HPV TESTING- WARTS 'N' ALL

Field T

Healthscope Pathology, Clayton, Victoria

Currently in Australia, the national cervical screening program targets the 18-69 year old demographic with a screening interval of 2 years using conventional Pap smear for the primary screen and reflex testing to HPV by molecular means for "test of cure". There is no facility for "self-collect" under the current program and the use of liquid based preparations is not funded under the MBS.

This will all change in 2017 when Australia adopts a new screening program that differs significantly to the current schedule. It is proposed that the new program will target the 25-69 year old demographic with an exit test for the 70-74 year old women. The screening interval changes to 5 years and will use HPV testing as the primary test with cytology as the reflex test. There will be a facility for self-collect of HPV sample for the under-screened or never screened women and a de-listing of the MBS item for pap smears. Liquid based preps will become the norm as these are required for the Molecular assays with cytology reflex. Self-collect samples will most likely be dry swabs for molecular testing however cytology reflex will not be an option for this type of collection and positive HPV patients will need to represent themselves for repeat testing using liquid based preparations.

This change will have an immense impact on the testing laboratories and requires careful and considered planning now to be able to accommodate and facilitate these changes. Molecular testing numbers will increase significantly as opposed to cytology testing numbers which will diminish proportionally with resultant staffing issues. There are many considerations including request forms, billing , reporting, lab logistics and equipment required just to name a few.

Equipment and testing platforms currently available in Australia are considered in this presentation discussing the essential criteria for HPV testing from the manufacturer and laboratory perspective. Points of discussion include regulatory approvals, clinical trials, adherence to Meijer criteria and utility for "test of cure" and primary screening. Compatibility with collection devices, workflow and key strengths are also discussed in conjunction with consideration of molecular targets currently in use.

MOLECULAR APPROACHES TO ENHANCE TESTING FOR GONOCOCCAL ANTIMICROBIAL RESISTANCE

This presentation was sponsored by Cepheid

Trembizki E on behalf of GRAND study investigators.

QPID laboratory, QCMRI, The University of Queensland, Australia

Neisseria gonorrhoeae antimicrobial resistance is a major global concern. There are limited new treatment options and as a result of increased use of nucleic acid amplification testing for diagnosis there is a lack of resistance data. Strain-typing and characterisation of associated resistance mechanisms is pivotal to understanding the development and spread of antimicrobial resistance. This NHMRC-funded GRAND (Gonorrhoea Resistance Assessment via Nucleic acid Detection) study was a national study aimed at determining the molecular basis of antimicrobial resistance in our local N. gonorrhoeae isolates with a view to implementing broad-based molecular surveillance. The study has allowed us to identify patterns and strains which may 'fly under the radar' of phenotypic resistance testing and has provided new insight into the spread of N. gonorrhoeae and resistance mechanisms in both remote and metropolitan populations in Australia. In addition, our molecular PCR assays provide promising steps towards individualised treatment of N. gonorrhoeae infections.

EVALUATION OF THE SEEGENE ANYPLEX™ II STI-7 DETECTION ASSAY FOR THE DIAGNOSIS OF SEXUALLY TRANSMITTED DISEASES (STD)

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Introduction:
The Anyplex TM II STI-7 detection kit represents a new type of multiplexed molecular test using Cyclic-Catcher Melting Temperature Analysis (CMTA), which allows the simultaneous amplification and detection of seven pathogens on a single channel detection. The assay uses the Tagging Oligonucleotide Cleavage and Extension (TOCE) method PCR for detection of Chlamydia trachomatis (CT), Neisseria gonorrhoeae (NG), Mycoplasma hominis (MH), Mycoplasma genitalium (MG), Ureaplasma urealyticum (UU), Ureaplasma parvum (UP) and Trichomonas vaginalis (TV) in urine, genital swabs and liquid-based cytology specimens.

Objectives:
We evaluated the performance characteristics of the Anyplex TM II STI-7 detection assay and determine its suitability for use in a high throughput, tertiary referral laboratory.

