Abstracts

P07.10 EVALUATION OF THE NEW BD MAX GC REAL TIME PCR ASSAY, ANALYTICALLY AND AS A SUPPLEMENTARY TEST TO THE BD PROBETEC GC QX AMPLIFIED DNA ASSAY, FOR MOLECULAR DETECTION OF NEISSERIA GONORRHOEAE

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Introduction The BD ProbeTec GC Qx Amplified DNA assay (Becton, Dickinson and Company) is used on the BD Viper System to detect Neisseria gonorrhoeae. However, subtype-specificity and cross-reaction with commensal Neisseria species have been described. Recently, the BD Max GC real time (rt) PCR assay was developed for the BD Max System (BD) as a supplementary test.

Methods We evaluated the performance of the new BD Max GC rt PCR assay by examining clinical specimens positive in the BD ProbeTec GC Qx Amplified DNA assay during July–October 2014 among 23815 screening or clinical patients (14846 females and 8969 males) as well as samples spiked with isolates of gonococci (n = 189), non-gonococcal Neisseria species (n = 261) and other closely related bacteria (n = 10).

Results Of 23815 patients tested with the BD ProbeTec GC Qx Amplified DNA assay, 85 (0.6%) females and 259 (2.9%) males were positive. Of these 344 positive specimens, 322 were tested with BD Max GC rt PCR assay. Sixty-nine (21%) of these samples were negative in BD Max GC rt PCR assay, a gonococcal dual target PCR and in the APTIMA Combo 2 (Hologic). These 69 specimens were obtained from pharynx (50%) of all screening positive pharyngeal specimens, urine (33%), vagina (11.4%), rectum (4.3%), and cervix (1.4%). In the analytical evaluation of the BD Max GC rt PCR assay, all gonococcal isolates were positive and all but one (N. cinerea) of the non-gonococcal isolates (99.4%) were negative. The N. cinerea isolate also cross-reacted in the BD ProbeTec GC Qx Amplified DNA assay.

Conclusion The BD ProbeTec GC Qx Amplified DNA assay had a suboptimal specificity for both urogenital and extra- genital clinical specimens. The new BD Max GC rt PCR assay showed a high clinical and analytical sensitivity and specificity, and might also be used for initial detection of N. gonorrhoeae.

Disclosure of interest Statement We are grateful to BD Diagnostics for providing the BD Max GC real time PCR tests. The isolate also cross-reacted with commensal Neisseria species. This interesting Neisseria sample was subject to analysis as part of Roche’s Global Surveillance Program. The isolate was tested by culture, phenotyping, whole cell MALDI-TOF MS and molecular methods such as 16S rRNA-based typing and whole genome sequencing.

Results Whole genome sequencing resulted in a complete genome assembly supporting phylogenetic analysis. Sequencing data assembled into a 2,882,113bp contig and genome closure, which identified the sample as a Neisseria commensal species clustering in the same clade with both N. sicca and N. macacae.

Based on the sequence information of the prophage/DR9 genetic signature of this newly characterised Neisseria strain revealed no matches to sequences in the public domain, suggesting that this strain is rare.

REFERENCE


P07.11 WHOLE GENOME SEQUENCING CHARACTERISATION OF A UNIQUE NEISSERIA STRAIN GENERATING A POSITIVE RESULT WITH THE COBAS® CT/NG TEST


Introduction Neisseria gonorrhoeae (NG) cause sexually transmitted bacterial infections. The Roche cobas® CT/NG Test is a qualitative Nucleic Acid Amplification test (NAAT) utilising a dual-target approach to detect sequences within the direct repeat (DR-9) region of the NG genome. To date, clinical performance data show excellent sensitivity and specificity for urogenital specimens. The test is validated for endocervical swabs, vaginal swabs and urine specimens.

Methods One clinical case report describes a positive test result for an oropharyngeal swab, an off-label specimen type for the cobas® CT/NG Test. This interesting Neisseria sample was subject to analysis as part of Roche’s Global Surveillance Program. The isolate was tested by culture, phenotyping, whole cell MALDI-TOF MS and molecular methods such as 16S rDNA-based typing and whole genome sequencing.

Results Whole genome sequencing resulted in a complete genome assembly supporting phylogenetic analysis. Sequencing data assembled into a 2,882,113bp contig and genome closure, which identified the sample as a Neisseria commensal species clustering in the same clade with both N. sicca and N. macacae.

Based on the sequence information of the prophage/DR9 genetic signature oropharyngeal swab specimens determined to be positive by the cobas® CT/NG Test are currently being evaluated for the presence of this sequence motif.

Conclusion The cobas® CT/NG Test on the cobas 4800 system is a reliable molecular method for detection of Chlamydia and Gonorrhoeae from genital specimens.

Disclosure of interest Authors of the abstract are employees of Roche Molecular Systems.

REFERENCE


P07.12 FACTORS INFLUENCING THE DETECTION OF NEISSERIA GONORRHOEAE FROM THE TONSILS AND POSTERIOR OROPHARYNX

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Background Limited data exists on the specific anatomical areas within the pharynx from which Neisseria gonorrhoeae can be detected. We examined factors influencing the detection of gonorrhoea from the pharynx.

Method Men who had sex with men (MSM) diagnosed with pharyngeal gonorrhoea by culture were recalled for repeat swabbing 7 days later: firstly from both tonsils then, using separate swabs, from the posterior oropharynx. These were tested for N. gonorrhoeae using culture and real-time PCR targeting the
gonococcal porA pseudogene and multi-copy opa genes. Cycle threshold (Ct) values obtained were used as semi-quantitative measures of gonococcal DNA. Sampling adequacy was assessed using a real-time PCR for human endogenous retrovirus 3 (ERV3).

