method

Methods:

After automated extraction of total nucleic acid using the Roche COBAS Ampliprep Instrument, a RT-PCR was performed in Roche LightCycler 480 instrument to amplify nucleotide 6-329 of the 5'-untranslated region of HCV. The RT-PCR product was further amplified using a semi-nested PCR (HCV Genotyping PCR) in Roche Light Cycler 480 instrument. The final product was analysed by melting curves using fluorescence resonance energy transfer (FRET) probes for genotype determination. Genotypes were compared in a blinded fashion with INNO-LiPA ™ test (Bayer Diagnostics) as a reference method on 209 EDTA plasma samples.

Results

A total number of 209 EDTA plasma samples were tested with both INNO-LiPA™ and SEALS Rapid HCV Genotyping Assay. SEALS Rapid HCV Genotyping PCR has a sensitivity and specificity of 98% and 97.7% respectively with the amplification failure rate of 9%. The amount of virus needed to be successfully genotyped by this method ranged between HCV RNA $\log_{10}$ 3.49 and 7.56 IU/mL. 102 of 103 samples identified as genotype 1 were concordant with the results of the comparison method (INNO-LiPA™). 86 of 87 samples identified as non-1 were concordant with the results of the comparison method (INNO-LiPA™). The melting curve analysis corresponding to genotype 1 was clearly separated from genotype non-1 (2a/c, 2b, 3a and 4).

Conclusions

The Rapid HCV genotyping assay developed by SEALS has the potential to accurately distinguish HCV genotype 1 and "non-1" in 98.5 % of times as compared with the INNO-LIPA assay with a turn-around time of 5 hours. This is a substantial improvement from the current situation where no suitable genotyping assay is available for the recipients eligible for HCV positive organ transplantation.

CADAVERIC BLOOD TESTING. CHALLENGES IN THE IVD FRAMEWORK

Leeman C

Serology Department – SydPath St Vincent's Hospital, Sydney

Introduction

In Australia donors of human tissues are subject to screening for infectious diseases. This is performed by a small number of laboratories under special license conditions with the Therapeutic Good's Administration (TGA).

Tissues may be retrieved from living or deceased donors. Testing specimens from deceased donors has many complications. Specimen collection is difficult and as a result specimen quality is often poor. The question remains "Will a sample collected post mortem be of sufficient quality to produce a reliable result?"

Manufacturers of Nucleic Acid Tests (NAT) for HIV, HBV and HCV have validated their assays for use on cadaveric bloods. However, manufacturers of serological assays have until now been reluctant to perform such validations. This has meant laboratories testing for HIV, HBV, HCV, Syphilis and HTLV I/II have had to complete extensive internal validations using cadaveric bloods in order to meet the regulatory requirements under the code of GMP (Good Manufacturing Practise).

The introduction of the new IVD framework has added further requirements for these laboratories to meet. Assays used 'off label' are recognised as in-house IVDs. The laboratory using an IVD 'off label' becomes an in-house manufacturer. Because all the infectious disease IVDs for screening are in Class 4, the requirements that a laboratory would have to fulfil as the manufacturer are impractical.

In 2012 the NRL (on behalf of the testing laboratories) and the Australasian Tissue and Biotherapeutics Forum (ATBF) approached IVD manufacturers to clarify their position on this impending issue.
Following this Abbott Diagnostics made clear their intention to perform validations of their infectious disease assays. This however has been no 'walk in the park'.

As recently as 1 July 2014 Abbott Diagnostics notified its Australian customer base that the cadaveric claim had been extended to almost all of their assays. This has relieved the burden of registering in-house Class 4 IVDs.

Despite these welcome advances there still remains a number of challenges for laboratories, regulators and manufacturers alike.

NRL EQAS: OBSERVATIONS FROM THE NRL SEROLOGY BLOOD SCREENING PROGRAM 2013

Vincini GA, Smeh K, Cabuang LM, Dimech W, Best SJ

NRL, Melbourne, Australia

Background

NRL has conducted external quality assessment schemes (EQAS) for blood screening laboratories for more than 20 years. In 2013, NRL introduced a 20-sample multi-marker serology EQAS designed specifically for the blood/donor screening laboratory (MMBS4320). MMBS4320 allows identification of aberrant results and improves understanding of testing strategies. Analysis of results provides valuable comparative data on the performance of assays as well as informing participants of their performance relative to others using the same assay (known as a peer group). The aims of the scheme were to identify sources of aberrant results and to review the serology testing strategies implemented by laboratories.

Methods

MMBS4320 2013 consisted of three 20-member panels. Each panel contained samples positive for one or more of anti-HIV, anti-HCV, anti-HBc total and HBsAg, as well as samples negative for all these markers, sourced from blood donors. In two samples, recombinant HIV-1 p24 antigen was spiked into normal human plasma to simulate early HIV infection. Participants were requested to test the samples in the same manner as their donor samples. Results were submitted to NRL using OASYS, an internet-based proficiency test application. The results were then compared with internally-generated reference results and with those obtained by the peer group.