Methods:
A total of 79 retrospective samples (69 urine samples and 10 genital swabs) were tested, 60 of which previously tested positive for UU, UP, MG, MH and TV using an in-house assay. A further 98 samples (58 urine samples and 40 genital swabs), tested in a second reference laboratory were simultaneously tested prospectively in parallel using the Anyplex TM II STI-7 assay.

Results:
Of the total of 177 samples tested both retrospectively and prospectively for Mycoplasma spp., the sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) achieved for the Anyplex TM II STI-7 kit was 97%, 99%, 97% and 99% respectively. The sensitivity, specificity, PPV and NPV for Ureaplasma spp. calculated based on 79 retrospective samples and the values achieved for the Anyplex TM II STI-7 were 98%, 96%, 98% and 96% respectively.

Conclusions:
The Anyplex TM II STI-7 assay offers rapid screening for seven common pathogens known to cause sexually transmitted diseases. The outcome of the evaluation revealed a good correlation with the retrospective and prospective analysis and justifies the manufacturer's claim. The routine use of this assay in the laboratory has improved the TAT of reporting of MG and TV in urine and genital swabs.

EVALUATION OF THE ABBOTT REAL TIME HCV GENOTYPE II ASSAY FOR RESOLVING INCONCLUSIVE HCV GENOTYPE BANDING PATTERNS FROM THE SIEMENS VERSANT HCV GENOTYPE 2.0 LIPA ASSAY

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Introduction:
The Siemens Versant HCV Genotype 2.0 assay is a line probe assay (LIPA) for the identification of HCV genotypes 1 to 6a-b, 6c-l and genotype 1 subtypes a and b. Banding patterns for the 5- UTR and the Core region are compared to reference banding patterns of known HCV genotypes supplied by the manufacturer. In contrast, the Abbott RealTime HCV Genotype II assay (HCV GT II) uses real-time PCR and fluorescent probes for the detection of HCV genotypes 1 to 6a-b and genotype 1 subtypes a and b. In the 2014 year we encountered 40 unresolved genotypes from HCV RNA positive samples (>50 IU/mL) using the LIPA method. The HCV GT II assay may be useful to resolve these inconclusive genotypes.

Objectives:
The objective of this investigation is to evaluate the use of the HCV GT II assay to resolve inconclusive LIPA results and assess the suitability of the HCV GT II assay for routine HCV genotype determination.

Methods:
A randomly chosen sub-set of inconclusive genotype samples (n=22) of known viral load, previously tested using the LIPA method, were tested retrospectively using the HCV GT II assay using the Abbott m2000 system.

Results:
The HCV GT II assay was able to resolve the HCV genotype for 20 samples tested. Two samples demonstrated a HCV GT II assay result of HCV Not Detected. These samples had a viral load of <500 IU/mL (reported lower limit of detection for the HCV GT II assay), however one sample in the subset was able to be genotyped with a viral load of 178 IU/mL. No samples required reflex testing to identify HCV genotype 6c-l. The HCV GT II assay identified HCV genotype 1a (n=3), 1a + 2 (n=1), 1a + reactivity with 3 (n=4), 1b + reactivity with 4 (n=1), 3 (n=8) and 4 (n=3).

Conclusions:
We conclude that the HCV GT II assay was useful to resolve inconclusive HCV genotypes generated by LIPA. In addition the HCV GT II assay may also be useful for resolving mixed infection. Cross-reactivity with 3 for genotype 1a is known to occur with the HCV GT II assay however this can be resolved with reflex testing if clinically required. The assay is performed on the automated Abbott m2000 system, is less labour intensive and is well-suited for routine diagnostic use. Furthermore the HCV GT II assay does not require manual interpretation of results which may be subjective. Further investigation regarding the analytical sensitivity and specificity is warranted particularly with the pending availability of reflex test for sub-types 6c-l.

