Efficacy of Duplicate Genital Specimens and Repeated Testing for Confirming Positive Results for Chlamydiazyme Detection of Chlamydia trachomatis Antigen

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In an attempt to increase Chlamydiazyme (Abbott Laboratories) detection of Chlamydia trachomatis antigen and to establish the reproducibility of positive results, we carried out an investigation into the usefulness of testing duplicate specimens, of more aggressive endocervical specimen collection by using cytobrushes instead of swabs, and of the repeated testing of both specimens from patients with one or two positive results. Duplicate endocervical (female) and urethral (male) specimens, including one swab and one cytobrush specimen from 1,331 nonpregnant women, were collected from symptomatic and asymptomatic patients. Specimens were transported and tested for C. trachomatis antigen as specified by the manufacturer. Tests on all specimens from patients with positive results were repeated. Antigen was initially detected in one or both specimens from 210 (10.7%) of 1,968 patients, and repetition of the tests confirmed its presence in 198 (10.1%) of the patients, including all 183 patients in whom it was initially detected in both specimens. Initial results from at least 8 of the 12 patients with irreproducible antigen detection were most probably falsely positive. Results from 21 (10.6%) of the 198 patients for whom antigen detection was confirmed were repeatedly positive on only one specimen (9 [4.5%] on the second of the two specimens collected). Of 115 women from whom one swab and one cytobrush sample were taken and who had repeatedly positive results, antigen was detected in 7 (6.1%) only on the swab sample and in 4 (3.5%) only on the cytobrush sample. Use of the cytobrush does not appear justifiable with the Chlamydiazyme assay, and collection of duplicate specimens provided only a modest increase in detection of C. trachomatis. However, repeated testing of specimens when results from only one of two specimens are positive appears to be of clinical value.

Materials and Methods

From 22 June 1987 until 9 March 1988, duplicate genital specimens for C. trachomatis antigen detection were obtained from asymptomatic and symptomatic patients suspected clinically of having sexually transmitted disease. If a specimen was to be collected for recovery of Neisseria gonorrhoeae, it was taken first. After removal of exocervical mucus, two consecutive STD-EZE cotton swabs (Abbott) were inserted into the endocervical canal of pregnant patients, rotated, and placed into their individual transport tubes, labeled no. 1 and 2 in order of collection. For nonpregnant females, a swab and then a cytobrush (Syva Co., Palo Alto, Calif.) were used to obtain endocervical specimens. The order of collecting the swab and cytobrush specimens was reversed partway through the study. For male patients, two consecutive STD-PEN cotton swabs (Abbott) were inserted 2 to 4 cm into the urethra, rotated, and placed into individual transport tubes with the order of collection specified. Specimens were stored in their transport tubes at 4 to 8°C and tested for C. trachomatis antigen within 72 h by using the Chlamydiazyme procedure (Abbott) as specified by the manufacturer. Specimen dilution buffer (1 ml) was added to each specimen, and after 10 to 15 min, 200 μl of the vortexed specimen and controls were reacted in sequence with beads containing antibody to C. trachomatis (37°C for 60 min), rabbit antibody to C. trachomatis (37°C for 60 min), horse-radish peroxidase-conjugated antibody to rabbit immunoglobulin G (37°C for 60 min), and O-phenylenediamine (25°C for 30 min). The reactions were terminated with 1 N sulfuric acid. Reaction beads were washed by using the Proquantum (Abbott), and the A492 was determined on the Quantumat spectrophotometer (Abbott). A result was considered positive when the A492 reading was greater than or equal to the mean of three negative controls plus 0.100 nm. Tests on all specimens from patients with positive or discrepant (one
TABLE 1. Relative costs per patient of different methods for genital specimen collection and testing by the Chlamydiazyme procedure

<table>
<thead>
<tr>
<th>Collection and testing method</th>
<th>Total technologist time (min)</th>
<th>Costs to hospital (dollars) for:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Labor</td>
</tr>
<tr>
<td>Single swab; results not confirmed by repeating tests(^a)</td>
<td>7</td>
<td>1.46</td>
</tr>
<tr>
<td>Duplicate swabs; tests selectively repeated(^b)</td>
<td>14.4</td>
<td>3.01</td>
</tr>
<tr>
<td>One swab and one cytobrush; tests selectively repeated(^b)</td>
<td>14.4</td>
<td>3.01</td>
</tr>
</tbody>
</table>

\(^a\) Method described in Chlamydiazyme product insert.
\(^b\) Tests on both specimens were repeated when the result of only one of two was positive.

positive, one negative) results were repeated (in duplicate when volume permitted) within 48 h for confirmation.

