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Positive Predictive Value of the Becton Dickinson VIPER system and the ProbeTec GC Q^x assay, in extracted mode, for detection of *Neisseria gonorrhoeae*.

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ABSTRACT

Objectives: Performance of the new Becton Dickinson ProbeTec GC Q^x assay on the BD VIPER platform, was evaluated to ascertain whether confirmatory testing is required in our clinical setting.

Methods: Positive predictive value (PPV) was determined by comparison with culture and a confirmatory NAAT based *N. gonorrhoeae* assay from genital and extragenital samples (rectal and pharyngeal) collected from a genito-urinary medicine clinic.

Results: Among 14,223 clinical genital samples, 149 (1.0%) specimens were positive using the ProbeTec GC Q^x assay, automated on the VIPER platform; 141 of these were confirmed either by culture or by a real time PCR targeting two gonococcal specific targets (PPV 94.6%; 95% confidence interval, 90%-98%). Among 840 pharyngeal samples, 26 (3.1%) were positive by the ProbeTec GC Q^x assay; 13 were confirmed (PPV 50%; 95% confidence interval, 30%-70%). Among 593 rectal samples, 17 tested positive by the ProbeTec GC Q^x assay; all were confirmed (PPV 100%; 80%-100%).

Conclusions: The lower 95% confidence interval of the PPV for the ProbeTec GC Q^x assay for genital specimens was >90%, in our GUM clinic population and therefore confirmatory testing for genital specimens is not required. Confirmatory testing of pharyngeal and rectal samples should continue in line with national guidelines.

Introduction

Testing of asymptomatic, sexually active people for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infection is widespread in Europe and North America. In most settings, gonorrhoea is less common than Chlamydia infection: in 2008 there were 209,000 new

diagnoses of Chlamydia in the UK compared to 16,629 new diagnoses of uncomplicated gonorrhoea.¹

The BD VIPER system (Becton Dickinson, New Jersey, USA) is an automated sample processor and fluorescent reader using strand displacement amplification (SDA) for qualitative detection of *N. gonorrhoeae* and *C. trachomatis* DNA. The previous BD assay was the ProbeTec ET GC assay, utilising similar technology but comprising a manual extraction via heating and lysis and a semi automated SDA procedure. The newer methodology has a fully automated extracted mode, which uses alkaline lysis of cells and binding of DNA to ferric oxide (FOX) to extract *N. gonorrhoeae* DNA and includes an extraction control to monitor for failed extraction and PCR inhibition.

The ProbeTec GC Q^x and ET GC assays target different regions of the *N. gonorrhoeae* pilin inverting protein homologue gene so there may be differences in the performance characteristics of these assays. The manufacturer claims sensitivity of 99.3% and specificity of 99.4% for genital samples.

Alternative commercial assays for the detection of *N. gonorrhoeae* are the Gen-Probe APTIMA Combo 2 assay on the Tigris platform (Gen-Probe, Inc., San Diego, USA), the ROCHE AMPLICOR CT/NG assay (Roche, Branchburg, NJ, USA) and the Abbott RealTime CT/NG assay on the *m2000* platform (Abbott Molecular Diagnostics, Illinois, USA). Each of these assays detect different targets. The APTIMA Combo 2 assay detects *N. gonorrhoeae* 16S rRNA using transcription mediated amplification and the *m2000* CT/NG assay detects the *N. gonorrhoeae* Opa gene using real time PCR.

For Chlamydia NAAT assays repeat testing using the same assay is considered adequate as this reduces inaccurate results due to processing errors but also reduces sensitivity in samples with a low pathogen load.² There have been reports of a relatively low specificity of the older ProbeTec ET GC assay for *N. gonorrhoeae* and the Roche COBAS AMPLICOR leading to unacceptably low positive predictive values (PPV) in a low prevalence population, therefore, repeat testing on the same platform is not considered adequate for GC due to cross reaction with commensal *Neisseria spp.*^{3 4} The Health Protection Agency and the Centers for Disease Control and Prevention recommend that for *N. gonorrhoeae* confirmatory testing, a different target should be considered if the positive predictive value (PPV) of an assay is < 90% to improve PPV.⁵⁻⁷ Our aim was to ascertain the PPV of the BD ProbeTec GC Q^x assay in our genito urinary medicine (GUM) clinic attendee population for genital and extragenital sites.

