Performance of the GeneXpert CT/NG Assay Compared to That of the Aptima AC2 Assay for Detection of Rectal Chlamydia trachomatis and Neisseria gonorrhoeae by Use of Residual Aptima Samples

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There are currently no commercially available molecular assays for the detection of Chlamydia trachomatis and Neisseria gonorrhoeae in rectal swabs with regulatory approval. We compared the Cepheid GeneXpert CT/NG assay with the GenProbe Aptima Combo2 assay, using 409 rectal swabs. Using Aptima as the gold standard, the sensitivity, specificity, and positive and negative predictive values of GeneXpert for the detection of C. trachomatis and N. gonorrhoeae were 86%, 99.2%, 92.5%, and 98.4% and 91.1%, 100%, 100%, and 98.6%, respectively. Despite significant dilution of samples prior to GeneXpert testing, the assay performed well with excellent specificity.

Chlamydia trachomatis and Neisseria gonorrhoeae are high-burden sexually transmitted infections (STIs) affecting significant numbers of the general population and high-risk subgroups. A total of 1,307,893 cases of chlamydia (426/100,000 population) and 309,341 cases of gonorrhea (100.8/100,000 population) were diagnosed in the United States in 2010 (3). The respective prevalences in England were 351 and 39.5 per 100,000 population, respectively, in 2011 (8). There has been a recent increase of 61% in cases of gonorrhea in men who have sex with men (MSM) in England. This could be explained by the increased use of nucleic acid amplification tests (NAATs) for extragenital samples (8). Management and control of these infections are heavily reliant upon highly sensitive and specific diagnostic techniques and tracing of sexual contacts.

There is now a wide body of evidence confirming that NAATs are significantly more sensitive than culture for detection of genital STIs. These assays have become a standard diagnostic technique and are now recommended for testing genital and extragenital sample types by various bodies, including the Centers for Disease Control and Prevention (CDC) and the United Kingdom Health Protection Agency (HPA) (4, 7). In the United Kingdom, 82% of sexual health clinics offer asymptomatic MSM screening by NAATs for chlamydia, and 23% are offered NAATs for gonorrhea (2). Several studies using NAATs for the detection of N. gonorrhoeae and C. trachomatis from rectal swab samples have shown a higher burden of disease than would have been detected with traditional assays, including culture (1, 6, 11–15).

Despite a high prevalence of self-reported anal sex in the general population and some high-risk groups, there are no commercially available tests for chlamydia or gonorrhea that have regulatory approval for use with rectal swab samples (1, 5, 9, 14). Problems of cross-reactivity with nongonococcal Neisseria species, which may frequently be found in the lower gastrointestinal tract, may have dissuaded commercial manufacturers from seeking regulatory approval for this sample type. Because of this, user verification is necessary to provide data to support their use. Few studies have evaluated the performance of NAATs for use with rectal samples; however, one such study tested self-collected rectal swabs with the Becton, Dickinson ProbeTec and Gen-Probe Aptima Combo2 assays (12). Based on a composite gold standard of culture or agreement in two or more NAATs, the authors found respective sensitivities of 77.1% and 84.3% for C. trachomatis and 49.0% and 81.8% for N. gonorrhoeae. A similar study using the same assays found respective sensitivities of 63% and 93% for C. trachomatis and 78% and 93% for N. gonorrhoeae (13). Neither of these two studies suggested that there was a significant problem with cross-reactivity of nongonococcal Neisseria species, with both reporting specificities in excess of 99.3% (12, 13).

The GeneXpert CT/NG assay (Cepheid, Sunnyvale, CA) is a newly developed real-time PCR-based assay for the simultaneous detection of C. trachomatis and N. gonorrhoeae. The assay uses the GeneXpert cartridge, which enables automated sample preparation, extraction, amplification, and detection in a closed system. There is nominal hands-on time, with results available in approximately 90 min. The assay has built-in target redundancy for N. gonorrhoeae (two different molecular targets must be detected for a positive result) and a sample adequacy control, which detects the presence of a single-copy human gene.

We aimed to validate this assay for use with rectal samples, comparing performance characteristics against those of the commonly used Aptima Combo 2 assay (GenProbe, San Diego, CA).