Results

A total of 116 donor screening laboratories from 23 countries participated in MMBS4320 2013 generating over 31,000 results from 113 assays. In the analysis of testing strategies, there were many instances where the testing strategy did not allow for adequate detection of HIV-1 p24 antigen positive samples. Many examples of aberrant test results were observed based on poor assay sensitivity, particularly where rapid tests were used or where third-generation tests were used in isolation. Further analysis of aberrant results revealed some of the errors identified were due to data entry.

Conclusions

Overall, the performance of all assays used was of a high standard. MMBS4320 provided participants with valuable information regarding their performance relative to their peers and created many opportunities to educate and facilitate continuous improvement in institutions delivering donor screening at a local, regional or national level.

BLOOD TRANSFUSION SYSTEM AND QUALITY ASSURANCE IN THAILAND

Tasanee Sakuldamrongpanich

National Blood Centre, Thai Red Cross Society

National Blood Centre, Thai Red Cross Society (NBC,TRCS) has been designed by the government to be responsible for National Blood Program in Thailand since 1966. Currently, The Blood transfusion system (BTS) consisted of a National Blood Centre (as headquarter), 12 Regional blood centres (RBCs) and 6 branches in Bangkok and 158 branches in the provinces. NBC and RBCs perform donor recruitment, blood collection, donor testing, processing into blood components and distribution for hospital used. The branches are hospital base blood banks, do the same functions as NBC but majority of them sent the donation samples to be tested at NBC or RBCs. In 2013, approximately 80% of whole country blood collection were tested by TRCS.

In order to ensure the safety and quality of blood products, experts from NBC, university hospitals and Ministry of Public Health (MOPH) drafted the first National policy on Blood Program and was announce in 1995 and the new version was launched in 2010. Standard for Blood Banks and Transfusion Services, Donor selection guideline, Donor blood collection guideline and National Guideline on clinical use of blood and blood component were also developed and used.

With the fundamental mission of providing good quality products and services, NBC has long been continued building up the establishment of Quality Assurance and Quality Management System (QMS). During 1997-1999, QMS initiated by WHO and ISO 9002 were started to implement at NBC. In 2000, NBC was certified for ISO 9002 and later on up graded to ISO 9001:2000. In 2001, WHO/SEAR designated NBC to be regional QMS training center and department of Medical Sciences, MOPH to be regional provider for EQAS on TTIs and blood group serology for member countries in order to strengthen the quality and safety of BTS. At present, all activities in NBC and 12 RNCs were certified ISO 9001:2008. January this year, the tests in donor screening lab, HLA lab and some tests in quality control lab were received the certification of ISO 15189:2007. This certification will be extended to 12 RBCs.

Due to the policy from MOPH for all hospitals to develop QMS, the hospital blood bank laboratories have also been implemented QMS and certified for either ISO or laboratory accreditations.

DEVELOPMENT OF WHOLE BLOOD EQAS FOR HIV POINT OF CARE TESTING

Braniff S-J, Vincini G, Wilson K and Best SJ

NRL, Melbourne, Australia

Introduction

The use of rapid Point of Care test (POCT) devices that operate with finger-prick blood as the sample source to screen for HIV is increasing in clinical and community care settings. Participation in an External Quality Assessment Scheme (EQAS) is recognised as critical for maintaining quality in pathology results. Therefore, testing sites offering HIV screening at point of care in Australia must participate in EQAS as required by the National HIV Testing Policy. Usually, HIV serology EQAS samples are serum or plasma supplied in tubes. In a POCT environment, whole blood samples more closely represent the actual samples tested at the site. In 2013 NRL committed to producing a whole blood HIV POCT EQAS for Australian participants beginning with a pilot program. Using information gathered from the pilot EQAS a three test event whole blood HIV POCT EQAS is currently underway in 2014.

Methods

Red blood cells were harvested from a whole blood donation and treated with stabilisation buffer. Aliquots of stabilised whole blood cells were re-constituted with plasma samples whose HIV reactivity was fully characterised and packaged in dropper bottles. Five-member panels were assembled and sent to participants along with handling instructions and results forms. Participants had 10 days to test the sample panel and return the completed results forms to NRL.

Results/Discussion

Seventeen HIV test sites around Australia participated in the pilot whole blood HIV POCT EQAS; gaining experience in EQAS testing and providing valuable feedback to NRL about the whole blood sample format. In general, all participants performed well with no aberrant results reported. Sample A exhibited some flow problems with the Alere Determine HIV Combo test and this necessitated a design review. The results of the pilot program were used to further improve the EQAS that is being provided as a full program in 2014.

Conclusions

NRL has delivered whole blood HIV POCT EQAS samples to participants around Australia for a pilot program in 2013 that has formed the basis for the full scheme (three test events) for 2014. Refinement of the whole blood sample type continues in order to provide an HIV POCT EQAS that is robust and compatible with POCT site procedures irrespective of the test that is in use.

