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°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 = 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 (10–1 to 10–9) from one colony of NG suspended in cobas® 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. 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 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© 4800 system, while the second complete set of samples were processed 24 hours later. Samples were tested using two different cobas© 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© 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.

Methods Retrospective review of women meeting CDC diagnostic criteria for "acute P.I.D." who completed a pain history questionnaire identifying symptoms strongly suggestive of endometriosis, namely:

1. Severe dysmenorrhoea interfering with schooling or work
2. Cyclical use of painkillers or heat application
3. Improvement on hormonal contraception
4. Cyclical dyschezia
5. Family history

Those who scored > 50% and whose symptoms failed to respond to hormonal treatment, were assessed by laparoscopy.

Results Of 149 women with high DSS, all tested negative for chlamydia by AptimaCombo2. 41 were referred to gynaecology, and 36 (aged 16–39, median 24y) had laparoscopy.

Of these, 23 had chlamydial antibody titre (CAT) measured, 4 were raised. 26/36 (72%) had endometriosis confirmed at laparoscopy including the four with raised CAT.

10/36 (28%) had no obvious signs of endometriosis or PID nor any other diagnosis.

Scores were similar in those with mild, moderate or severe endometriosis and the apparently disease-free group (mean score 87% & 85% respectively).

Conclusions

1. DSS is a simple means of excluding PID in women with acute pelvic pain and filtering appropriate referrals to gynaecology with high rates of endometriosis disease finding.
2. Laparoscopy may not identify exclusively uterine or rectovaginal endometriosis and negative cases remain under review.
3. DSS cannot predict disease extent due to "high end failure" as genuinely severe endometriosis is uncontrolled by hormonal contraception.
4. Dysmenorrhoea symptom scoring reliably identifies women who are likely to be given antibiotics for PID when they actually require hormones for endometriosis, and could improve specificity in patient selection for PID research.

P2.056 PREVALENCE OF *NEISSERIA GONORRHOEAE* SPECIMENS CONTAINING *POR A* PSEUDOGENE DELETION AMONG GONOCOCCAL RESISTANCE TO ANTIMICROBIALS SURVEILLANCE PROGRAMME (GRASP)SPECIMENS AT THE HEALTH PROTECTION AGENCY

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Background There has been an emergence of *Neisseria gonorrhoeae* strains which although phenotypically are indistinguishable from *N. gonorrhoeae,* vary in their genotype and require heightened surveillance. Isolates of *N. gonorrhoeae* were identified in Scotland, Australia and Sweden which lacked sequences in the *porA* pseudogene *(PAP)* and consequently gave false negative results in the *PAP* real-time polymerase chain reaction (RT-PCR) for *N. gonorrhoeae*. In 2011 two *PAP* negative isolates were found in England. We sought to determine the prevalence of *PAP* negative isolates amongst those received through the national surveillance programme, GRASP.

Method A screening protocol was devised which entailed using initial *PAP* testing followed by repeat *PAP* and confirmatory *opa* RT-PCR testing. Lysates prepared from isolates received for GRASP during 2011 were used. Any lysate with an initial *PAP* negative result was serially diluted to check for inhibition, then repeated on the original lysates and if still negative confirmed on a freshly prepared isolate direct from the archived isolate.

Results Of 156 GRASP lysates tested 146/156 (94%) were *PAP* positive, 10/156 (6%) samples were initially found to be *PAP* negative. On repeat testing however only a single isolate remained PAP negative when repeat *PAP* testing was performed on samples prepared from fresh culture.

Conclusion A single *PAP* negative specimen has been identified to date within GRASP, which potentially is carrying the menginococcal PorA. However confirmation by meningococcal PCR will be necessary.

P2.057 VERY EARLY INFANT DIAGNOSIS AND ART OUTCOMES IN KENYA

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Background In resource limited settings, effectiveness of PMTCT programmes and ART outcomes in HIV infected neonates remains poorly documented. The study aimed at evaluating the efficacy of PMTCT programmes in 10 maternities in Kenya and to describe outcomes in HIV-infected neonates.

Methods HIV-exposed neonates were screened at birth at week 6. Heel prick samples of blood on DBS were used for DNA real time PCR testing. HI-RNA viral load and ARV drug resistance genotyping were done accordingly.

Results Between 2008 and 2011. 1,000 exposed neonates were screened for HIV infection. 60% were born from mothers on Tritherapy, 20% from mothers receiving dual AZT/sdNVP therapy, and 12% to mothers receiving only sdNVP. 70% of neonates received sdNVP at birth. All neonates were formula fed exclusively. Seven were diagnosed HIV+ at birth (Utero transmission rate = 0.91%). 55% were lost of 5 of follow up and 5 died before week 7. 15/900 were diagnosed positive at week 7 (peri partum transmission rate = 1.80%). 17/24 infected neonates started ART. Virological follow-up indicated that 8/11 reached undetected VL whereas 4/13, representing resistance to RTIs (one pre-ART, 2 Post ART), were in treatment failure. 9/22 (40.1%) infected-neonates were successfully treated.

Conclusion The study highlights the feasibility and interest of the very early infant diagnosis, illustrates the efficacy of PMTCT interventions and clearly points out the difficulties faced to treat effectively infected neonates.

P2.058 RAPID HIV TESTING IN THE PUBLIC HEALTH SETTING IN NORTH RHINE-WESTPHALIA, 2011–2012

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Background North Rhine-Westphalia is the federal state with the highest number of HIV infections in Germany. The Landeszentrum Gesundheit (lzg.nrw) organises and supports anonymous HIV testing by 53 local public health authorities (LPHA). Aim of this study was to assess if offering additional rapid testing in the LPHA could attract hard-to-reach risk groups to HIV testing.

Methods After counselling, 24 LPHA offered their clients a rapid assay (RA; Vikia HIV 1/2, bioMérieux) alternatively to routine testing by a 4th generation HIV test (chemiluminiscent microparticle immunoassay, CMIA, Abbott) in a private laboratory (Labor Krone, Bad Salzuflen). Reactive tests were confirmed by immunoblot analysis and/or RT-PCR.

Results In 2011–2012, 24,623 clients were tested by CMIA in all 53 LPHA and 21,513 by RA in 24 LPHA. Among clients tested by CMIA there were 48.8% women, 50.5% men, median age was 31 years, 12.9% were men who have sex with men (MSM), 13.2% female sex workers (FSW). Among RA clients there were 39.2% female, 60.5% male, 73.9% belong to the age range of 20–39 years, 13.9% MSM, 0.7% FSW. In the CMIA, 1.2% of the samples were reactive versus 0.6% in RA. Overall, 0.8% of LPHA clients were