HCV GENOTYPING EXTERNAL QUALITY ASSESSMENT SCHEMES, 2012-2014

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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 is 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 HCV Genotyping (HCVG) with respect to ability of participants to detect different HCV genotypes.

Methods:
Participants' data from nine HCVG EQAS test events over three years were analysed. These panels were composed of well-characterised plasma samples of known (but undisclosed) concentrations and genotypes. All stock samples were assigned a genotype/subtype by nucleic acid sequencing of the HCV core gene by an external laboratory. Genotyping results reported by each participant were evaluated by comparing the genotypes reported with the reference result. Differences in subtype reported were not evaluated. Unless stated otherwise, a result was considered aberrant if the genotype reported by the participant differed from the reference result.

Results:
An average of twenty-five laboratories participated in the HCVG EQAS program over the three years.
The table below shows the average percentage of participants who were able to correctly identify different HCV genotypes over all three test events for each year and the percentage distribution of each HCV genotype for each year.

	GT6		GT5		GT4		GT3	
	% distributed	% detected	% distributed	% detected	% distributed	% detected	% distributed	% detected
2012	14	78	14	96	7	93	29	92
2013	31	70	15	92	N/A	N/A	15	76
2014	14	64	22	100	N/A	N/A	22	97
	GT2		**GT1/2**		**GT1**		**GT1 (1 log lower)**	
	% distributed	% detected	% distributed	% detected	% distributed	% detected	% distributed	% detected
2012	22	96	N/A	N/A	14	98	N/A	N/A
2013	23	94	8	58	8	88	N/A	N/A
2014	21	100	N/A	N/A	7	92	14	83

The majority of participants were able to detect most of the different HCV genotypes. Lower detection numbers were seen for HCV genotype 6 and HCV dual genotype 1/2. The main reason for lower detection numbers for HCV genotype 6 was due to the choice of assay combinations.

Conclusion:
Participation in EQAS allows laboratories to assess the performance of HCVG assays in correctly identifying different HCV genotypes.

DIENTAMOEBA FRAGILIS: AN INNOCENT CARRIER OR A SNEAKY PATHOGEN?

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Introduction:
Since the introduction of multiplex protozoa PCR (Giardia, Dientamoeba, Cryptosporidium and Entamoeba histolytica) as a routine screening test at St Vincent's Pathology in early 2014, we have found an increased number of positives as compared to previous years. We have analysed these positives in order to establish the significance of D. fragilis and the possible clinical implications.

Methods:
Protozoa PCR was performed using the LightMix® kit on LightCycler® 480. All results of protozoa PCR from February 2014 to May 2015 were analysed for type of faeces specimen, age and any clinical notes which were provided on the request slip along with the specimen.

Results:
We found increased positive PCR results for all four parasites tested. The D. fragilis rates were very high as compared to previous years. There was a higher positive rate in children. This data will be presented along with a discussion on what it means clinically.

Discussion/Conclusion:
The role of D. fragilis as a pathogen is highly doubtful. The increased rates of D. fragilis by PCR compared to microscopy (gold standard) have been observed by a number of laboratories worldwide. We analysed the positives in terms of the consistency of the faecal specimens and the clinical notes provided and found that most specimen were non-diarrhoeal (formed or semiformed) and the clinical indications for testing were vague and non-specific. This is possibly leading to 'over treatment' of this protozoa with no good clinical outcome. We now alert the physician (by a comment on the pathology report) of the doubtful nature of this protozoa and advice not to treat non-diarrhoeal patients with formed faeces. We are considering not testing/reporting D.fragilis as a routine, without discussion with the laboratory.

PRELIMINARY RESULTS OF THE ROCHE GASTRO MULTIPLEX PCR KITS FOR THE DETECTION OF GASTROINTESTINAL PATHOGENS OF BACTERIAL AND PARASITIC AETIOLOGY COMPARED TO RESULTS OF BACTERIAL CULTURE AND IN-HOUSE PCR ASSAYS.

