Low Positive Predictive Value of a Nucleic Acid Amplification Test for Nongenital Neisseria gonorrhoeae Infection in Homosexual Men

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The diagnosis of nongenital Neisseria gonorrhoeae infection by the Becton Dickinson ProbeTec ET Chlamydia trachomatis and N. gonorrhoeae Amplified DNA assay had low positive predictive value among an Australian community-based sample of homosexual men. Only 30.4% of oropharyngeal samples and 73.7% of anorectal samples were positive for N. gonorrhoeae by the porA assay. The accuracy of nucleic acid amplification tests in this context is compromised without supplemental testing.

Nucleic acid amplification tests (NAATs) for the detection of Neisseria gonorrhoeae have provided clinical laboratories with advantages over traditional culture methods; such advantages include increased sensitivity with regard to limit of detection, allowing high throughput without the need for viable organisms, and the ability to perform multiplex assays for the simultaneous detection of Chlamydia trachomatis [1]. However, potential drawbacks of NAAT include increased cost, inability to determine antimicrobial sensitivities, the possibility of contamination, and the presence of interfering or inhibiting substances. In addition, NAATs are validated for use only with genital samples, principally because of potential cross-reactivity with commensal Neisseria species, which are more common at nongenital sites [1]. For this reason, US and Australian guidelines recommend that culture for N. gonorrhoeae be performed on samples from the pharynx or anorectum [2, 3]. However, for community-based screening, barriers to specimen collection and transportation delays may mean that diagnosis by NAAT is preferred by some clinicians [1].

The Becton Dickinson ProbeTec ET C. trachomatis and N. gonorrhoeae Amplified DNA assay (BD ProbeTec) uses strand displacement amplification and targets the multicopy pilin gene-inverting homologue of N. gonorrhoeae [4]. The BD ProbeTec assay has been shown to cross react with commensal Neisseria species, such as Neisseria flavescens, Neisseria lactamica, Neisseria subflava, and Neisseria cinerea, which leads to false-positive results for samples obtained from nongenital sites [1]. Several supplemental assays for both genital and nongenital samples have been developed with a target sequence different from that of the screening assay. Targets have included the orf1, ompIII, and opa genes, which have also shown cross-reactivity with Neisseria species other than N. gonorrhoeae [1]. In some populations, cppB assays failed to detect N. gonorrhoeae because of certain strains lacking the target sequence [1]. The porA pseudogene has been shown to be a suitable target for diagnosis of both genital and nongenital gonorrhea [5, 6]. Almost 100% concordance was observed between results of rectal and pharyngeal porA testing and results of N. gonorrhoeae culture among an Australian clinical population predominantly consisting of homosexual men [5]. Of the small number of discrepant nongenital specimens identified, all had positive results of 3 additional gonococcal NAATs, suggesting that these culture-negative, porA-positive specimens yielded true-positive results [5].

To determine the number of false-positive results of N. gonorrhoeae testing of samples obtained from nongenital sites, we performed supplemental testing using the porA pseudogene for oropharyngeal and anorectal samples collected from participants in the Health in Men Study that were positive for N. gonorrhoeae by screening with the BD ProbeTec assay.

Methods. The Health in Men Study was a community-based cohort study that commenced in 2001 and concluded in July 2007. A total of 1427 HIV-negative men who reported having sex with other men in the past 5 years were recruited primarily through gay community events, institutions, and networks in Sydney. Health in Men Study participants were offered annual screening for sexually transmissible infections, including those due to C. trachomatis and N. gonorrhoeae, with use of
urine specimens, oropharyngeal swabs specimens, and self-collected anorectal swab specimens [7]. Uptake was high, with 90.2% of participants agreeing to screening throughout the period of the study.

Samples were screened using the BD ProbeTec assay according to the manufacturer’s instructions [8]. This assay includes an amplification control to monitor for interfering and/or inhibiting substances. Result interpretation was performed as detailed by the manufacturer, with method-other-than-acceleration scores >2000 considered to indicate positivity for N. gonorrhoeae DNA.

Automated nucleic acid extraction was performed using the Roche MagNA Pure LC. Stored swab specimens were processed with the addition of 450 μL of PCR-grade water, 200 μL of which was used for nucleic acid extraction and 200 μL of which was spiked with a low concentration of N. gonorrhoeae DNA (obtained from N. gonorrhoeae culture isolates) and was processed to monitor for interfering and/or inhibiting substances. Two hundred microliters of BD ProbeTec–processed extracts were also used for automated nucleic acid extraction with the MagNA Pure LC. Another 200 μL was spiked with a low concentration of N. gonorrhoeae DNA and was processed. The final elution volume for all specimens was 50 μL. Extracts were then analyzed or stored at −20°C for analysis at a later date.

The LC porA assay was used as described elsewhere [9]; each capillary was loaded with 2 μL of 10 × master reagent (LightCycler FastStart DNA Master Hybridization Probes kit; Roche Diagnostics), 4 mmol/L of magnesium chloride (Roche Diagnostics), 0.4 μmol/L of forward primer, 0.6 μmol/L of reverse primer, 0.2 μmol/L of each hybridization probe (Promega), and 5 μL of DNA extract. The final volume was adjusted to 20 μL with PCR-grade water. Each run contained 1 positive control and at least 1 no-target control. The LightCycler instrument was used for amplification and detection, with conditions described elsewhere [9]. Samples were considered to be positive for N. gonorrhoeae DNA if they showed exponential amplification and a melting peak of ~65°C.

