Effect of Endocervical-Specimen Adequacy on Detection of Chlamydia trachomatis by the APTIMA COMBO 2 Assay

C. K. Rogers, 1* B. J. Wood, 2 P. Rizzo, 2 and C. A. Gaydos 2

Wyoming Public Health Laboratory, 517 Hathaway Bldg., 2300 Capitol Ave., Cheyenne, Wyoming 82002, 1 and Johns Hopkins University, 1159 Ross Bldg., 720 Rutland Ave., Baltimore, Maryland 21205 2

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Six hundred one endocervical specimens were analyzed for Chlamydia trachomatis by the APTIMA Combo 2 assay and evaluated for columnar epithelial cell adequacy by direct fluorescent-antibody staining. With 5.5% positive adequate and 7.8% positive inadequate specimens (P = 0.27), the study suggested no difference in positivity rates due to specimen adequacy when this amplified technology was used.

Chlamydia trachomatis infection is the most common bacterial sexually transmitted disease in the United States (7). In women, symptoms of C. trachomatis infection are usually mild or absent (15). The Centers for Disease Control and Prevention (CDC) has recommended widespread screening of women in an effort to control this epidemic (3). Many improvements in test technologies have evolved, including nucleic acid amplification tests (NAATs) (2, 5, 6, 8, 14, 16). However, since chlamydiae are intracellular organisms which infect columnar epithelial cells, inadequate specimens with few or no columnar cells have substantially impacted the sensitivity of testing for C. trachomatis antigens (9, 11). The value of increased sensitivity when using amplified testing to overcome inadequate specimens has not been confirmed in other studies with older NAATs (1, 9–13, 18). As a result, CDC has recommended monitoring of columnar cell content in endocervical specimens to assess specimen quality (4).

This study assessed whether specimen adequacy, based on the traditional presence or absence of columnar epithelial cells, significantly affected the detection of C. trachomatis by a more recent NAAT, the APTIMA Combo 2 assay (Gen-Probe, Incorporated, San Diego, CA). We believed the increased sensitivity of this second-generation amplification test, APTIMA Combo 2, could potentially overcome the necessity to measure cellular adequacy.

Six hundred one specimens were collected from women at two family planning clinics, two sexually transmitted disease clinics, and a community health center (sites A, B, and C). Asymptomatic and symptomatic females who presented at the clinics, and a community health center (sites A, B, and C). A specimen was graded as adequate if it contained any columnar epithelial cells, with or without other cells (9–11). As a result, CDC has recommended monitoring of columnar cell content in endocervical specimens to assess specimen quality (4).

Specimen adequacy by clinic site averaged 70%. When excluding the positivity rates of adequate versus inadequate specimens was found (P = 0.27). Only 7 of 601 (1.2%) were DFA slide positive, demonstrating typical fluorescent elementary bodies. These 7 were included in the 37 Combo 2-positive results. There were no discordant specimens in which Combo 2 was negative and DFA was positive. All 37 (100%) Combo 2 positives repeated positive. Thirty-four aliquots of the Combo 2-positive specimens were available and sent for testing with the stand-alone APTIMA CT assay. All 34 aliquots confirmed as positive for C. trachomatis by the APTIMA CT assay. A remnant sample was unavailable for 3 of the 37 Combo 2-positive specimens.

Specimen adequacy by clinic site averaged 70%. When examining the positivity rates of adequate versus inadequate groups within individual sites, numbers in each category were...
too small for statistical analyses. Site C routinely uses a large proctoscopic-type swab for a more thorough removal of exocervical mucus, exposing more of the cellular components. There was no increase in specimen adequacy resulting from this practice.

The accepted definition of adequacy has previously been based upon columnar epithelial cell presence (1, 9, 10, 18). However, the presence of erythrocytes (RBCs) may indicate cervical friability, a common finding for *Chlamydia trachomatis* infection (9, 18). Given this scenario, the study looked at the additional presence of RBCs in adequate and inadequate groups. There was no significant change in positivity for groups containing RBCs from that for groups without RBCs (P = 0.28; P = 0.48).

The use of two swabs rotated simultaneously during collection eliminated the discrepancy which can occur when a single swab is used to collect a specimen for two testing platforms. Additionally, this collection method resulted in a total sample positivity of 6% (37/601), which is similar to the general 6% prevalence rate in the current population.

CDC has suggested supplemental testing after an initial positive NAAT screening in certain cases and in populations with a low prevalence of infection (4). One such approach suggested by CDC involves repeating the original test on the original specimen. In this study, all 37 Combo 2-positive specimens were repeat positive by the original test and the original specimen, thus confirming positive results by repeat testing. Another CDC option suggested additional testing of the original specimen with a different NAAT or one that uses an alternative target or format. Of the 34 Combo 2 samples with sufficient quantity for allocation and testing with the stand-alone APTIMA CT assay, all 34 (100%) verified as positive with this ligase chain reaction and ligase chain reaction for the detection of *Chlamydia trachomatis*. Int. J. STD AIDS 8:731–738.

Table 1. *Chlamydia* positivity in adequate versus inadequate specimens (n = 601)

<table>
<thead>
<tr>
<th>Adequacy</th>
<th>No. (%) of specimens tested</th>
<th>Result by APTIMA Combo 2</th>
<th>Positivity rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. positive</td>
<td>No. negative</td>
<td></td>
</tr>
<tr>
<td>Adequate</td>
<td>422 (70)</td>
<td>23</td>
<td>399</td>
</tr>
<tr>
<td>Inadequate</td>
<td>179 (30)</td>
<td>14</td>
<td>165</td>
</tr>
<tr>
<td>Total</td>
<td>601</td>
<td>37</td>
<td>564</td>
</tr>
</tbody>
</table>

* Aliquots from 34 Combo 2-positive specimens were available for confirmatory testing. All 34 confirmed positive.

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REFERENCES


