Positive Predictive Value of Gen-Probe APTIMA Combo 2 Testing for *Neisseria gonorrhoeae* in a Population of Women with Low Prevalence of *N. gonorrhoeae* Infection

Matthew R. Golden,² James P. Hughes,² Linda E. Cles,² Karen Crouse,² Katherine Gudgel,² Jinxin Hu,² Paul D. Swenson,² Walter E Stamm,¹ and H. Hunter Handsfield³

¹Division of Infectious Diseases and ²Department of Biostatistics, University of Washington, and ³Public Health–Seattle and King County, Seattle, Spokane Regional Health District Laboratory, Spokane, and ⁴Washington State Department of Health, Olympia, Washington

We collected specimens from women who tested positive for *Neisseria gonorrhoeae* using the Gen-Probe APTIMA Combo 2 (AC2), and we performed confirmatory tests using a nucleic acid amplification test (NAAT) that targets alternate gonococcal nucleic acid sequences. Among 59,664 specimens, 280 (0.47%) had positive results using AC2; 265 of these specimens were tested using the confirmatory NAAT, of which 258 yielded positive results (positive predictive value, 97.4%; 95% confidence interval, 95.1%–98.8%). Routine confirmatory testing of specimens with positive AC2 gonorrhea test results is not indicated.

Programs to test asymptomatic, sexually active young women for genital tract infections with *Neisseria gonorrhoeae* and *Chlamydia trachomatis* are widespread in many industrialized countries. In most settings, gonorrhea is much less common than chlamydial infection; 5.6% of female family planning clinic patients aged 14–24 years in the United States tested positive for *C. trachomatis* in 2002, whereas the prevalence of *N. gonorrhoeae* in that population was <1% in 2000 [1, 2].

Nucleic acid amplification tests (NAATs) are commonly used to test for *C. trachomatis* and gonorrhea [3, 4]. The Gen-Probe APTIMA Combo 2 (AC2) assay detects both *C. trachomatis* and *N. gonorrhoeae*. The AC2 package insert states that the test for *N. gonorrhoeae* has a sensitivity of 99.2% and a specificity of 98.7% when cervical swab specimens are used and a sensitivity of 91.3% and a specificity of 99.3% when urine specimens obtained from women are used. These estimates are comparable to those for other NAATs [5] and, if correct, would mean that the positive predictive values (PPVs) for the test in a population with a prevalence of *N. gonorrhoeae* of 1% would be 43% and 57% for cervical and urine specimens, respectively. Most clinicians would not consider this PPV adequate for a test result to be regarded as diagnostic, and the Centers for Disease Control and Prevention (CDC) recommends that confirmatory testing “be considered” when nonculture tests have a PPV of <90% [6]. However, estimates of the sensitivity and specificity of NAAT are not sufficiently precise to confidently define the PPV of these tests for *N. gonorrhoeae* in the very low-prevalence populations in which they are typically used.

To assess whether there is a need to routinely perform confirmatory tests for specimens that test positive for *N. gonorrhoeae* using AC2, we used the commercially available Gen-Probe APTIMA GC (AGC) assay to retest all specimens obtained from women that tested positive for *N. gonorrhoeae* by AC2 in a low-prevalence population. Because the study design we employed is not typically used to assess new diagnostic tests, we also calculated the number of specimens we would have had to test using both assays to obtain a similar estimate of the PPV had we enrolled patients in a study designed to precisely define the AC2’s sensitivity and specificity and then used those parameters to calculate the PPV.

**Methods.** Study specimens included consecutive urine and cervical swab specimens obtained from women who tested positive for *N. gonorrhoeae* in 3 public health laboratories and 1 academic laboratory in Washington state in 2002 and 2003. Specimens from sexually transmitted disease clinics were excluded. All specimens were collected as part of routine clinical care.

AC2 tests were initially performed on pooled lots of 4 or 5 specimens. AC2 testing was done according to the manufacturer’s instructions for individual specimen testing. If a pool yielded positive results, specimens were tested individually. Some pools contained both urine and cervical swab specimens. Only specimens that tested positive individually were regarded as positive; these specimens were frozen at −20°C for up to 30 days and at −70°C thereafter for future evaluation.

AGC was used to test all specimens that initially yielded positive AC2 results, as well as to test a sample of consecutive individual specimens at each laboratory that originally tested
negative for *N. gonorrhoeae*. AGC is similar to AC2 except that it uses oligonucleotides that target 16S rRNA sequences different from those of AC2.

Ninety-five percent confidence intervals for the estimated PPV were calculated using an exact binomial method [7]. The sample size for the confirmatory testing design in table 1 was determined by finding the smallest N that consistently (i.e., 95% of the time) gave a lower bound of a 95% CI for PPV of ≥0.90. Technically,

$$N_{\text{confirm}} = \min_n \sum I(9 < \text{CI}[\alpha,v,n,x]) \left( \frac{n}{x} \right) v^*(1-v)^{n-x} \geq 0.95,$$

where $I(B)$ is the indicator function with value 1 if B is true and is 0 otherwise, and $\text{CI}[\alpha,v,n,x]$ is an $\alpha$ level (exact) confidence interval for the PPV, $v$, based on a sample of size $n$ with $x$ true positives. $N_{\text{dual}}$ and $N_{\text{confirm}}$ were related by

$$N_{\text{dual}} = \frac{N_{\text{confirm}}}{p \times \text{sensitivity} + (1-p) \times (1-\text{specificity})},$$

where $p$ is the disease prevalence. PPV and specificity were related using

$$\text{PPV} = \left( 1 + \frac{1-\text{specificity}}{\text{sensitivity}} \frac{1-p}{p} \right)^{-1}.$$
on all specimens that yield positive results for a defined period of time. Results from such studies could guide a decision on whether routine confirmatory testing is warranted [9].