Results 100 MSM with culture positive pharyngeal gonorrhoea were included. Isolation rates by culture from the tonsils and posterior oropharynx were 62% and 52% respectively (p = 0.041). PCR was significantly more sensitive than culture at both the tonsils (84% vs. 62%; p < 0.001) and oropharynx (81% vs. 52%; p < 0.001). Culture positivity was greater with higher gonococcal DNA loads at both the tonsils (p = 0.001) and oropharynx (p < 0.001). At the oropharynx, higher ERV3 DNA load was associated with improved gonococcal detection using culture (p = 0.013) as well as PCR (p = 0.045). At the tonsils, higher ERV3 DNA load was associated with improved gonococcal detection by PCR (p = 0.040).

Conclusion Neisseria gonorrhoeae can be cultured from the tonsils as well as the posterior oropharynx with greater isolation rates where gonococcal loads are higher. While PCR is substantially more sensitive than culture at each site, like culture, PCR is dependent on the adequacy of sampling.

Disclosure of interest statement None to disclose.

P07.13 DETECTION OF TREPONEMA PALLIDUM DNA FROM WHOLE BLOOD AND EARLOBE SPECIMENS IN PATIENTS FROM TWO STI CLINICS IN LIMA, PERU

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Background Prior studies have reported PCR detection of Treponema pallidum DNA from whole blood and earlobe scrapings. Factors affecting efficiency such as PCR target gene and clinical stage of syphilis have been studied. We aimed to determine other factors associated with detection of T. pallidum DNA from whole blood and earlobe samples.

Methods Data were obtained from baseline samples collected for a prospective cohort study of 401 men who have sex with men (MSM) and transgender women (TV) in Lima, Peru. Participants were assessed for HIV using rapid HIV testing (Alere Determine, MS, USA) and OraQuick Advance (OraSure, Otsuka Pharmaceutical, Tokyo, Japan). Whole blood samples and earlobe capillary blood was collected from patients with a positive RPR and TPPA. DNA extraction was performed using the QIAamp mini kit (Qiagen, Valencia, CA), and samples were then concentrated. Uchosing specific primers for the TpN47 gene, an aliquot of the DNA sample was amplified using conventional PCR for participants with high RPR titers (≥1:16). Positivity was determined by visual detection of PCR product on 1% agarose gel.

Results 56 participants had RPR titer ≥1:16 (1:16(n = 18), 1:32(n = 16), 1:64(n = 17), 1:128(n = 3), and 1:256(n = 2)). A total of 7 (12.5%) participants had T.pallidum DNA detected from whole blood, and 6 (10.7%) from earlobe capillary blood (10 participants total). Of these 10 participants, 5 were HIV positive, and 5 were HIV negative.

Conclusion Our relatively low efficiency of detection from both whole blood and earlobe samples suggest that neither HIV status nor high RPR titer (≥1:16) seem to predict high likelihood of DNA detection. Further study is ongoing to determine if these or other characteristics are associated with positive detection of T. pallidum DNA.

Disclosure of interest statement The Picasso study is funded by a grant from the United States NIAID and was implemented by the Universidad Peruana Cayetano Heredia in collaboration with the University of California, Los Angeles. The molecular part of the study was implemented under the supervision of the University of Washington. No pharmaceutical grants were received during the development of this study.

P07.14 APTIMA TV NAAT TEST PERFORMANCE IN GUM CLINICS AND PRIMARY CARE IN THE UK


Introduction The Aptima TV NAAT for the detection of Trichomonas vaginalis (TV) is ~100% sensitive compared to wet mount microscopy (50%) or culture (73%). Asymptomatic women attending genitourinary medicine (GUM) clinics and patients in primary care are often not tested for TV, as the prevalence and sensitivity of current tests is assumed to be too low for testing to be cost effective.

Our aim was to determine how many additional cases were identified with the new test and whether self-taken and clinician-taken vaginal swabs are of equivalent sensitivity in symptomatic GUM patients.

Methods Patients were tested using the Aptima TV NAAT alongside existing testing methods.

Results The positivity of TV determined by TV NAAT was 4.8% (26/543) and 1.8% (26/1593) in women with and without symptoms attending GUM and 2.7% (35/1295) and 1.1% (41/3593) respectively in primary care.

TV NAAT significantly outperformed our existing testing methods. In a subset of 491 GUM patients in whom all tests were performed, 14/23 (61%) TV NAAT positive patients were identified on wet prep or culture (p = 0.004). In a subset of 2930 primary care patients in whom all tests were performed, 19/74 (26%) TV NAAT positive patients were identified on wet prep or culture (p < 0.0001). These results may reflect deterioration of specimens in transport to the laboratory.

Self-taken vaginal swabs were equivalent in sensitivity to clinician taken swabs; of the 26 patients who tested positive on either NAAT test, 25 tested positive on self-taken swab and 21 tested positive on clinician taken swab (p = 0.51).

Conclusion Testing women attending GUM clinics and in primary care with the Aptima TV NAAT test will identify additional cases and should replace conventional microbiological testing methods if found to be cost-effective.

Disclosure of interest statement Hologic provided the tests for the Aptima TV NAAT research study and have sponsored the authors to present this data at ISSTDR.