Conclusions TB screening in HIV-1 patients by serial QFT-GIT assays may be of clinical value due to its excellent reproducibility. However, QFT-GIT conversions and reversions should be interpreted carefully as their clinical significance remains to be established.

P2.046 EVALUATION OF MATRIX-ASSISTED LASER DESORPTION/IONIZATION TIME-OF-FLIGHT MASS SPECTROMETRY (MALDI-TOF-MS) FOR SPECIES IDENTIFICATION WITHIN THE NEISSERIA GENUS - EFFECTIVE ALTERNATIVE TO CONVENTIONAL TECHNIQUES?

doi:10.1136/sextrans-2013-051184.0311

[1]**D Golparian**, [2]B Dauphin, [1]B Hellmark, [3]B Claesson, [1]M Unemo. *[1]WHO Collaborating Centre for Gonorrhoea and other STIs, Department of Laboratory Medicine, Microbiology, Örebro University Hospital, ÖREBRO, Sweden; [2]Assistance Publique-Hôpitaux de Paris, Paris, France; [3]Department of Clinical Microbiology, Skaraborg Hospital, Capio Diagnostic AB, Skövde, Sweden*

Background Highly specific and sensitive discrimination between closely related pathogenic and commensal Neisseria spp is crucial because these species frequently colonise the same anatomical sites. Herein, two commercially available Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF-MS) platforms and one independent software and database (Andromas) was compared to conventional phenotypic and genetic tests routinely used for identification of Neisseria spp.
Methods The performance of each platform, analysing 129 pathogenic isolates (Neisseria gonorrhoeae and Neisseria meningitidis) and 69 commensal Neisseria isolates (15 different species), was determined by deposition of one single culture colony to the MALDI plate and analysed in Microflex (Bruker, Germany) and VITEK MS (bioMérieux, France) according to the manufacturer's instructions. Subsequently, the acquired data from Microflex was submitted for analysis in the Andromas software and database (Andromas, France), which uses a different algorithm for species identification. Unfortunately, VITEK MS data is not compatible with Andromas. Phenotypic and genetic (16S rRNA gene sequencing) methods were used for final discrepancy analysis (still pending).
Results Microflex correctly identified all (100%) N. gonorrhoeae and N. meningitidis, however, four commensal isolates were indicated as possible N. meningitidis. Three of these four isolates were N. kochii. The VITEK MS misidentified 1 N. gonorrhoeae, 1 N. meningitidis and 2 commensal isolates (both N. kochii) were indicated as possible N. meningitidis. Finally, Microflex data analysed in the Andromas software and database correctly identified all (100%) pathogenic and commensal strains.
Conclusion This study shows that both Microflex and VITEK MS discriminate pathogenic Neisseria species from commensal Neisseria species with a high, but not ideal, specificity. Furthermore, an optimal MALDI-TOF-MS platform should be compatible with secondary softwares and databases for confirmation. Importantly, the Microflex results analysed in the secondary software and database Andromas correctly identified all (100%) pathogenic and commensal strains.

P2.047 EVALUATION OF A MULTIPLEX REAL-TIME PCR ASSAY FOR RAPID DETECTION OF *C.TRACHOMATIS* AND *N. GONORRHOEAE* FROM GENITAL CLINICAL SPECIMENS

doi:10.1136/sextrans-2013-051184.0312

[1]**M Screm**, [1]M Di Santolo, [1]C Scarparo, [1,2]A Arzese. *[1]Microbiologia, Azienda Ospedaliero-Universitaria, Udine, Italy; [2]Department of Experimental Clinical Medicine, University of Udine, Udine, Italy*

Background Sexually transmitted diseases (STDs) are among the most common causes of illness in the world, being associated to acute disease, infertility, long term disability and death. In particular the prevalence of *C.trachomatis* and *N. gonorrhoeae* infections appear to remain steady or increasing, although both diagnostic and therapeuthical tools are available, at least in developed countries. Standard diagnostic protocols can be complex, time-consuming, and sensitivity of culture methods affected by specimen characteristics. In addition, certified methods are today required, especially in the management of cases of sexual abuse.
Methods Multiplex real-time PCR method (Xpert CT/NG, *Cepheid*) has been tested versus *N. gonorrhoeae* standard culture and *C. trachomatis* molecular assay (*Artus C. trachomatis TM PCR Kit,* Qiagen). 45 clinical samples (female and male urogenital swabs, ocular infections, first void of urine, seminal fluid, and external quality control samples - UK NEQAS) were selected and stored at -20°C. Xpert CT/NG allowed to perform in one-step extraction, amplification and detection of *C.trachomatis* and *N. gonorrhoeae* DNA directly from all the variety of selected clinical specimens, within 60 min.
Results 100% results agreement was found between Xpert CT/NG and standard protocols, including expected results by UK-NEQAS quality assessment specimens. However, batch PCR is a multi-step time-consuming process, the turn-around-time (TAT) required from 8 to 48 hrs versus the single technical and computer-assisted interpretation step by Xpert CT/NG, resulting in a < two hrs TAT.
Conclusions Xpert CT/NG test is a easy, rapid, accurate and certified method in routine diagnostics of *N. gonorrhoeae* and *C. trachomatis* infections.