EXTERNAL QUALITY ASSESSMENT FOR HIV CO-RECEPTOR TROPISM TESTING

Land S[1], Vincini G[1], Swenson LC[2], Harrigan PR[2]

[1]NRL, Melbourne, Australia; [2]BC Centre for Excellence in HIV/AIDS, Vancouver, British Colombia, Canada.

Background

Chemokine receptor 5 [CCR5] inhibitors are appropriate for treatment of individuals infected with HIV-1 CCR5 strains. Many laboratories use genotypic methods, developed in-house, to determine HIV-1 co-receptor tropism (tropism). This External Quality Assessment Scheme [EQAS] aims to investigate protocols and outcomes of laboratories performing tropism testing; the long term objective is to assist in standardisation of testing outcomes and develop a collaborative international testing network.

Methods

Laboratories followed their standard protocol and reported tropism and False Positive Rate [FPR] values. Details regarding protocols were gathered by questionnaire. Panel I samples [n=12] were chromatograms of the HIV-1 *env* gene [V3 loop]; tropism was determined by a reference laboratory. Panel II required tropism determination from 10 DNA samples derived from clones [n=2] and HIV-1 infected individuals [n=8]; shipped ambient to participants; tropism was determined by consensus.

Results

Laboratories [n=24] from 15 countries participated in the EQAS. Most [18 of 24] routinely performed triplicate testing; most of these [15 of 18] determined tropism from the lowest FPR value derived from triplicate testing. Various software programs were used to assemble and edit sequence, the most common being ReCall followed by ChromasPro, Segman, SeqScape and Staden Package. All but one used the online algorithm Geno2pheno to determine tropism from viral sequence. Geno2pheno FPR cut-offs varied between 5% and 20%. Nearly half [11 of 23] the laboratories testing Panel I reported discordant results for at least one of 10 samples; including three laboratories reporting CXCR4-using [X4] virus as CCR5 [R5]. Most discordant tropism calls were not a consequence of inter-laboratory difference in FPRs; they were more likely due to differences in sequence interpretation. All laboratories [n=17] testing Panel II reported the reference tropism [X4] for both clone-derived samples; three reported X4-using virus as R5 and none reported R5-using virus as X4 for samples derived from patient material.

Conclusion

In a group of international laboratories, quality assessment revealed inter-laboratory variability in HIV co-receptor tropism determination by genotypic methods. This highlights the need to quality assure laboratories' test outcomes to ensure competency, consistency and comparability. Experience has shown continued participation in EQAS can assist laboratories to reach concordance in genotypic test outcomes derived using various protocols, including those developed in-house [Land et al. 2013 and Tu et al. 2013]. NRL continues to offer EQAS for genotypic tropism testing to assist in improvement of inter-laboratory concordance and to encourage a collaborative testing network.

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SUMMARY OF HIV-1 VIRAL LOAD NUCLEIC ACID TESTING EXTERNAL QUALITY ASSESSMENT SCHEMES, 2013-2014

Taqi RS, Cabuang LM, Best SJ

NRL, Melbourne, Australia

Introduction

NRL provides Quality Assurance (QA) programs to laboratories that test for blood-borne infectious diseases by serology and/or nucleic acid testing (NAT). One of the components of NRL QA are External Quality Assessment Schemes (EQAS). Participation in EQAS gives participants a means of assessing independently their laboratory performance and comparing their results with those of others in their peer group. It also offers a means of examining the performances of different assays.

Objective

To examine the performance of HIV-1 viral load (VL) testing with respect to inter-run reproducibility and linearity of detection by analysis of results reported by EQAS participants.

Methods

Participants' data from four HIV-1 viral load (VL) EQAS test events (TE1, TE2, TE3 and TE4) were analysed. Each test event consisted of five well characterised plasma samples. For three of these test events, some of the samples that contained HIV RNA (group M, subtype B) were common between the test events, or were presented in tenfold dilution series. The fourth EQAS panel consisted of samples that all contained HIV RNA (group M, Subtype AE) presented as a ten-fold dilution series. The panels were constructed in this way to allow analysis of inter-run reproducibility and assessment of the linearity of detection. Log_{10} transformed results reported by participants were grouped by assay (peer groups) for analysis.

Results

Fifty laboratories participated in TEs 1-3 and their results fell into 13 peer groups. Analysis showed that the results of inter-run reproducibility for replicate samples provided over the test events differed by no more than $0.28 log_{10}$ copies/mL. The difference between results for ten-fold diluted samples did not differ by more than $1.0 \log_{10} \pm 0.3 \log_{10}$ copies/mL.

Fifty-nine laboratories participated in TE4 and their results fell into 11 peer groups. One of the subtype AE samples in the panel was provided at a viral load that was close to the lower limit of quantification for some assays. Fifteen percent of participants reported either "Not Detected" or "Below Linear / Detection Limit" for this sample. One participant reported "Not Detected" for all of the subtype AE samples presented in the panel.

Conclusion

NRL EQAS results indicate that HIV-1 VL testing provides linear and reproducible quantification of HIV RNA. Participation in EQAS allows laboratories to assess the performance of viral load assays in different HIV-1 subtypes.