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Gastrointestinal illness has many aetiological agents including bacteria, viruses and parasites. Correctly identifying the cause of infection is important for effective treatment. The current gold standard for identification is Standard Culture Methods and Microscopy. Both are slow and subjective. Polymerase chain reaction (PCR) is an alternative that is more rapid, sensitive and specific. The multiplexing of PCR reactions allows for the detection of several targets in a single reaction. The Roche Gastro Multiplex PCR kit is a hexaplex that allows for the identification of up to five different targets as well as an internal control. Bacteria Campylobacter, Salmonella, Shigella, Yersinia and Aeromonas. Parasites Blastocystis spp, Dientamoeba fragilis, Giardia intestinalis, Entamoeba histolytica and Cryptosporidium spp.

The Roche Bacterial and Parasite Gastro Multiplex PCR kits were compared to standard culture methods and in house PCR for the detection of Campylobacter spp, Salmonella spp, Shigella spp and Yersinia enterocolitica and Blastocystis spp, Dientamoeba fragilis, Giardia intestinalis, Entamoeba histolytica and Cryptosporidium spp in stool specimens. A total of 318 specimens, previously analysed by microscopy and culture for the routine diagnosis of gastroenteritis, were tested in this study. A true positive result was considered to be any specimen that was positive by any two of the three methods used.

The Roche Gastro Multiplex PCR detected Campylobacter in 72/74 (97.3%) specimens of which 73 were previously detected by culture. Salmonella was detected in 75/75 (100%) of culture positives, with a further 2 positives detected, 1 by each PCR. The Shigella PCR tested positive for 3/3 (100%) of culture positive specimens. For Yersinia enterocolitica 1/1 (100%) culture positive was detected by the PCR as well as 1 positive not detected by culture.
Blastocystis spp was detected in 52/52 (100%) specimens of these 23 had been detected by culture, For Dientamoeba fragilis 1/1 (100%) culture positive specimens were detected plus a further 24 culture negatives by both PCR's. Giardia intestinalis detected 21/21 (100%) culture positive specimens. A further 2 specimens were detected by both PCR methods. The Entamoeba histolytica PCR tested positive for 5/5 (100%) of culture positive specimens. Finally Cryptosporidium spp detected 3/3 (100%) of the culture positives as well as 4 more samples positive by both the Roche PCR and In-house PCR.

Further studies are to be carried out but the preliminary data indicates a possible underreporting of incidences of bacterial and parasitic gastrointestinal illness by current methods of identification. The Roche Gastro Multiplex PCR assay showed improved performance when compared to bacterial culture. Its performance is comparable to the in-house PCR assays. However it does have the advantage of being a multiplex and thus is more time efficient and cost effective than the Singleplex in-house assays.
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HIV DRIED TUBE SAMPLES: A NOVEL APPROACH FOR HIV VIRAL LOAD EQAS PROVISION

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Introduction:
NRL has conducted EQAS for HIV-1 RNA viral load testing since 2005, providing participating laboratories with 5-member panels of frozen HIV positive plasma samples. The provision of frozen samples can limit the participation in EQAS of laboratories in resource-limited settings due to the high cost of shipping samples on dry ice and a lack of reliable storage facilities (-20°C) at the testing site.

Ramos et al described the use of dried tube samples (DTS) as an alternative method for the delivery of proficiency panels. DTS would eliminate the need for shipping EQAS panels on dry ice and allow more testing laboratories to participate in EQAS.

Objective:
To examine the design, stability and transport of HIV Viral Load DTS as an alternative sample type for NRL EQAS.

Methods:
There were two parts to this project. In part one, four volumes (20, 30, 40 and 50µL) of HIV infected plasma were air-dried in a class two biosafety cabinet. Once dried, each sample was reconstituted with 1mL of nuclease-free phosphate buffered saline (PBS) and tested on the Abbott RealTime HIV Assay (0.6mL initial volume). The data from part one were used to determine the optimum volume in the preparation of future DTS. The second part of the project determined the stability of the samples at four temperatures (-70°C, 4°C, Room Temperature (RT°C) and 37°C) and four time points (2, 6, 24 and 52 weeks after manufacture).