The Z test for differences in proportions was used for statistical analysis of results (3). Confidence intervals for a true response probability were calculated by using equation 1 of Simon (18).

RESULTS

Material and labor costs associated with the Chlamydiazyme assay when different collection and processing procedures were followed (as compared with the procedure recommended by the manufacturer) are shown in Table 1. Duplicate genital specimens were obtained from 1,968 patients, who ranged in age from 3 to 79 years (mean, 24.7 years; median, 23 years). C. trachomatis antigen was initially detected in specimens from 210 (10.7%) of the patients (in both specimens from 183 [87.1%] of these patients and in only one of two specimens from the remaining 27 [12.8%]). The age range of these patients was 13 to 49 years (mean, 20.7 years; median, 20 years). Repetition of the test confirmed the presence of antigen in 198 (10.1%) of the patients; in 21 (10.6%) of these, repeated detection of antigen occurred for only one of the two specimens (Table 2). One or both specimens were repeatedly positive for 42 (9.1%) of 460 asymptomatic females, 151 (10.4%) of 1,454 symptomatic females, and 5 (9.2%) of 54 symptomatic males.

Repeatability of results. Among the 210 patients in whom antigen was initially detected, results for both specimens were repeatedly positive for 177 (84.3%) patients and results for one specimen were repeatedly positive while those for the other were repeatedly negative for another 15 patients (7.1%) (Fig. 1). Of the 15 repeatedly positive specimens from the latter patients, 5 were collected first, 7 were collected second, and the order of collection was unknown for the remaining 3. For the remaining 18 (8.6%) patients with initially positive results, the results for one of two duplicate specimens changed to negative (their optical densities [ODs] dropped to 4.8 to 93.3% of the cutoff values) when the tests were repeated. However, antigen was detected reproducibly in the duplicate specimen from 6 (33.3%) of these 18 patients (twice each in specimens collected first and second and twice in specimens with the collection order unknown). For these six patients, the final OD readings for the specimens with results which changed from positive to negative ranged from 62.4 to 88.6% of the cutoff values. For all 12 of the remaining patients (age range, 17 to 49 years; mean, 26.1 years; median, 23 years) with irreproducible C. trachomatis antigen in one specimen, the duplicate specimens lacked detectable antigen; seven (58.3%) of these specimens were collected second. For 8 of these 12 patients, the final OD readings for the specimens (four of which were collected first) that reverted to negative results were <40.8% of the cutoff values.

A decrease of 5 to 6% in the mean \(A_{492}\) was observed when tests on specimens with positive results were repeated (0.055 with swabs collected first, 0.070 with swabs collected second, and 0.056 with the cytobrushes). All results which were initially negative remained negative when tests on specimens from the 27 patients with discrepant results were repeated.

Relative value of the cytobrush and of duplicate specimens. Of 115 female patients from whom samples were taken with both a swab and a cytobrush and from whom repeatedly positive results were obtained, positive results from 4 (3.5%) were obtained only with the cytobrush, whereas positive results from 7 (6.1%) were obtained only with the swab (not significant) (Table 3). However, antigen was detected only in the specimen collected second from at least 9 (4.5%; 95% confidence limit, 2.4 to 8.4%) and as many as 14 (7.1%; 95% confidence limit, 4.8 to 11.3%) of the 198 patients with confirmed positive results (depending on the true order of specimen collection from the 5 patients who had only one positive result and for whom the actual order of duplicate specimen collection could not be determined). Therefore, the use of a second specimen collection device resulted in an increase of at least 4.8% (9 of 189) to 7.6% (14 of 184) in the number of patients with repeatedly positive results. The mean \(A_{492}\) associated with positive results was similar among the specimen collection devices (1.125 for swabs collected first, 1.141 for swabs collected second, and 1.132 for cytobrushes).

DISCUSSION

Aggressive endocervical specimen collection with a scraper has been reported to significantly increase the recovery of C. trachomatis in culture (5). However, no increase was reported in numbers of patients with positive direct fluorescent-antibody smears when cytobrushes were used rather than swabs (although more elementary bodies were frequently detected from cytobrush specimens) (12). For Chlamydiazyme detection of C. trachomatis antigen, the results of the current study indicated no advantage of a cytobrush over a swab which would offset the disadvantages
of using a cytobrush, including increased cost (Table 1) and potential risks to pregnant patients (12).

The relative value of multiple genital specimens for detection of C. trachomatis appears to be related, at least in part, to the number of specimens collected and to the type of assay system used. Reported increases in the number of infected patients detected by the use of multiple specimens have ranged from 5% to as high as 69% when cultures were inoculated (5, 6, 15, 19) but from 0% to only 9% (8, 17) when the Chlamydiazyme method was used. Multiple specimens may be more effective for detection of C. trachomatis when used with culture because of the amplification effect of culture and its greater ability to detect very small numbers of organisms in a specimen (4, 9, 17, 20). The second specimen collected in the current study was the only repeatedly positive specimen from at least 9 (4.5%) and as many as 14 (7.1%) of the 198 patients with repeatedly positive results.