A total of 427 specimens obtained from oropharyngeal and anorectal sites were tested with the LC porA supplemental assay. Samples composed of 158 processed swab specimens that were initially reactive for N. gonorrhoeae DNA with the BD ProbeTec assay (136 oropharyngeal swab specimens and 22 anorectal swab specimens) were stored at −70°C before supplemental testing. The remaining samples tested were BD ProbeTec extracts, which were stored at −70°C before processing for supplemental testing. This group included 154 BD ProbeTec ET extracts (extracted from 140 oropharyngeal swab specimens and 14 anorectal swab specimens) that were initially reactive for N. gonorrhoeae DNA with the BD ProbeTec assay and 50 each of oropharyngeal and anorectal extracts that were negative for N. gonorrhoeae DNA with the BD ProbeTec assay. Positive predictive values (and negative predictive values) were calculated for samples obtained from each site as the proportion of specimens that were initially reactive (or negative) with the BD ProbeTec assay and that tested positive (or negative) with the LC porA assay.

**Results.** A total of 327 samples that were initially reactive for N. gonorrhoeae DNA with use of the BD ProbeTec assay were tested with the LC porA supplemental assay. Because there was no statistically significant difference between the results obtained with the processed swab specimens and those obtained with BD ProbeTec ET extracts for either oropharyngeal (P = .17) and anorectal (P = .27) specimens, data for the swab specimens and the extracts were combined. Eighty-eight of 289 oropharyngeal samples and 28 of 38 anorectal samples tested positive for N. gonorrhoeae with the LC porA assay. Thus, the positive predictive value of the BD ProbeTec assay for the detection of N. gonorrhoeae was 30.4% (95% CI, 25.2%–36.1%) for the oropharynx specimens and 73.7% (95% CI, 56.9%–86.6%) for the anorectum specimens. Negative predictive values were 100% for both types of specimens, because all 50 oropharyngeal and 50 anorectal extracts that were negative for N. gonorrhoeae DNA with the BD ProbeTec assay were also negative with the LC porA assay. All samples that were negative with the LC porA assay had N. gonorrhoeae DNA detected in their spiked specimens, indicating that there were no false-negative results attributable to interfering and/or inhibiting substances.

**Discussion.** Only one-third of oropharyngeal and three-quarters of anorectal specimens that were initially reactive for N. gonorrhoeae DNA with the BD ProbeTec assay had positive LC porA assay results. Thus, supplemental testing of nongenital specimens that were initially reactive for N. gonorrhoeae with the BD ProbeTec in this community-based cohort of homosexual men resulted in greatly improved accuracy of gonorrhea diagnosis at these sites. Currently, there are no commercially available N. gonorrhoeae NAATs that are validated for use with oropharyngeal and anorectal samples; however, in situations in which they are used, it is recommended that nongenital samples undergo supplemental testing by NAAT targeting genes different from those used in the screening assay [3]. False reactivity of nongenital specimens with use of commercially available gonococcal assays appears to be a general problem when NAAT assays are used. The results of supplemental testing for N. gonorrhoeae DNA in specimens obtained from the oropharynx were positive for as few as 5.6% of these specimens when another commercially available assay was used [10].

For genital specimens collected in a clinical setting, previous studies have shown that the BD ProbeTec assay for detection of N. gonorrhoeae has a positive predictive value of 80%–100% and a negative predictive value of 98%–100% [11]. Our data demonstrate that the positive predictive value of the assay is
substantially less for pharyngeal swab specimens but only slightly less for anorectal swab specimens. Positive predictive value is higher when the prevalence of disease is relatively high, such as in our population [7]. In populations with a low prevalence of gonorrhea, the positive predictive value of the BD ProbeTec assay for specimens from nongenital sites will be even lower than what we have reported.

Because of financial and laboratory constraints, our study did not use 3 separate assays with distinct targets on the gonococcal gene, as is recommended for nongenital *N. gonorrhoeae* diagnosis by NAAT [3]. Nonetheless, we sought to improve diagnostic precision by using a supplemental *porA* NAAT that has been reported elsewhere to have excellent sensitivity, specificity, and discriminatory capacity for *N. gonorrhoeae* in nongenital samples in an Australian population [5]. It is possible that the sensitivity of the BD ProbeTec assay was higher than that of the *porA* assay for some samples, leading to misclassification of some test results as false positive. However, because of the mobility of the gay population in Australia, it is unlikely that the excellent *porA* assay sensitivity obtained in another eastern Australian city [5] would be substantially less among Health in Men Study participants tested in Sydney.

Community-based screening of homosexual men could play an important role in the control of sexually transmitted infections and may reach groups who would otherwise not access clinical services for sexually transmitted infection testing. Accurate, noninvasive diagnostic techniques are pivotal to the success of such screening programs. False-positive results may lead to substantial adverse social, sexual, and economic consequences because of the requirements of partner notification, claims of infidelity, unnecessary clinic visits and pathology tests, and unwarranted treatment. Epidemiological studies that have used reported results of the BD ProbeTec assay for nongenital sites without supplemental testing by another NAAT [12] will substantially overestimate gonococcal prevalence, particularly at the pharyngeal site.

The use of NAAT for the diagnosis of gonorrhea remains problematic [1], and appraisal of gonococcal NAATs for testing of specimens obtained from nongenital sites requires further validation in different populations in diverse geographical locations. Our results suggest that a positive result of a single NAAT cannot be considered to be reliable for diagnosis of pharyngeal gonorrhea in Australian homosexual men. More research involving a larger number of specimens is required to determine the performance characteristics of the BD ProbeTec assay for anorectal samples. Nonetheless, we believe that using a *porA* supplemental assay enables a diagnosis of oropharyngeal and anorectal *N. gonorrhoeae* infections to be made with confidence.

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