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INVESTIGATIVE REPORT

Genital and Extra-genital Screening for Gonorrhoea using the BD Probetec ET System with an In-house PCR Method Targeting the *porA* Pseudogene as Confirmatory Test

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Diagnosing gonorrhoea from extra-genital as well as genital sites is important in managing this sexually transmitted disease. In this study we evaluated a screening procedure for *Neisseria gonorrhoeae* (GC) from all sample sites in a low-prevalence setting. A total of 69,252 specimens submitted for *Chlamydia trachomatis* testing were also examined for GC on the BD Viper™ platform using the BD Probetec ET system. In order to avoid false-positive results all GC BD reactive samples were re-tested using a PCR method with the *porA* pseudogene as target. Using this method we screened 170% more samples for GC than in the previous year, in the same population, and diagnosed more than twice as many GC-positive episodes. The BD system can be used successfully to screen extra-genital as well as genital specimen types for GC in a low-prevalence area if it is combined with a validated confirmatory PCR test. ***Key words: Neisseria gonorrhoea; diagnosis; NAAT; pharyngeal infection; rectal infection.***

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Gonorrhoea is a sexually transmitted disease (STD) caused by the bacterium *Neisseria gonorrhoeae* (gonococci = GC). During the 1970s until the early 1990s a declining incidence of gonorrhoea was observed in many high-income countries, but for the last 10 years there has been a slow increase in incidence in several countries (1–3). A number of explanations for the increase in diagnosed gonorrhoea have been suggested, including unsafe sexual behaviour amongst young people, and amongst men who have sex with men (MSM), more active diagnostic screening programmes and more sensitive testing methods (1, 4). Although genital infections are most common, GC may cause infections in the pharynx, rectum and eyes. Pharyngeal and rectal infections are mostly asymptomatic, but constitute reservoirs for disease transmission and are under-diagnosed in general practice (GP) (2, 5, 6). Many MSM are infected at extra-genital sites (7), and it has been shown that more than two-thirds of pharyngeal and rectal infections detected by site-specific testing would have been missed if urine and urethral samples alone were used (8). A study in our population, performed 10 years ago with GC culture, found that half of the pharyngeal and rectal GC-positive patients (13 out of 25 patients) were not positive in the urethra (6).

Detection of GC by culture is a state-of-the-art procedure with high specificity, but there can be problems with sensitivity because of the fastidious nature of GC. For pharyngeal and rectal samples, the sensitivity of culture detection is also low because there are fewer GC at these sites, and growth competition from other bacteria even on selective media (8–12).

Nucleic acid amplification tests (NAATs) for detection of GC have been shown to have increased sensitivity, primarily in specimens from extra-genital sites. However, commercial GC NAATs have not been validated and approved for extra-genital samples, and some GC NAATs have significant specificity problems due to cross-reactivity with commensal bacteria. The US Centers for Disease Control (CDC) has recommended confirmatory testing when the positive predictive value (PPV) of a test is < 90% (13), but few companies supply a true confirmatory test. Many clinical microbiology laboratories that can afford NAATS serve low-prevalence populations, in which specificity problems are particularly troublesome, as the low prevalence inherently leads to a low PPV (14, 15).

Based on these considerations, we validated a sensitive, specific and affordable diagnostic procedure for GC testing of all samples, urethra, cervix, rectum and pharynx, from the Copenhagen area, which is a low-prevalence setting. We screened all samples for GC using the BD Probetec ET system (BD Diagnostics, Copenhagen, Denmark) in combination with the BD Viper™ instrument, and confirmed all BD GC-reactive samples with an in-house PCR targeting the *porA* pseudogene. We present here data on GC testing only. Information on *Chlamydia trachomatis* (CT) testing is given in Table SI (available from: http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1192).

METHODS

Background and data collection

Our laboratory covers the municipalities of Copenhagen and Frederiksberg for all GC and CT diagnostics, a population of approximately 600,000 people. Samples are submitted from GPs and specialists, four hospitals, the sexual health clinic (Sex og Samfund) and the STD clinic in Copenhagen. Since 2004 the BD Viper™ has been used for detection of CT on cervical, urethral and urine specimen with a throughput of approximately 100,000 samples in 2007. Diagnosis of GC has been performed by culture, with a throughput of approximately 30,000 samples in 2007. On 1 April 2008 our laboratory introduced the BD Viper™ for the simultaneous analysis of GC and CT in all STD samples. Thereafter, all samples submitted primarily for CT testing were also screened for GC.