P2.048 EVALUATION OF TWO COMMERCIAL REAL-TIME PCR ASSAYS FOR DETECTION OF MYCOPLASMA GENITALIUM IN UROGENITAL SPECIMENS

doi:10.1136/sextrans-2013-051184.0313

[1,2]C Le Roy, [1,2,3]S Pereyre, [1,2,3]**C Bébéar**. *[1]University of Bordeaux, Bordeaux, France; [2]Institut National de la Recherche Agronomique, Bordeaux, France; [3]Centre Hospitalier Universitaire de Bordeaux, Bordeaux, France*

Objectives Mycoplama genitalium is a sexually transmitted organism associated with non-gonococcal urethritis in men and several inflammatory reproductive tract syndromes in women. Nucleic acid amplification tests are currently the only available methods for detection. The first commercially available real-time (RT-) PCR kits have been recently developed. We compared the TIB MOLBIOL LightMix® Kit Mycoplasma genitalium and the Diagenode Mycoplasma genitalium real-time PCR kit to the in-house TaqMan RT-PCR used routinely for the M. genitalium diagnostic.
Methods DNA extracts from 50 M. genitalium-negative and 53 M. genitalium-positive urogenital specimens collected between January 2010 and May 2011 at the Bordeaux University hospital, France, were retrospectively and systematically selected and thawed. DNA had been extracted using the MagNA Pure DNA isolation kit I (Roche Diagnostics). DNA extracts were evaluated by the TIB-MOLBIOL LightMix® Kit Mycoplasma genitalium and the Diagenode Mycoplasma genitalium real-time PCR kit (DIA-MG-050 vs2) in comparison with a M. genitalium in-house RT-PCR targeting the MgPa adhesin gene using the cobas z480 analyser (Roche Diagnostics).
Results The in-house PCR was first evaluated using two thermal cyclers, LC480 and cobas z480 (Roche Diagnostics). As no significant difference was noted, the cobas z480 was used in the rest of the study. The clinical sensitivity was 98%, 92% and 100% for the LightMix® Kit Mycoplasma genitalium, the Diagenode Mycoplasma genitalium real-time PCR kit and the in house RT-PCR, respectively. The clinical specificity was 100% for both kits and 94% for the in house RT-PCR. There was no statistically significant difference between the clinical sensitivity and specificity of these 3 methods. Moreover, there was no statistically significant difference between

the cycle threshold mean of the in-house assay and those of both commercial kits.

Conclusion Both commercial kits allowed prompt and specific results, validated by the use of an internal amplification control.

P2.049 NAATS FOR GONORRHOEA DIAGNOSIS IN WOMEN: EXPERIENCE OF A TERTIARY CARE HOSPITAL IN NORTH INDIA

doi:10.1136/sextrans-2013-051184.0314

[1]**S Sood**, [1]R Verma, [1]S Mir, [1]M Agarwal, [1]N Singh, [2]H K Kar, [1]V K Sharma. *[1]All India Institute of Medical Sciences, New Delhi, India; [2]Dr. R M L Hospital, New Delhi, India*

Background Gonorrhoea is among the most frequent of the estimated STIs and health implications related to morbidity and mortality especially in women and children are significant. The use of nucleic acid amplification tests (NAATS) has been shown to provide enhanced diagnosis of gonorrhoea in female patients & are considered the standard for diagnostic purposes currently. However, it is recommended that an on-going assessment of the test assays should be performed to check for any probable sequence variation occurring in the targeted region. In the present study, an in-house PCR targeting *opa*-gene of *Neisseria gonorrhoeae* was used in conjunction with 16S ribosomal PCR to determine the prevalence of gonorrhoea in female patients attending the tertiary care hospitals

Methods Endocervical samples collected from 250 female patients attending the Dermatology and Gynaecology OPD of AIIMS and STD clinic of Dr. R.M.L. Hospital, New Delhi, India were tested using *opa* and 16S ribosomal assay. Additionally, they were processed by conventional methods. True positives were defined as ones which were positive by culture and/or positive by both 16S ribosomal gene and *opa*- gene based PCR.

Results Of the 250 female patients, only 1 was positive by conventional methods, i.e., microscopy and culture. Based on PCR results, *opa*-gene based PCR was positive in 17 patients who were also positive by 16S ribosomal PCR. However, 16S ribosomal gene based PCR picked up 8 extra positives. Overall, 17 patients were found to be true positives.