Results:
For part one, it was determined that 50µL of plasma was the optimum volume for the preparation of the DTS. The viral load results showed that 50µL gave the least variation when testing replicate samples after reconstitution (0.19 log) and that the viral load of the dried sample was within 1 log of the liquid plasma sample, once dilution factors were accounted for. Part two of the project showed that long term storage of HIV RNA is optimal at -70°C (0.17 log difference between the mean values for samples stored between two and 52 weeks after manufacture). Samples stored at 4°C showed a difference of 0.67 log between the mean viral load at two and six weeks after manufacture. While each of the storage temperatures showed a difference of less than 1 log between the mean values two and six weeks after manufacture, a difference of greater than 1 log was demonstrated between the mean values for samples stored at -70°C and those stored at RT°C and 37°C six weeks after manufacture.

Discussion:
Although nuclease activity is likely to degrade RNA in sample not stored at -70°C, our results showed that it is feasible to ship and store DTS at 4°C for EQAS purposes. Increasing the concentration of the initial stock sample should counter RNA degradation and allow for analysis of reproducibility and the ability to demonstrate log10 differences in ten-fold dilutions. The current NRL EQAS allows for a three-week testing window. Therefore the six week stability at 4°C would allow for chilled shipment, storage and testing within the test event time period. As the sample type is quite different to the NAT EQAS that NRL currently offers, any DTS EQAS would need to be analysed separately and not compared with results from the frozen, liquid sample.

Conclusions:
In order to maintain RNA integrity over the long term (irrespective of whether the samples are provided as a frozen, liquid sample or a DTS); samples should be stored at -70°C. However, differences in quantification owing to the short term shipping and storage could be negated by using an initial stock sample with a high viral load and by only analysing results from EQAS within peer groups and sample type. A pilot HIV DTS EQAS should allow for further analysis of the extent of variation in the results, actual shipping conditions and ease of use. Information gathered from the pilot will highlight improvements to the EQAS and help define evaluation criteria in the future.

[1]Ramos A, Nguyen S, Garcia A, Subbarao S, Nkengasong JN, Ellenberger D. (2013). Generation of dried tube specimen for HIV-1 viral load proficiency test panels: A cost-effective alternative for external quality assessment programs. J. Virol. Methods. 188; 1-5.

ASSESSMENT OF VIVEST™ SAMPLE STORAGE AND TRANSPORTATION DEVICE

Land S

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Introduction:
Infectious diseases proficiency monitoring often requires transport of samples over long distances in climatic conditions that can cause deterioration in sample integrity. To preclude deterioration, samples intended for External Quality Assessment Schemes [EQAS] for nucleic acid amplification tests are shipped frozen by NRL to participants. Transportation of frozen samples is expensive and this cost can prevent participation in EQAS.

ViveST™ is a biological sample transport system offering preservation of sample integrity during ambient transport and storage. We summarise the outcomes of the manufacturer's, others' and our assessment of the ViveST™ device as a sample format option when HIV RNA is the analyte such as in EQAS for HIV viral load and genotypic drug resistance testing.

Method:
The difference between HIV viral load in the liquid sample and post elution from the ViveST™ device was quantified. HIV RNA stability was compared in the frozen liquid sample and the ViveST™ device at relevant viral loads, time periods and temperatures: 4.7 and 5.7 log10 copies/mL; 2, 6, 10 and 21 days, and 6 months; -70oC, ambient and 46oC.

Results:
Compared with the frozen liquid sample a decrease of approximately 1 log10 in HIV RNA in samples eluted from the ViveST™ device stored at -70oC for up to 6 months was demonstrated; a 1.4 log10 decrease when stored ambient for 21 days ; a 1.9 log10 decrease at 46oC for up to 10 days.