METHODS

Traditionally diagnostic assessments are made by comparing the test to a reference assay for all samples. This would represent an expensive and time consuming approach. As this was a service evaluation, we used a methodology similar to that reported by Golden *et al* who determined the PPV of a NAAT for *N. gonorrhoeae* by confirming only the samples testing as positive in a larger number of specimens without performing reference tests in all samples.⁸

There is currently no clear consensus on standards for evaluations of NAATs. Choice of ‘gold standard’ becomes complicated because NAATs are likely to be more sensitive than culture based methods and genuine positives may not be recognised. We calculated PPV by

comparing screening NAAT results to a composite 'gold standard' consisting of bacterial culture and two confirmatory real-time PCR results.

Specimen Collection

Between 23rd June 2009 and 15th January 2010, patients attending the Courtyard (GUM) clinic at St George's NHS Healthcare Trust were tested for infection with *N. gonorrhoeae* and *C. trachomatis* using the ProbeTec CT/GC Q^x assay, automated on the VIPER platform as per the manufacturer's instructions.

Consecutive routine specimens of urine samples, endocervical, urethral, rectal and pharyngeal wet swabs were included in this service evaluation as outlined below. All male patients were tested with a first void urine for Chlamydia/gonorrhoea NAAT. In addition, clinically indicated swabs for gonococcal culture and the ProbeTec GC Q^x assay were taken from urethral, rectal and pharyngeal sites. Male patients giving a history of dysuria or urethral discharge had a urethral swab collected for Gram stain and culture for *N. gonorrhoeae*. Men who have sex with men had culture and NAAT, performed on rectal and pharyngeal swabs based on history of anal and/or oral sex. Three swabs were taken simultaneously from the throat by sticking them together with tape and rotating them. One swab was for *N. gonorrhoeae* culture, one for BD ProbeTec GC Q^x and one for BD ProbeTec ET GC (used for confirmatory testing at STBRL). Rectal swabs were collected without a speculum unless rectal symptoms were present. The culture swab was taken first, followed by the GC Q^x and the ET GC swab. Urethral swabs were taken from female patients for gonorrhoea culture. After inserting a speculum endocervical swabs were collected for culture and BD ProbeTec GC Q^x in that order.

Testing by ProbeTec GC Q^x

Samples positive for *N. gonorrhoeae* in the initial ProbeTec GC Q^x assay were retested using the same sample and a fresh extraction using the same assay. A NAAT was considered positive if positive on the repeat run. Samples that did not repeat as positive were considered equivocal as this may have resulted from a processing error and a repeat sample was requested, these samples were not used in the analysis of PPV. The confirmatory testing method is shown in figure 1.

Confirmation by real-time PCR

All samples testing positive for *N. gonorrhoeae* by the ProbeTec GC Q^x assay were forwarded to the Sexually Transmitted Bacteria Reference Laboratory (STBRL) where they were examined blind using two *N. gonorrhoeae* specific real-time PCRs. Some negative samples were also sent.

Upon receipt, 200µl of ProbeTec ET GC transport buffer was extracted using the MagNAPure Compact (Roche Diagnostics, Indianapolis, USA) and examined using a modified version of a previously published *N. gonorrhoeae* real-time PCR assay which targets the *porA* pseudogene.⁹ Briefly, PCR was performed using 25 µl reactions containing 5µl of extracted DNA, 4mM MgCl₂, 1U Taq DNA polymerase (Applied Biosystems, California, USA), 250nM of forward and reverse primers and 500nm of probe (Table 1). Also included within this PCR were 80mM of primers and probe of a previously described internal control which targets the Human RNase gene, to monitor for both inhibition and specimen quality.¹⁰ PCR was performed using a Rotor-Gene 6000™ (Qiagen, Hilden, Germany) using cycling conditions of: 10mins at 95°C, followed by 40 cycles of 95°C for 30 seconds and 60°C for 60 seconds.

Specimens negative using this assay were re-examined using a further assay which targets the *Opa* gene.¹¹ PCR was performed using 20µl reactions containing 5µl of extracted DNA, 5mM MgCl₂, 1U Taq DNA polymerase (Applied Biosystems) and 250 nM of forward and reverse primers and 500nM of probe (Table 1). PCR samples were run on a Rotor-Gene 6000™ using cycling conditions of: 10mins at 95°C, followed by 50 cycles of 95°C for 15 seconds and 60°C for 60 seconds.

