Objective

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 = 57).

Method

DNA was extracted from all samples using the NorDiag Bullet robot (NorDiag ASA Company) and kept in −20°C prior to testing. All samples were tested on LightCycler 2.0 (Roche Molecular Systems Inc.) by adding 10 µl of DNA to 30 µl NG PCR kit (GeneProof) reagent mix.

Result

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 = 6) 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.

Poster presentations

P2.054

OPTIMAL PROCESSING OF CHLAMYDIA TRACHOMATIS SIMULATED SAMPLES FROM PROFICIENCY TESTING PANELS BY DILUTION WITH COBAS® 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® 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® 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® 4500 system, while the second complete set of samples were processed 24 hours later. Samples were tested using two different cobas® 4500 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® PCR media, showed no significant difference between target and IC Ct values indicating incubation period in cobas® PCR media does not impact performance. Simulated Urine Sample dilution of 1:5 in cobas® PCR media using the 400ul sample input volume produced similar IC Ct values (mean Ct = 35.5) to cobas® 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® PCR media.

P2.055

P.L.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.L.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 dysmenorrhea symptom scoring (DSS) as more probably having endometriosis than PID.