The manufacturer of the ViveST™ device has reported a decline in HIV RNA in plasma stored on the device at 4oC, ambient and 40oC/75% relative humidity over 2 months by 0.84, 0.91 and 1.69 log10 copies/mL, respectively.

When samples were kept on the ViveST™ device in conditions that mimicked the pre-test handling and shipping conditions of EQAS samples [-70oC / 2 weeks; ambient / 2 weeks; refreezing at -70oC] the viral load declined by 1.3 log10. The viral load declined a further 0.2 log10 when samples were held at 46oC for 2 days prior to refreezing, which may occur during a shipping delay.

EQAS for genotypic drug resistance testing requires nucleic acid sequence amplification and detection of mutations associated with drug resistance (DRMs). In comparison with the frozen liquid sample format, sequence was amplified from 97% of samples eluted from ViveST™ and >98% of the DRMs assessed as part of the EQAS were detected. Kantor et al. reported 98% sequence concordance between plasma and ViveST™ frozen plasmas [n=34].

Conclusion:
The ViveST™ sample format could be a viable option for shipping HIV RNA EQAS samples at ambient temperature thereby improving access for laboratories in remote and resource limited settings. The likely decrease in viral load must be taken into account when preparing the EQAS samples and the inclusion of a temperature monitoring device should be considered because of the deleterious effect of high temperature on viral load. The outcome of introducing the ViveST™ device sample format will be evaluated in future EQAS.

MULTIPLEXED DETECTION OF INFECTIOUS DISEASE PATHOGENS USING MNAZYME QPCR: A NOVEL REAL-TIME TECHNOLOGY WITH A SUPERIOR CAPACITY FOR MULTIPLEXING

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SpeeDx Pty Ltd, National Innovation Centre, Eveleigh, Australia, 2015

Introduction:
The pressure for faster, cheaper, better sample testing has resulted in an increased need for highly sensitive and specific multiplexed assays. Current real-time chemistries are expensive and/or difficult to multiplex. MNAzyme qPCR is a novel real-time technology that provides a powerful tool for molecular diagnostics with multiple advantages over other real-time chemistries. MNAzyme qPCR panels work on any real-time machine and take advantage of all available channels.

Methods:
MNAzyme qPCR panels have been developed for the multiplexed detection of DNA ore RNA from a variety of pathogens associated with respiratory illnesses (10 targets/3 wells), atypical pneumonia (5 targets/1 well), viral or bacterial meningitis (10 targets/4 wells), sexually transmitted infections (STIs) (5 targets/1 well), enteric viral infections (8 targets/3 wells), and other infectious diseases. MNAzymes are comprised of two DNA partzymes, which come together only in the presence of PCR amplicons, to form active enzymes that modify universal reporter probes to produce fluorescent signals that are monitored in real-time. Since signal production requires the binding of two partzymes and two PCR primers to a target, this approach has higher specificity than other real-time chemistries, making it ideal for molecular diagnostics. The use of a series of well-characterized, universal probes provides many advantages over target specific probes. They result in reliable, consistent performance when coupled to any target and facilitate rapid and simple development of multiplexed qPCR tests.

Results:
All panels demonstrate robust performance in both singleplex and multiplex, and have high analytical specificity and sensitivity. The R2 for all targets was above 0.99 and PCR efficiencies range from 90% to 110%. No inter-panel cross-reactivity was observed and no cross-reactivity was detected using a wide range of non-target organisms. Comparison with competitors' assays showed the MNAzyme qPCR panels outperformed these with respect to both sensitivity and specificity.

Conclusion:
MNAzyme qPCR provides a superior approach to real-time PCR with the advantages of greater specificity, robust performance in multiplex, reduced cost, and amenability to rapid development of multiplexed panels compared to other technologies.

Disclosure of Interest Statement:
SpeeDx is the developer and manufacturer of the assays evaluated in this study.