Culture

VCAT (containing vancomycin, colistin, amphotericin and trimethoprim) selective culture plates (Biomérieux, Marcy l'Etoile, France) were inoculated in the GUM clinic and incubated (48 hours, CO₂). Presumptive colonies were Gram stained, oxidase tested and subcultured onto a purity plate. *N. gonorrhoeae* was confirmed by MicroTrak *N. gonorrhoeae* culture confirmation fluorescent antibody test (Trinity Biotech Plc, Co. Wicklow, Ireland) and API NH (BioMérieux, Marcy l'Etoile, France). Corresponding culture plates for all ProbeTec GC Q^x extragenital samples testing as positive were incubated for a total of 72 hours to maximise growth of *Neisseria spp.*

Analysis of test performance

A sample was treated as a true positive if positive by either culture or by confirmatory *PorA* or *Opa* real time PCR. Stata 8.0 (College Stn. Tx, USA) was used to determine PPV and 95% confidence intervals. A limitation of this methodology is that sensitivity and specificity were not determined because negative samples were not repeated by confirmatory NAAT and were not compared to culture result. However we may estimate the specificity if we assume a value for the sensitivity of the assay, based on the following relationship¹²:-

$$PPV = \frac{(sensitivity) \times (prevalence)}{((sensitivity) \times (prevalence)) + ((1 - specificity) \times (1 - prevalence))} \quad \text{[Equation 1]}$$

RESULTS

PPV and prevalence for genital samples

In total 14,223 genital specimens (5851 male urine samples, 8075 cervical and 297 male urethral swabs) were tested. Two samples that tested as positive in the initial ProbeTec GC Q^x assay did not repeat as positive on the repeat ProbeTec GC Q^x run. These two samples were reported as equivocal and excluded from the analysis. Of these 14, 221 samples, 150 (1.1%) samples from 149 patients tested positive for *N. gonorrhoeae* using the ProbeTec GC Q^x assay. Of these positive samples, 141/149 (94.7%) were confirmed by either culture or HPA confirmatory PCR⁹⁻¹¹ giving a PPV of 94.6% (95% CI, 90%-98%) (Table 2). For the remaining eight patients with unconfirmed positive genital specimens, three patients had a confirmed *N. gonorrhoeae* infection at another site, suggesting that these are genuine positive samples; raising the PPV of genital samples to 96.7% (95% CI, 93% - 100%). Of the confirmed positive genital samples four (2.7%) were culture positive but not detected by confirmatory PCR.

Based on the relationship described in equation 1, if we assume a sensitivity of 99.3% then the estimated specificity will be 99.96%. Even if we assume a much lower sensitivity e.g. 50% the estimated specificity remains at 99.96%.

PPV and prevalence for extragenital samples

In total 593 rectal and 840 pharyngeal swab samples were tested. Of these specimens 17/593 (2.87%) rectal swabs and 26/840 (3.10%) pharyngeal swabs tested positive for *N. gonorrhoeae*, using the ProbeTec GC Q^x assay. Of these positive samples, all 17 rectal swabs were confirmed positive giving a PPV of 100% (95% CI, 80%-100%). Of the 26 pharyngeal samples 13 samples were confirmed by either GC culture or in house HPA confirmatory PCR with a PPV of 50% (95% CI, 30%-70%) (Table 2). The number of samples testing as positive by the ProbeTec GC Q^x assay, confirming by NAAT and culture are shown in Table 2. For positive results, which were unconfirmed, two of the thirteen pharyngeal samples had one or more samples confirmed positive for *N. gonorrhoeae* from another site and these were therefore considered to be likely genuine positive samples. If these samples were considered true positives the PPV would be 57.7% (95% CI 39% -77%) for pharyngeal samples. Of the confirmed positive samples one (3.8%) pharyngeal swab and one (5.9%) rectal swab were culture positive but not detected by confirmatory NAAT. Nine samples were confirmed by NAAT but were not confirmed by culture. Four of the unconfirmed pharyngeal samples grew *Neisseria meningitidis* upon culture. All extragenital samples were positive upon retesting via ProbeTec GC Q^x.