In this study, we employed such an approach and demonstrated that AC2 has a PPV of 97% in a population of women in which the prevalence of *N. gonorrhoeae* infection is 0.5%. Although future studies should seek to replicate our results, on the basis of our findings, we do not believe that use of the AC2 for detection of *N. gonorrhoeae* requires routine confirmatory testing.

Our study has 4 limitations. First, we studied a single NAAT in women. Our findings should not be extrapolated to indicate that confirmatory testing is not needed when using other NAATs, nor do our findings address the issue of AC2 performance in men or on vaginal swab specimens. Second, our laboratories initially tested pooled specimens, a standard procedure in the 4 study laboratories that is neither approved by the US Food and Drug Administration nor recommended by APTIMA’s manufacturer. Because we initially tested pooled specimens, all study specimens were tested twice before confirmatory testing was performed, a procedure that might affect test specificity and, consequently, PPV. However, none of our pooled specimens yielded positive results and then failed to yield a positive result for an individual specimen, a finding that might have raised concerns that repeated testing improved specificity. Increased false-positive testing could be more common when testing individual specimens, because the number of tests performed increases, or could increase with pooling due to increased specimen handling and opportunity for contamination. Thus, we cannot say with certainty how specimen pooling might have affected our estimate of PPV. Third, we assumed that specimens that yielded negative AGC results had false-positive AC2 results. If AGC is less sensitive than AC2, the true PPV of AC2 may be higher. Fourth, and most importantly, the approach we used would not have ruled out all false-positive results. False-positive NAAT results can occur in 3 ways. First, carry-over amplicon can contaminate samples from a previous reaction. Because the AGC assay does not amplify or detect AC2 amplicon, the confirmatory approach we used excludes false-positive results stemming from amplicon contamination. Second, if the rRNA sequences targeted by AC2 and AGC were both present in an organism other than *N. gonorrhoeae*, some specimens we classified as having confirmed positive results may have had false-positive results with both tests. The AC2 package insert [10] information and unpublished data suggest that both AC2 and AGC are highly specific (J. Schachter and D. V. Ferrero, personal communication). Although we cannot completely exclude the possibility that we overestimated the PPV for AC2 because of some shared non-specificity of AC2 and AGC, routine confirmatory testing using either AGC or another NAAT would not necessarily overcome this limitation. Third, false-positive test results can result from mislabeling of specimens or specimen contamination with organisms. Elimination of such false-positive results would require patients to provide additional specimens.

In summary, we have shown that the AC2 does not require routine confirmatory testing, even in populations with a very low prevalence of *N. gonorrhoeae* infection. However, clinicians should be aware that all tests can have false-positive results, and repeated or confirmatory testing may be indicated when

### Table 2. Outcomes of *Neisseria gonorrhoeae* confirmatory testing study among women tested outside of sexually transmitted disease clinics.

<table>
<thead>
<tr>
<th>Laboratory or specimen type</th>
<th>No. of specimens tested during study period</th>
<th>No. (%) of positive AC2 results</th>
<th>No. (%) of AC2-positive specimens tested using AGC and AGC test results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>All</td>
<td>Positive</td>
</tr>
<tr>
<td>Laboratory</td>
<td></td>
<td>Spokane Public Health</td>
<td>8483</td>
</tr>
<tr>
<td></td>
<td></td>
<td>UWa</td>
<td>13,962</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PHSkCa</td>
<td>4024</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WA State</td>
<td>33,195</td>
</tr>
<tr>
<td></td>
<td></td>
<td>All</td>
<td>59,664</td>
</tr>
<tr>
<td>Specimen type</td>
<td></td>
<td>Swab</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Urine</td>
<td>NA</td>
</tr>
</tbody>
</table>

**NOTE.** AC2, APTIMA Combo 2 (Gen-Probe); NA, not available; AGC, APTIMA GC (Gen-Probe); PHSkCa, Public Health Seattle & King County (Washington); UW, University of Washington (Seattle).

* Some specimens were tested at both the UW laboratory and the PHSkCa laboratory. In these instances, confirmatory tests were performed only at the PHSkCa laboratory. Twenty-one specimens originally tested at the UW laboratory had confirmatory testing done at the PHSkCa laboratory.

* All specimens were also culture positive for *N. gonorrhoeae*.  

---

**BRIEF REPORT • CID 2004:39 (1 November) • 1389**

---

Downloaded from https://academic.oup.com/cid/article-abstract/39/9/1387/406425 by guest on 15 February 2019
specific clinical circumstances lead clinicians to doubt laboratory results. The study design we used should be considered for wider application in evaluating new diagnostic tests for use in low-prevalence populations.

Acknowledgments

We thank Dr. Craig Hill for comments on this manuscript.

Financial support. National Institutes of Health (grant NIH K23 AI01846; to M.R.G.) and an unrestricted grant from Gen-Probe.

Potential conflicts of interest. M.R.G. and P.S. received recent research funding from Gen-Probe (submitted article only). All other authors: No conflict.

References