HOW DO YOU SOLVE A PROBLEM LIKE GONORROHOEA WITH QA?

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Introduction:
Neisseria gonorrhoeae is a gram-negative, oxidase-positive, intracellular diplococcus and is the causative agent of the sexually transmitted disease gonorrhoea. Definitive diagnosis is imperative for patient management, public health and medicolegal purposes. In order to assist laboratories assess performance of Nucleic Acid Testing (NAT) for the detection of N. gonorrhoeae, RCPAQAP introduced a Quality Assurance Program in 2002 with two surveys per year each containing six lyophilised specimens simulating clinical samples. This module has evolved with the introduction of new technology and assays.

Objective:
Here we aim to 1) review participant performance in the N. gonorrhoeae QA module, focusing on recent surveys, challenges and changes, and assess how these may assist participants to review testing methods, performance and issues and 2) to discuss the challenges of providing a QA program for N. gonorrhoeae and review some recent changes to the program.

Methods:
Results for the N. gonorrhoeae QA module were reviewed from 2002 to 2015, with a particular focus on recent surveys.

Results:
In 2002, 41 participants returned results (29 Roche COBAS Amplicor, 7 BD ProbeTec, 4 Abbott LC and 1 in-house PCR). In 2015, 63 participants returned results (28 Roche COBAS 4800, 7 Abbott RealTime, 1 AusDiagnostics, 7 BD Viper, 6 in-house Realtime PCR, 5 BD Probetec, 2 Gen-Probe APTIMA, 1 artus, 1 Cepheid, 1 Diagenode Diagnostics, 1 InterLabService, 1 Sacace Biotechnologies, 1 Seegene and 1 Siemens Versant kPCR). In 2004, the use of 'confirmatory' tests was performed by 9 (16%) participants, in 2015 supplementary tests were performed by 28 (44%) participants. Over the years, low level CFU/mL specimens have been included and have tested the lower detection limit of assays. Overall, the inclusion of more clinically relevant (higher) levels have been better detected by participants. Other challenges have included the addition of cppB-negative N. gonorrhoeae in 2010/11 and porA negative N. gonorrhoeae in 2013/14/15. The addition of other Neisseria strains including N. lactamica and N. macacae in 2014/15 have highlighted issues with testing of off-label sites and importance of supplemental testing and final result interpretation.

The challenges for the QAP in terms of providing samples for N. gonorrhoeae are numerous. The sample volume required was identified in 2002 as an issue for some assays and continues in 2015 with several assays requiring larger volumes for testing. Assays detecting RNA verses DNA have provided challenges in terms of ensuring RNA degradation is minimised, and this has prompted work on the provision of liquid samples which appears promising. The difficulties in assessing discrepancy in screening and supplementary assays has prompted the recent introduction of a final result interpretation screen.

Conclusions:
Overall the testing of N. gonorrhoeae is satisfactory and has improved over the years. However, there are some areas for further consideration including the testing off-label specimens and the use of supplementary testing. The RCPAQAP will continue to develop and evolve this module to provide specimens that are of interest and allow participants to assess their performance.

SOGAT AND THE STANDARDISATION OF TESTING

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In 1994, a workshop hosted by the UK National Institute for Biological Standards and Control (NIBSC) and the European Plasma Fractionation Association (EPFA - now International - IPFA) primarily to discuss the advent and introduction of nucleic acid amplification techniques to screen blood donations for the presence of viral infection. The main focus was to ensure standardisation of these techniques across tests and jurisdictions around the world. To do this, a committee of experts, manufacturers and regulators was formed to meet in 1995 to discuss the standardisation of genome amplification techniques (SoGAT) under the remit of the WHO and the result was the introduction of the first International Standards for NAT. This group has met annually over the past 20 years, has seen the standardisation of blood virology in the donor setting, and has expanded to include clinical virology (in 2008) and serology (in 2013). A brief introduction to the work of SoGAT, and the expected aims of SoGAT in the future will be presented.