DISCUSSION

The ProbeTec GC Q^x assay is FDA licensed and CE marked for use with urethral, endocervical and self collected vulvo-vaginal swabs plus male and female urine samples. This assay is not licensed for use on pharyngeal, rectal or eye swabs. For genital samples we found a PPV of 94.6% (95% CI, 90%-98%) in our clinic population, with a confirmed *N. gonorrhoeae* rate of 1.0%. As the lower CI is 90%, it is reasonable to use the ProbeTec GC Q^x assay without routine confirmatory testing in this setting. Current national

guidelines state that confirmatory GC NAATs are required if the PPV is less than 90% and confirmatory testing is unnecessary for genital specimens in our GUM population.^{5 6} However, in populations where the prevalence of *N. gonorrhoeae* is low e.g. primary healthcare settings, the PPV may be lower and confirmatory testing by a second NAAT, with a second genetic target, may be required.

Our routine practice is to retest all NAAT positive samples on the same platform, although this is not explicitly recommended by the manufacturer. This is to reduce inaccurate results due to sample processing errors. This practice of repeat testing of positive samples on the same platform is not universally practiced but there were only two specimens, neither extra-genital, that did not retest as positive. The inclusion of this small number of specimens would not alter these findings.

During the evaluation period no culture positive samples were ProbeTec GC Q^x negative from the same site. This supports the manufacturer's claim that the assay has a high sensitivity of greater than 99.5%.

Akduman *et al* found that the ProbeTec ET GC assay had a PPV of 84.9% for testing of urine samples.¹³ In a large multicentre study the PPV of the assay for female urine samples varied from 54.8% to 100%. The site reporting the lowest PPV of 54.8% had a *N. gonorrhoeae* prevalence of 1.2%, which is similar to our reported prevalence.¹⁴ The PPV reported here demonstrates that the ProbeTec GC assay, in extracted mode, has a higher PPV than the ProbeTec ET GC assay and is likely to have a higher specificity than that reported of the ProbeTec ET GC assay.

There are concerns regarding the use of NAATs for the detection of *N. gonorrhoeae* from rectal and pharyngeal samples, due to lower specificity. Although NAATs are the most sensitive assays available,¹⁵⁻¹⁷ the risk of false positive results is high due to the genetic similarity between *N. gonorrhoeae* and commensal *Neisseria spp.* leading to cross reactions. Optimal methods for detection of *N. gonorrhoeae* infection at extragenital sites are uncertain and no commercial NAAT is licensed for detection of *N. gonorrhoeae* from extragenital sites. Here, we found that our NAAT assay identified more pharyngeal GC infections than culture in keeping with previous studies.¹⁸⁻²⁰ Indeed NAATs are redefining the epidemiology of gonococcal infection. Out of 26 pharyngeal samples initially testing as positive 13 (50%) were not confirmed either by culture or by confirmatory NAAT. Two possible explanations are possible; the first is that these samples had a low inoculum of *N. gonorrhoeae*. The second is that cross reactivity resulted in an increased number of false positive ProbeTec GC Q^x results. The ProbeTec ET GC and Roche COBAS AMPLICOR CT/NG assays are known to cross react with strains of *N. subflava* and *N. cinerea* resulting in false positive results^{21 22} and these species may be present in the oropharynx as commensal bacteria in up to a third of individuals.²³ Such cross reactivity has not yet been reported with the APTIMA Combo 2 test.²⁴ Specificities of the APTIMA Combo 2 and ProbeTec ET GC for oropharyngeal swabs have been found to be similar, and have been reported to be greater than 99.4%.¹⁶ However Ota *et al* determined the PPV of the ProbeTec GC ET assay to be only 82.6% for pharyngeal swabs.¹⁵ It is likely that the low PPV determined here reflects lower specificity of this assay.

A further limitation may be the order in which swabs were taken. For rectal and endocervical swabs culture swabs were taken before swabs for NAAT testing and therefore

the NAAT assay may have been disadvantaged and may result in an underestimate of PPV. The pharyngeal swabs were collected for all three samples simultaneously, by taping them together, as we considered it unpleasant for patients to undergo three successive samplings. Whilst practitioners rotated the triple swab, there may well have been sampling differences.