The small drop (5 to 6%) in A492 frequently observed when tests on specimens with positive results were repeated was most probably due to instability of the C. trachomatis antigen during storage. Repeating a Chlamydiazyme test was of no diagnostic value for 183 (87.1%) of the 210 patients for whom positive results were initially obtained. Results with both specimens from these 183 patients were positive when initially tested, and results with at least one of the two specimens remained positive when the tests were repeated.

**FIG. 1.** Chlamydiazyme detection of C. trachomatis antigen following initial testing of all specimens and repeated testing of specimens from patients with positive results.

<table>
<thead>
<tr>
<th>Patients (no.) and collection device</th>
<th>No. (%) of patients with repeatedly positive results</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>Swab, swab (583)</td>
<td>78 (13.4)</td>
</tr>
<tr>
<td>Swab, brush (748)</td>
<td>68 (9.1)</td>
</tr>
<tr>
<td>Brush, swab (583)</td>
<td>47 (8.1)</td>
</tr>
<tr>
<td>Male</td>
<td></td>
</tr>
<tr>
<td>Swab, swab (54)</td>
<td>5 (9.2)</td>
</tr>
<tr>
<td>Total (1,968)</td>
<td>198 (10.1)</td>
</tr>
</tbody>
</table>

* Devices are shown in order of collection.

b One or both specimens from each patient were repeatedly positive.

c Order of collection could not be determined.
Repeating a test appeared to be justified only for specimens from the remaining 27 (12.8%) of the patients whose initial results were positive, i.e., patients with discrepant results from the duplicate specimens. These patients represented only 1.4% of the total number of patients tested. For these 27 patients, the specimen which was initially positive was repeatedly positive and _C. trachomatis_ antigen was considered present in 15 (55.6%). For 8 (29.6%) of the 27 patients, however, results with one of the two specimens were initially positive (A_{95}^2 range, 121.6 to 1.180.4% of the cutoff values) but fell well below the cutoff (A_{95}^2 range, 4.8 to 40.8% of the cutoff values), with a drop in OD ranging from 72.9 to 99.6%, when the tests were repeated. Because of these observations and the repeatedly negative results (A_{95}^2 range, 4.8 to 35.6% of the cutoff values) obtained with tests on the duplicate specimens from these patients, the initial positive results from these eight patients were most probably falsely positive as a result of human or technical errors during the assays. The initial positive results from the remaining 4 (14.8%) of the 27 patients with discrepant results were considered inconclusive. Although the single initial positive result from each patient (A_{95}^2 range, 7.2 to 122.5% above the cutoff values) was not repeatable, the level of the negative results (A_{95}^2 range, 66.7 to 93.3% of the cutoff value; drop in OD ranging from 12.6 to 67.8%) obtained when tests on these specimens were repeated indicated that a low level of antigen might be present and that additional specimens from these patients should be collected and tested. Therefore, repeated testing of duplicate specimens appeared helpful in defining the clinical diagnosis in at least 23 (11.0%) of the 210 patients from whom positive Chlamydia results were initially obtained.

In summary, collection of endocervical specimens with a cytobrush does not appear to be cost-effective when the brushes are assayed by the Chlamydiazyme system. Collection of a second specimen from each patient provided a modest increase (≥4.8%) in detection of patients with Chlamydiazyme-positive results when large numbers of patients were studied. Because of that increase, as well as the value of the second specimen from four additional patients in indicating that the initially positive (nonrepeatable) results from the first specimen were probably falsely positive, this laboratory routinely requests duplicate endocervical swabs. Individual laboratories can determine the cost-effectiveness of duplicate specimens in their own patient populations. Repeated Chlamydiazyme testing of both specimens was clinically helpful and appeared cost-effective only when results of one of the two specimens were positive. Although the reactivity associated with most of these initially positive specimens was confirmed, results with one of two specimens from 18 (8.6%) of the patients with initially positive results changed to negative when the tests were repeated. Test repetition identified 8 (3.8%) of 210 patients for whom initially positive results were most probably falsely positive. Because of the medical and social implications associated with false-positive results for _C. trachomatis_ from genital specimens, the use of a blocking antibody (when available) to confirm the specificity of a positive Chlamydiazyme result would be a highly desirable alternative to such test repetition, especially in low-risk and pediatric patient populations.

**LITERATURE CITED**