Conclusion The clinical sensitivity of conventional methods for the detection of *N. gonorrhoeae* in female patients is low. The gonococcal detection rates increased significantly when molecular method was used giving a prevalence rate of 6.8%. However, it is pertinent to mention that the widespread use of NAATs might result in lack of isolates and ignorance of possible emergence of resistant organisms.

P2.050 DEVELOPMENT OF MOLECULAR BEACON BASED DIAGNOSTIC ASSAY FOR DETECTION OF NEISSERIA GONORRHOEAE AND CHLAMYDIA TRACHOMATIS

doi:10.1136/sextrans-2013-051184.0315

D Sachdev, A L Patel, I Kumari, D Saluja. *Dr. B.R. Ambedkar Center for Biomedical Research, University Of Delhi., Delhi, India*

Background Due to absence of cost effective and rapid diagnostic test syndromic management of Neisseria and Chlamydia was recommended in developing countries. Being nonspecific such a strategy has resulted in over treatment leading to increased drug resistance. It also misses out on asymptomatic patients resulting in increase in disease burden. The study describes the development and evaluation of a low cost duplex PCR method (dPCR) for co-detection of Neisseria and Chlamydia. Using molecular beacons we further provide a method for quantitative and easy detection of the two pathogens.

Methods Endocervical swabs were collected from patients visiting gynaecology department of various hospitals in Delhi. We standardised and evaluated in-house uniplex PCR (uPCR) for diagnosis of Neisseria against Roche Amplicor Micro Well Plate CT/NG kit. Method was modified to co-detect N. gonorrhoeae and C. trachomatis in single test. Further we developed visual assay for detection of Chlamydia and Neisseria using molecular beacon probe.

Results Clinical samples (n = 412) were used to validate in-house uPCR assay for Neisseria. The PPV and NPV were found to be 86.77% and 97.2%. We further modified our uPCR to dPCR for simultaneous detection of Neisseria and Chlamydia. The overall infection rate was found to be 27.8% and 26.3% for Neisseria and Chlamydia respectively while 11.3% of patients were co-infected. The in-house dPCR was found to be 85.7% sensitive and 97% specific. To further enhance the sensitivity and specificity of our test, molecular beacon were designed against the amplicons. Use of molecular beacons also reduced the detection time as amplicons could be directly visualised under dark reader.

Conclusions The in-house dPCR assay is rapid and as sensitive as commercially available tests. Use of molecular beacons provides a highly specific and easy to use detection method, making it a better option for routine diagnosis of genital infection in developing countries.

P2.051 A COST-EFFECTIVE AND SIMPLE ALTERNATIVE TECHNIQUE FOR RESUSCITATION OF FREEZE-DRIED CULTURES OF NEISSERIA GONORRHOEAE

doi:10.1136/sextrans-2013-051184.0316

M Bala, V Singh, M Kakran, V Ramesh. *VMMC & Safdarjung Hospital, New Delhi, India*

Background Freeze drying (lyophilization) of bacteria is a very well established method for the archiving and long-term storage. The recommended medium for resuscitation of freeze-dried cultures of Neisseria gonorrhoeae is 1 ml of a nutrient blood broth or a rich peptone broth supplemented with 10% blood or nutrient broth. Sheep blood or horse blood is not easily available in most of the labs. and human blood is not recommended. Normal saline (0.9% W/V Sodium chloride) is mostly available in all the labs. This study compared the nutrient blood broth and normal saline for resuscitation of freeze-dried cultures of reference and clinical strains of N. gonorrhoeae and evaluated their performance characteristics for the growth of N. gonorrhoeae strains.

Methods A prospective study was undertaken between January 2011 and December 2012. Ninety three N. gonorrhoeae lyophilized strains including 83 clinical isolates, ATCC 49226 and nine WHO reference strains were tested using both the methods. Reconstituted material from both the techniques was subcultured on to chocolate agar and was incubated for 24–48 hrs at 36°C in a moist atmosphere containing 5 to 10% carbon dioxide. The results were recorded in terms of viable gonococci, colony morphology and colony size.

Results N. gonorrhoeae was successfully isolated from 89 (95.7%) lyophilized strains by both the techniques used for revival. In other 4 cases, it was difficult to state with absolute certainty that the gonococci were nonviable or that unsatisfactory lyophilization practises were responsible for these results as the quality of the lyophilsed strains also determined the growth on revival.

Conclusion Preliminary data suggest that nutrient blood broth and normal saline were equal in performance for revival of lyophilized strains and normal saline could be recommended as convenient and inexpensive alternative for universal use.

P2.052 ANALYTICAL EVALUATION OF THE SPECIFICITY AND NEISSERIA GONORRHOEAE DETECTION OF THE NOVEL GENEPROOF N. GONORRHOEAE DUAL-TARGET PCR ASSAY

doi:10.1136/sextrans-2013-051184.0317

B Hellmark, D Golparian, M Unemo. *WHO Collaborating Centre for Gonorrhoea and other STIs, Department of Laboratory Medicine, Microbiol, Örebro, Sweden*