We found a PPV of 100% (95% CI 80-100%) for rectal swabs, out of 17 samples testing as positive all were confirmed by either culture or the second NAAT assay. However this is too small a number to give sufficient confidence in this estimate and larger numbers are required to confirm that the lower CI is greater than 90%. Others have also demonstrated the high PPV of rectal swabs for detection of *N. gonorrhoeae* but sample sizes were also small.^{15 16} In compliance with national guidelines we recommend that extragenital samples testing positive by NAAT should be confirmed. Samples testing as positive which are not confirmed should be reported as equivocal and a repeat sample requested.⁵⁻⁷

Discrepant analysis of samples which are reference standard negative will increase the PPV. As this was a service evaluation, partner status was not included: the result is that the PPV reported here may be an underestimate of the true value. However for unconfirmed ProbeTec GC Q^x positive samples a partial discrepant analysis was performed, samples testing as positive in the ProbeTec GC Q^x assay were considered a true positive if confirmed positive results were available from one or more alternative sites.

We used *N. gonorrhoeae* specific real-time PCRs to act as confirmatory assays. Interestingly, six specimens which were both ProbeTec GC Q^x and culture positive, were not confirmed by the STBRL real-time PCR assays. There maybe numerous reasons for

this including DNA degradation, as the confirmatory testing was performed off site, and therefore specimens incurred a period of transportation delay before testing.

It is possible, that some culture negative samples testing as positive in the ProbeTec GC Q^x assay may not have been confirmed even if genuinely positive. Choice of confirmatory assay is important as this should detect a different target to the screening NAAT and have good sensitivity and specificity. These could include real time PCR assays available from reference laboratories or developed in house e.g. PorA/Opa PCR or reciprocal arrangements with a laboratory with a different platform.²⁵

In summary, we have demonstrated that the ProbeTec GC Q^x assay can achieve a high PPV when testing genital specimens in our GUM clinic population and therefore in accordance with national guidelines confirmatory testing is unnecessary. However testing with a second supplementary NAAT assay may still be desirable in lower prevalence populations.

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Key Messages

Testing of genital specimens by the ProbeTec GC Q^x assay does not require confirmatory testing in our GUM clinic population.

Confirmatory testing of pharyngeal swabs, testing as positive, should continue as recommended by national guidelines.

The PPV for rectal swabs looks promising but larger numbers are required to confirm that the lower CI is >90%.

Declaration of Interest

We acknowledge the support of Becton-Dickinson in the article – who provided some technical staff time and provided some testing kits. There were no other conflicts of interest.

Author Contribution

KC, TP, PH conceived the evaluation and designed the testing. PH and TS coordinated sample collection in the GUM clinic. SA and CI provided real time PCR confirmatory testing for the samples and helped revise the manuscript. SA, PH and TS contributed to the methods section of the manuscript. CP wrote the of the paper, coordinated sending of samples to STBRL, collected data and performed statistical analysis with TP. PH, TP, TS and JD discussed results and revised manuscript. All authors contributed to the manuscript and approved the final version.

