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.

**Methods** Further provide a method for quantitative and easy detection of Neisseria and Chlamydia. Using molecular beacons we increased in disease burden. The study describes the development of resistant organisms. It also misses out on asymptomatic patients resulting in over treatment leading to increased drug resistance. It has resulted in over treatment leading to increased drug resistance.

**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 16S ribosomal 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 hospital.

**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 16S and 16S ribosomal assay. Additionally, they were processed by conventional methods. True positives were defined as those which were positive by culture and/or positive by both 16S ribosomal and 16S-16S 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, 16S-16S 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.

**Development of Molecular Beacon Based Diagnostic Assay for Detection of Neisseria Gonorrhoeae and Chlamydia Trachomatis**

**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 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.

**Cost-effective and Simple Alternative Technique for Resuscitation of Freeze-dried Cultures of Neisseria Gonorrhoeae**

**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 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. 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 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 lyophilized 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.

**Analytical Evaluation of the Specificity and Sensitivity of Neisseria Gonorrhoeae Detection of the Novel GeneProof N. Gonorrhoeae Dual-target PCR Assay**

**Background** Gonorrhoea and other STIs, Department of Laboratory Medicine, Microbiol, Örebro, Sweden
Poster presentations

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 (*podA* 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).

Methods 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.

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 = 5) 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 emphasizes 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 ranging from 73.5% for asymptomatic patients consulting at CDAG to 95.8% for symptomatic patients consulting at CIDDIST. Concerning 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® PCR MEDIA


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Background Proficiency materials are designed to resemble real 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.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 dysmenorrhea symptom scoring (DSS) as more probably having endometriosis than PID.