We present here data from the introduction of the analysis from 1 april 2008 until 7 January 2009, and compare it with the results of GC culture. These results are also compared with the results of GC culture in the laboratory one year previously.

Specimen collection

Asymptomatic and low-risk patients were sampled with one BD ProbeTec ET Wet Swab taken from each relevant specimen site, e.g. the urethra, rectum, pharynx and cervix. In general practice, first-void urine from men was the recommended specimen type, using the BD ProbeTec Urine Preservative Transport Kit (UPT). Self-collected vaginal specimens using chlamydia swabs transported in 2 SP medium (SSI Diagnostica, Hillerød, Denmark) were also included in this study.

For GC culture, specimens from symptomatic and/or high-risk patients were collected with charcoal swabs and transported in Stuart's medium, after sampling for NAAT testing. The culture samples were stored at 5ºC until transport and inoculated onto chocolate agar and a *Neisseria*-selective chocolate agar with antibiotics (SSI Diagnostica, SSI-703) within 24 h and incubated in carbon dioxide (CO_2) for 48 h. In symptomatic patients microscopy was performed on methylene-blue stained urethral, cervical and rectal discharge, at the STD clinic.

NAAT testing, confirmatory testing and interpretation

In the laboratory all samples were tested for both GC and CT in the same run on the BD Viper™ instrument. The BD ProbeTec ET system utilizes homogeneous strand displacement amplification (SDA), with the pilin-inverting gene as the target for GC. Positive and negative controls for GC and CT were included for each run, as provided by the manufacturer. All results were interpreted according to the manufacturer's instructions. All GC SDA-reactive samples (positives and low-positives, as defined in the manufacturer's instructions) were re-tested with a confirmatory test. The confirmatory test was an in-house real-time PCR targeting the *porA* pseudogene, using the same primers as described and validated by Hjelmevoll et al. (16, 17). A TaqMan™ probe, FAM-CAGCAAGTCCGCCTATACGCCTGCTACTT-BHQ-1 was used, and an internal amplification control was included in the master mix, as described previously (18). Total nucleic acid was extracted from patient samples with the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche). Prior to this study, in-house validation of the PCR method was performed. The limit of detection was determined to be less than 50 colony-forming units (CFU)/ml in the BD collection kit (unpublished data). Only confirmed SDA-reactive samples were interpreted as true-positive (NAAT-positive), with GC SDA-reactive and PCR-negative samples interpreted as SDA false-positive (NAAT-negative). To specify our case definition: a positive GC sample is a sample that is both SDA-reactive (positive or low-positive) and is confirmed positive by PCR. When both criteria are fulfilled this is referred to as a NAAT-positive result.

A positive episode of gonorrhoea infection was defined as one or more NAAT-positive results or culture-positive results within 30 days. When comparing a NAAT-positive result with the culture result, a window of ± 30 days was used. If the patient sample was cultured more than 30 days before or after the NAAT-positive result it was interpreted as two different episodes.

When the laboratory reported a NAAT-positive result, the clinicians were advised to recall patients for GC cultures and antibiotic susceptibility testing before starting treatment.

The laboratory participates in the NEQAS QC NAAT programme for GC and CT.

RESULTS

The final material comprised 69,252 samples from 45,082 individuals. Demographic data and numbers of samples and exclusions (2.5%) are reported in Table SII and SIII (available from: http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1192). Rectal and pharyngeal samples were predominantly from the STD clinic. All urine and vaginal samples were from GPs or home sampling.