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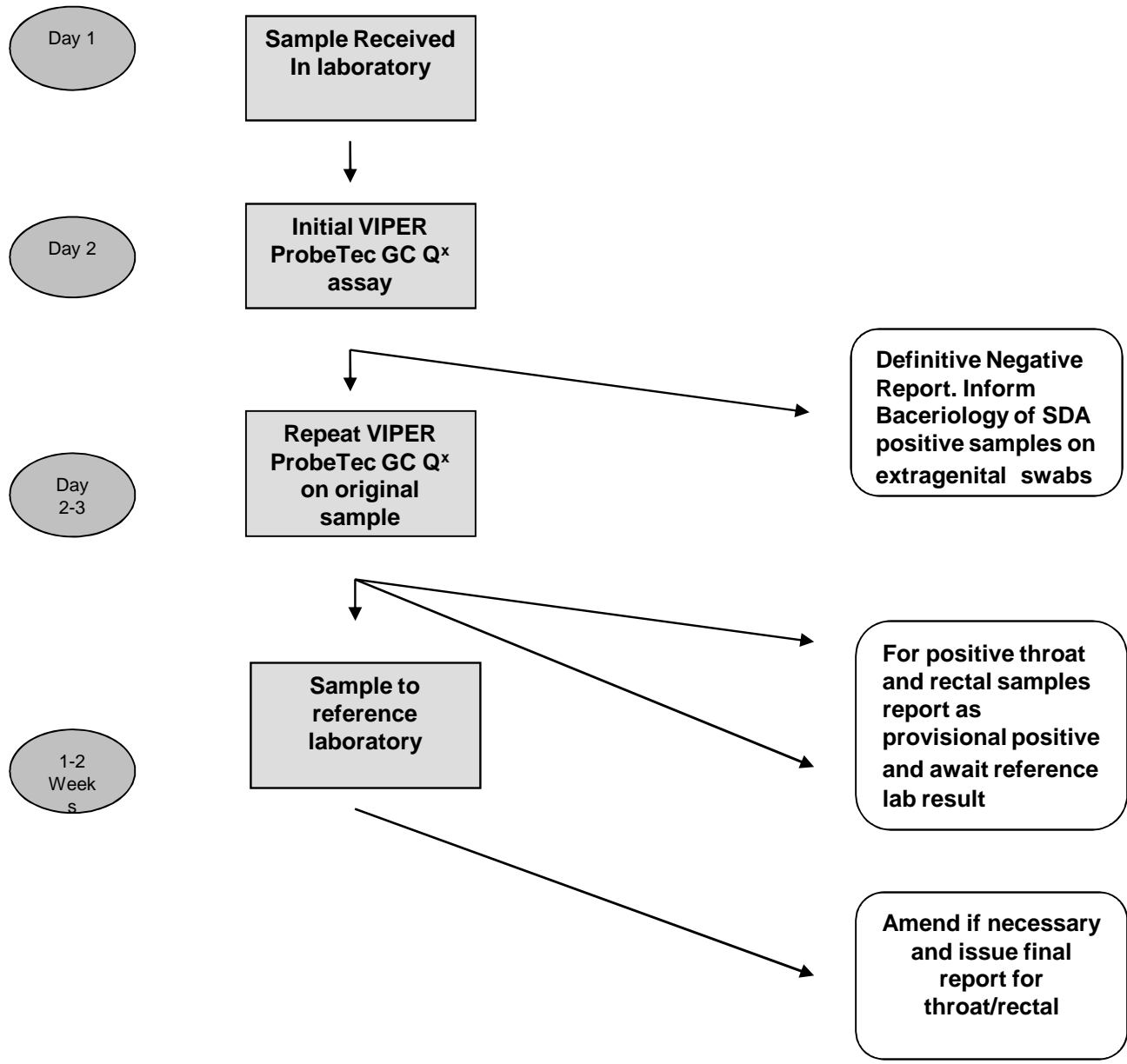


Figure 1 Outline of confirmatory testing protocol

Name	Sequence (5'-3')	Reference
papTM-F	5'CAGCATTCAATTTGTTCCGAGTC 3'	9
papTM-R	5'GAACTGGTTTCATCTGATTACTTTCCA 3'	9
papTM-P	FAM-CGCCTATACGCCTGCTACTTTTACGC-BHQ1	9
Gcopa-LNA	FAM-CCGATATAATC+CGTC+CTTCAA+CATCAG-TAMRA	11
GcopaF-	TTGAAACACCGCCCGGAA	11
GcopaR-	TTTCGGCTCCTTATTCGGTTTAA	11

Table 1 Primer and Probe nucleic acid sequences for PorA and Opa real time PCR assays

Specimen Type	No. of specimens tested	No. (%) of positive VIPER GC Q ^x Results	No (%) and result of ProbeTec Q ^x specimens tested using confirmatory NAAT and culture					
			Pos by NAAT	Neg by NAAT	Pos by Culture	Neg by Culture	Confirmed Pos by culture and/or NAAT	Neg by both culture and confirmatory NAAT
Genital	14,223	149(1.0)	134 ^a (89.3)	15 (10)	131 (87.9)	18 (12.0)	141	8
Pharyngeal	840	26(3.1)	13 (50)	13 (50)	5 (19.2)	21 (80.8)	13	13
Rectal	593	17 (2.87)	9 ^a (52.9)	1 (5.9)	8 (47.1)	5 (29.4)	17	0

^a One sample was inhibitory by confirmatory NAAT but positive by culture.

Table 2 Numbers of ProbeTec GC Q^x positive samples confirming by supplementary NAAT and culture for different sample types.