MATERIALS AND METHODS

We undertook an evaluation of the diagnostic accuracy of the Cepheid CT/NG assay using rectal swab specimens, comparing its performance with that of the Aptima Combo2 (followed by either the Aptima CT or GC assay as necessary for confirmation).
TABLE 1 Distribution of results for samples tested with each assay for chlamydia and gonorrhea

<table>
<thead>
<tr>
<th>Organism</th>
<th>GeneXpert result</th>
<th>Aptima Combo2 result (plus confirmation for positive results)</th>
<th>No. (%) of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Chlamydia trachomatis</em></td>
<td>Negative</td>
<td>Negative</td>
<td>363 (88.8)</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>Positive</td>
<td>37 (9)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td>6 (1.5)</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>3 (0.7)</td>
</tr>
<tr>
<td><em>Neisseria gonorrhoeae</em></td>
<td>Negative</td>
<td>Negative</td>
<td>353 (86.3)</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>Positive</td>
<td>51 (12.5)</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>5 (1.2)</td>
</tr>
</tbody>
</table>

* Samples with positive results in the Aptima Combo2 assay were confirmed using the Aptima CT or Aptima NG assay as necessary.

RESULTS

It was immediately apparent that residual Aptima samples were incompatible with the GeneXpert CT/NG assay; when used neat, these produced consistently invalid results. This was resolved with dilution into GeneXpert swab sample buffer, and a series of dilutions was tested to determine the lowest dilution ratio producing a valid result.

A total of 409 rectal swab samples were tested using both assays. A total of 9 (2.2%) and 5 (1.2%) samples gave discordant results for *C. trachomatis* and *N. gonorrhoeae*, respectively. The distributions of results obtained using both assays are shown separately for *C. trachomatis* and *N. gonorrhoeae* in Table 1. All samples with positive results using the Aptima Combo2 test were confirmed positive using the Aptima CT and/or Aptima GC.

A total of 43 (10.5%) samples were positive for *C. trachomatis* using Aptima, six of which (1.5%) were not detected by GeneXpert; this resulted in an estimated sensitivity of 86% (95% confidence interval [CI], 72.1 to 94.7%). GeneXpert produced a presumed false-positive result in three (0.7%) samples that were negative by Aptima, resulting in a specificity of 99.2% (95% CI, 97.6 to 99.8%). These samples were not tested further and it is possible that these were false negatives for the Aptima assay. A total of 56 (13.7%) samples were positive for *N. gonorrhoeae* using Aptima, five of which (1.2%) were not detected by GeneXpert, resulting in an estimated sensitivity of 91.1% (95% CI, 80.4 to 97%). GeneXpert did not produce any additional positive results, resulting in a specificity of 100% (95% CI, 99 to 100%).

Using Aptima as the gold standard, GeneXpert gave a positive predictive value (PPV) and a negative predictive value (NPV) of 92.5% (95% CI, 79.6 to 98.4%) and 98.4% (95% CI, 96.5 to 99.5%), respectively, for *C. trachomatis* and a PPV and an NPV of 100% (95% CI, 98.6% to 99.6%), respectively, for *N. gonorrhoeae*. The overall accuracy was 97.8% (95% CI, 95.9 to 99%) and 98.5% (95% CI, 96.8 to 99.6%) for *C. trachomatis* and *N. gonorrhoeae*, respectively. Kappa statistics were calculated and found to be 0.88 (95% CI, 0.8 to 0.96) for *C. trachomatis* and 0.95 (95% CI, 0.9 to 0.99) for *N. gonorrhoeae*, indicating a high level of agreement between the assays.

Seven samples were positive for both *C. trachomatis* and *N. gonorrhoeae* using the Aptima test; these samples were also positive for both targets using GeneXpert.

DISCUSSION

The incompatibility of Aptima buffer with the GeneXpert CT/NG test is presumed to be due to an interfering or inhibiting substance, although it is not clear what this may be. This necessitated diluting residual Aptima buffer into GeneXpert swab sample buffer at a ratio of 1:15, which may have resulted in the observed reduction in sensitivity compared with the Aptima assay. Despite this, these preliminary data show promise, with excellent specificity for both targets. Since residual samples were anonymized following testing with the Aptima assay, it was not possible to correlate test results with clinical findings.

The specificity of the GeneXpert assay for *N. gonorrhoeae* is similar to that found for other NAATs in previously published studies (>99.3%), suggesting that cross-reactivity is not a significant problem for this target in this high-prevalence population. This may be due to the target redundancy of the GeneXpert assay and suggests that a confirmatory test is not required for MSM.

The GeneXpert CT/NG test is well suited for near-patient testing due to its rapid turnaround time and ease of use. Such a practice may result in significant changes to the patient pathway and may aid in enabling immediate treatment of infections and prompt contact tracing. Cost-effectiveness studies are clearly needed. An additional advantage of the test is the inclusion of a target detecting human DNA to ensure sample adequacy. This may be a useful feature given the trend toward using self-collected samples (10). The GeneXpert CT/NG assay appears to be sensitive and specific for use with rectal swabs. However, further study using primary samples collected directly into GeneXpert buffer is required.

ACKNOWLEDGMENTS

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REFERENCES