Strand displacement amplification vs. PCR results

Of the 69,252 samples SDA tested for GC, 541 were SDA-reactive and were tested by the confirmatory *porA* PCR. Of these, 339 were PCR-positive, giving a GC sample-positive rate of 0.49%. A total of 423 samples were SDA-positive, with 333 (79%) confirmed PCR-positive. A total of 118 samples (46 cervical, 2 vaginal, 3 rectal, 56 pharyngeal, 6 urethral and 5 urine) were SDA low-positive, with only 6 (5%) (3 cervical, 1 vaginal and 2 pharyngeal) confirmed positive, and the remaining 112 (95%) PCR-negative. The proportion of confirmed positive results for each specimen type is shown in Table I. The proportion of SDA-positive results confirmed by PCR ranged from 31% to 100%. The best performance of SDA was found for urine samples, whereas the poorest performance was found for pharyngeal samples. For both males and females the highest positive rate of GC was in the pharyngeal samples.

Nucleic acid amplification tests-positives vs. culture results; window of 30 days

The 339 confirmed positive samples (333 SDA-positives and 6 SDA low-positives) represented 198 episodes from 188 males and 57 episodes from 55 females. For male episodes 44% (87) were culture-positive, 30% (60) were culture-negative, and 26% (51) were not cultured within ± 30 days. For female episodes 30% (17) were culture-positive, 31% (18) were culture-negative and 39% (22) were not cultured.

Table I. *Proportion of polymerase chain reaction (PCR)-confirmed results among the 423 strand displacement amplification (SDA)-positive specimens in relation to sampling site and sex*

	Female		Male	
Sample site	PCR/SDA positive (%)	Positive rate (%)	PCR/SDA positive (%)	Positive rate (%)
Cervical	46/56 (82)	49[a]/36,727 (0.13)		
Vaginal	0/0	1[a]/760 (0.13)		
Rectal	3/6 (50)	3/477 (0.63)	31/36 (86)	31/1,042 (3.0)
Pharyngeal	8/26 (31)	8/490 (1.6)	54/92 (59)	56[a]/1,076 (5.2)
Urethral	30/42 (71)	30/14,960 (0.20)	94/98 (96)	94/4,180 (2.2)
Urine	4/4 (100)	4/483 (0.83)	63/63 (100)	63/9,055 (0.70)

[a]A further six nucleic acid amplification tests (NAAT)-positive samples were identified among 118 SDA low-positive samples.

Culture-positives vs. nucleic acid amplification tests results

Looking at the GC culture-positive samples in the study period we found 132 culture-positive samples from 90 males and 18 females. Of the culture-positive samples, 110 (83%) were found to be NAAT-positive from the same site within 30 days. Four (3%) of them were found to be SDA-negative at the same site and 18 (14%) were not screened by SDA from the same site within 30 days. Of the four culture-positive and SDA-negative samples, three were from a male urethra and one was from a male pharynx. SDA testing was performed on the same day as the positive culture for two of the incongruent results. One SDA test was performed 14 days after the positive culture, when treatment had been completed. The SDA-negative result from the pharynx had positive cultures from the same day in both urethra and pharynx, together with a confirmed positive SDA from the urethra with SDA testing 8 days later from pharynx, urethra and rectum, which were all negative; there was no information on treatment status.

Consequences of the GC NAAT screening procedure

Comparing the results from the current study period with the previous year, we found a 92% reduction in GC cultures handled in the laboratory, with an 11-fold higher culture-positive rate. (Previous year: 25,481 cultures with 143 culture-positives; current results: 2,116 cultures with 132 culture-positives)

A total of 14,526 samples from females were tested (cultured) for GC in the previous year compared with 53,899 samples tested (SDA) in the study period. For males, 10,955 samples were cultured before implementation of SDA, compared with 15,353 samples tested with the new procedure. In total, 172% more samples were tested for GC.

In the study period we found 339 samples that were NAAT-positive and 132 samples positive by culture. This represented a total of 265 positive episodes, with 10 episodes diagnosed by culture alone. During the same time-period in the previous year there were 143 positive GC culture samples, representing 117 positive episodes. The increase in diagnosed GC episodes was 126%.

The total laboratory expenses were almost the same, with increased spending on SDA reagents for GC tests, and confirmatory PCR being balanced by equivalent savings as a result of the reduction in GC samples for culture.

