

**Objectives** Highly sensitive and specific assays for diagnosis of *Neisseria gonorrhoeae* (NG) are imperative. Unfortunately, several commercial and in house NG nucleic acid amplification tests (NAATs) have shown suboptimal specificity. The *Neisseria gonorrhoeae* PCR kit (GeneProof) is a novel NG dual-target (*porA* pseudogene and 16S rRNA gene) real-time PCR. Herein, the analytical sensitivity and specificity of the NG PCR kit (GeneProof) were evaluated using a collection of well-characterised gonococcal isolates (n = 105), with a global representativeness, and non-gonococcal *Neisseria* isolates (n = 149; 21 different species and subspecies), as well as specimens positive with three other commercially available NAATs (n = 37).

**Methods** DNA was extracted from all samples using the NorDiag Bullet robot (NorDiag ASA Company) and kept in  $-20^{\circ}\text{C}$  prior to testing. All samples were tested on LightCycler 2.0 (Roche Molecular Systems Inc.) by adding 10  $\mu\text{l}$  of DNA to 30  $\mu\text{l}$  NG PCR kit (GeneProof) reagent mix.

**Results** All 105 gonococcal isolates, including three *porA* mutants, were detected and none of the 149 non-gonococcal *Neisseria* strains were false positive. Accordingly, the assay displayed 100% analytical sensitivity and specificity. The analytical sensitivity was 1–10 genome copies per reaction. All positive samples from the Abbott RealTime PCR CT/NG (Abbott Laboratories) (n = 5) and COBAS 4800 (Roche Molecular Systems Inc.) systems (n = 8) were verified. However, for the BD ProbeTec ET/Qx *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Amplified DNA (Becton Dickinson) only eight out of 24 low-positive samples could be verified as true positive.

**Conclusions** This study shows that the GeneProof NG PCR kit is analytically highly specific and sensitive for detection of *N. gonorrhoeae*. This study also emphasises the importance of verifying *N. gonorrhoeae* NAAT positive specimens, particularly specimens that are low positive or from extragenital sites, with an alternative NAAT using a different target.

**P2.053 CHLAMYDIA TRACHOMATIS/NEISSERIA GONORRHOEAE SCREENING IN DUPLEX: SHOULD WE VERIFY N. GONORRHOEAE POSITIVE RESULTS?**

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The objective of this study was to assess the utility of a supplementary PCR test following a positive Abbott m2000 PCR test result for *Neisseria gonorrhoeae* (NG) issued from urogenital specimens tested in the Department of Bacteriology at the Bordeaux University Hospital, France in 2011–2013.

All NG-positive PCR specimens either negative with NG culture or without culture result, were retrieved and tested with two CT/NG PCR tests: Cepheid GeneXpert CT/NG and Roche cobas 4800 CT/NG (both targeting two genes). Analytical sensitivity of the three tests, Abbott, Cepheid and Roche for NG detection, was tested on serial dilutions (10<sup>-1</sup> to 10<sup>-9</sup>) from one colony of NG suspended in cobas<sup>®</sup> PCR medium (Roche).

Of 11.010 specimens tested on Abbott m2000, the global prevalence of NG was 2.5% (277/11.010) and varied with the origin of the patient, from 0.8% at a free and anonymous STI screening centre (CDAG) to 6% at STI clinics (CIDDIST). Out of 215 specimens analysed, 112 were confirmed by culture or with a second PCR-positive sample, and 103 were tested with the two other PCR assays. A total of 197/215 NG-positive Abbott PCR results were confirmed. The overall positive predictive value (PPV) of the CT/NG Abbott test was 91.6%, ranging from 73.5% for asymptomatic patients consulting at CDAG to 95.8% for symptomatic patients consulting at CIDDIST. Concern-

ing the analytical sensitivity, the Cepheid test was 10 and 100 times more sensitive than the Abbott and the Roche tests, respectively.

In populations where the prevalence was < 1%, the Abbott CT/NG test had a PPV < 90% and therefore required confirmation testing. When NG screening is associated with that of CT in populations with variable prevalence, it should be recommended to either use a NG PCR test with two targets or confirm a positive result by another PCR test with a different target.

**P2.054 OPTIMAL PROCESSING OF CHLAMYDIA TRACHOMATIS SIMULATED SAMPLES FROM PROFICIENCY TESTING PANELS BY DILUTION WITH COBAS<sup>®</sup> PCR MEDIA**

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**Background** Proficiency materials are designed to resemble true clinical samples, yet challenges exist in procuring sufficient quantity of patient material. Simulated samples are often provided for this testing. Matrix effect with simulated samples can confound molecular assessment, having negative consequences for the laboratory through failed proficiency testing. This study was conducted to evaluate simulated urine samples provided for proficiency testing which generate invalid results with the cobas<sup>®</sup> CT/NG test.

**Methods** Simulated urine proficiency panels were acquired from a commercial proficiency testing provider. Panels were evaluated in triplicate by routine procedure at neat concentrations and processed with cobas<sup>®</sup> PCR media at the following dilutions 1:1, 1:5, 1:10, 1:20, 1:50, 1:100, 1:200, and 1:500. The samples were held at room temperature (1 complete set) for 1 hour prior to loading on the cobas<sup>®</sup> 4800 system, while the second complete set of samples were processed 24 hours later. Samples were tested using two different cobas<sup>®</sup> 4800 workflows (400ul vs. 850ul of sample). Internal control and target Ct values were assessed for each sample to determine success of amplification.

**Results** Invalid results due to internal control failures were observed at neat concentrations of simulated urine samples. Incubation of samples for 1 hour or 24 hours in cobas<sup>®</sup> PCR media, showed no significant difference between target and IC Ct values indicating incubation period in cobas<sup>®</sup> PCR media does not impact performance. Simulated Urine Sample dilution of 1:5 in cobas<sup>®</sup> PCR media using the 400ul sample input volume produced similar IC Ct values (mean Ct = 35.5) to cobas<sup>®</sup> PCR media tested alone (mean Ct = 36), and produced a robust target signal (mean Ct = 22).

**Conclusions** Proficiency testing materials may require optimization for use on commercially available systems. Optimal processing of simulated urine specimens can be achieved by dilution in cobas<sup>®</sup> PCR media.

**P2.055 "P.I.D." OR ENDOMETRIOSIS? LAPAROSCOPIC ASSESSMENT, CHLAMYDIAL ANTIBODIES AND DYSMENORRHEA SYMPTOM SCORING IN WOMEN WITH ACUTE PELVIC PAIN**

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**Background** Most women with endometriosis receive unnecessary antibiotics for "P.I.D." because both conditions present with pelvic pain and dyspareunia.

We used negative chlamydial antibody titre and laparoscopy to confirm diagnoses in women identified by dysmenorrhoea symptom scoring (DSS) as more probably having endometriosis than PID.