DISCUSSION

For several reasons, as mentioned in the introduction, laboratories serving clinics and hospitals with a low prevalence of GC face difficulties when they want to perform sensitive, specific and affordable GC diagnostics from all possible specimen sites. This study is the largest validation study of NAAT (BD, SDA) used for GC screening in a low-prevalence setting. Screening with SDA can give false-positive test results, presumably if there is cross-reaction with commensal bacteria and, as expected, the SDA test was found to have a low PPV in this setting, even for some categories of genital sample types, confirming the previous recommendations by the CDC for confirmatory GC testing with an alternative target. We chose the *porA* pseudogene as target for the confirmatory test, based on its high sensitivity and specificity, and, so far, this target has not been found to cross-react with any other *Neisseria species* (11, 16, 17). We considered confirmed test results to be accurate. There is no indication that we have over-diagnosed gonorrhoea. Despite finding many more cases in the community setting using NAATs, it is interesting that we have had no complaints from GPs or patients with positive GC findings.

For this study we screened almost 70,000 samples and found a GC sample-positive rate of 0.5%. Pharyngeal and rectal swabs (3,085 samples) could be confirmed only at low rates (31–86%) and resembled the results by McNally et al. (19) who used the same target, *porA* pseudogene, in the confirmatory test. Others have found better performance for both pharyngeal and rectal specimens, especially in MSM settings (8, 10, 20, 21). The results are difficult to compare, however, because of different settings regarding sample collection, NAATs used, confirmatory test chosen, definition of "a true-positive" and GC prevalence. Differences may also reflect the natural variability of *Neisseria spp*. in different populations (15).

For genital samples the importance of the confirmatory test is recognized in our setting, with only 82% of the cervical samples being confirmed. Only for male urethral samples (from an STD clinic) and for first-void urine, could the necessity of the confirmatory test be considered. The excellent performance of SDA on urine, with a 100% concordance between SDA and PCR results, was in a population prevalence group of less than 1%, with nearly 10,000 tests performed. One other study also found no false-positive SDA test on urine samples (22), although false-positives have been published (23–26). None of the studies used the *porA* pseudogene as target in the confirmatory tests.

A survey of NAAT platforms in England and Wales revealed that only one out of 19 SDA laboratories confirmed positive GC by an alternative target (27). Rectal swabs were tested by 10 of these laboratories and throat swabs by nine. As most GC testing is presumably done in low GC prevalence areas, based on our results, a large number of patients will be given a false-positive test result. This is of major concern and needs to be addressed by laboratories and healthcare providers.

False-negative test results are also possible with the SDA (15). We did not test any SDA-negative results by PCR and therefore cannot exclude this possibility. Compared with the results of GC culture, there were SDA false-negatives, but we consider these to be at an acceptably low rate. Low rates of false-negative SDA results have also been found by others (8, 10, 22, 28) or not at all (19). During the study period we did not control for inhibition, but in the most recent version of the SDA test, inhibition control is now included.

Using the BD Viper platform makes it possible to perform high-throughput screening for GC on all specimen types at the same time as CT diagnostics is performed. This could be performed with limited extra work compared with performing CT testing alone, with extra cost only for the GC test reagents and the confirmatory PCR. Using the validated in-house PCR made it possible to perform the confirmatory test on the same clinical specimen without the need to inconvenience the patient by taking new samples. The SDA/PCR expenditures were balanced by the expense saved through the reduction in GC cultures. In the laboratory, repetitive work reading negative GC agars was reduced, and with a positive rate of 6.2% this is now a more interesting job. The in-house confirmatory test delivered rapid results compared with out-sourcing to a reference laboratory.

Our guideline, which recommends GC cultures from GC-positive patients, worked to some degree, with 71% of all NAAT-positive episodes having cultures performed within 30 days. Of these, 57% were positive. The 43% culture-negative samples may be explained as due to a combination of primary antibiotic treatment in symptomatic patients and the lower sensitivity of culture detection. To survey GC antimicrobial resistance development we believe that culture isolations from approximately 40% of the NAAT-positive episodes are sufficient. In cases of treatment failure new cultures from each case will be examined.

In conclusion, we were able to screen 170% more samples for GC compared with the same time-period the year before, and to diagnose more than twice as many GC-positive episodes without increasing the costs. We suggest that NAATs can be also used when screening for GC on extra-genital specimens in low-prevalence settings. However, an independent assay should always be applied for confirmatory testing of all SDA-positive results.

The authors declare no conflicts of interest.

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