Direct Fluorescent-Antibody Confirmation of Chlamydial Antigen below the Detection Threshold of the Chlamydialzyme Enzyme-Linked Immunosorbent Assay

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The sensitivity of the Chlamydialzyme enzyme-linked immunosorbent assay (ELISA) (Abbott Laboratories, North Chicago, Ill.) for detection of Chlamydia trachomatis in endocervical specimens has frequently been reported to be significantly lower than that of cell culture (1, 8). Reduced detection of the pathogen has been associated, in part, with poor technique in collection of specimens (2, 3, 5, 6). While these poorly collected specimens may contain levels of antigen below the Chlamydialzyme threshold of detection, they may have a sufficient number of viable Chlamydia cells to permit reproduction of the organism to a detectable level in culture.

The manufacturer's instructions indicate that a Chlamydialzyme result should be considered positive only if the Absorbance (A492 nm) reading is greater than or equal to the mean result of three negative controls plus 0.100. Improved Chlamydialzyme sensitivity, however, has been previously reported to be a result of performing direct fluorescent-antibody (DFA) tests on duplicate specimens whenever the ELISA-to-cutoff ratio of any original specimen ranged from 0.3 to 1.0 (7). The Chlamydialzyme blocking reagent (Abbott Laboratories) has been shown, with DFA tests, to reliably confirm true-positive Chlamydialzyme results and to identify false-positive results when the ELISA result is above the manufacturer's recommended cutoff (4). There are no published studies, however, that have indicated that the blocking assay is equally reliable when the ELISA result is below the Chlamydialzyme cutoff. The purpose of the current study was to evaluate the ability of the Chlamydialzyme blocking reagent either to confirm or to eliminate the possibility of chlamydial antigen whenever the A492 was below, but within about 70% of, the cutoff. The hypothesis was that a specimen whose Chlamydialzyme result fell within that low borderline range but whose repeated ELISA result was not blocked by ≥50% with the blocking reagent might be considered truly negative for C. trachomatis antigen without any DFA testing. If the hypothesis were correct, the selective use of the DFA procedure only on those specimens with borderline Chlamydialzyme results which could be blocked by ≥50% might provide a cost-effective means of significantly increasing the detection and confirmation of chlamydial antigen by the Chlamydialzyme procedure.

Specimen collection and testing. Duplicate endocervical swabs for detection of C. trachomatis antigen were obtained from asymptomatic females attending a prenatal clinic and from females with symptoms suggestive of sexually transmitted diseases. Specimens were collected, combined, and tested with the Chlamydialzyme ELISA as previously described (2). Positive ELISA results (above the cutoff) were confirmed by repeating the test and by demonstrating a reduction of ≥50% in the A492 with the blocking reagent (3, 4). All specimens with negative ELISA results but with A492 ranging from 0.30 to the cutoff were retested with both the blocking reagent and the DFA test (MicroTrak; Syva Co., Palo Alto, Calif.). For the latter procedure, 0.3 ml of each specimen in dilution buffer was centrifuged at 10,000 × g for 5 min. Approximately 30 μl of resuspended pellet solution was added to a slide which was then stained with the DFA reagent following the manufacturer's instructions. All fields of each smear were screened by microscopy at a magnification of ×500. Detection of three or more chlamydial elementary bodies was considered to be a positive result (7) and was confirmed at a magnification of ×1,000. Confidence intervals around the single-point proportion estimates were calculated as described by Wassertheil-Smoller (9).

Detection of chlamydial antigen. From 1 September 1991 until 30 November 1992, endocervical swabs were collected from 4,007 patients (age range, 13 to 78 years; mean, 25.2 years; median, 23 years). The specimen volume was insufficient to perform all of the necessary tests for seven of the patients. Positive Chlamydialzyme results with A492 above the manufacturer's cutoff were obtained with specimens from 237 (5.9%) of the 4,000 patients studied. Chlamydial antigen was confirmed in 233 (5.8%) of the specimens by repeating the ELISA and using the blocking reagent. Only 202 (5.0%) of the 4,000 specimens tested had A492 which ranged from 0.30 to the ELISA cutoff. The activity of only 66 (32.7%) of these borderline Chlamydialzyme reactions was blocked by 50% or more by the ELISA blocking reagent, but three or more chlamydial elementary bodies were detected with the DFA in 34 (51.5%) of these 66 specimens (Table 1). As can be seen from the table, a wide range of elementary body counts was observed in these specimens, with a trend towards higher counts as the

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Chlamydiazyme result approached the manufacturer’s cutoff. In contrast, three or more fluorescent chlamydial elementary bodies could not be found in any of the remaining 136 specimens with borderline Chlamydiazyme results (ranging from 0.030 to the ELISA cutoff) when the ELISA activity was blocked by less than 50%.

In all, antigen of *C. trachomatis* was confirmed in 267 (6.7%) of the specimens tested. Patients with positive results ranged in age from 13 to 34 years (mean, 20.1 years; median, 20 years). Since only 233 of the specimens from patients with confirmed positive results were associated with *A*₄₉₂>S greater than or equal to the ELISA cutoff, selective use of the blocking reagent and DFA below the cutoff increased the number of patients found to have confirmed chlamydial antigen by 14.6% (95% confidence interval, 10.1 to 19.1%).

Rapid assays for detection of chlamydial antigen in endocervical specimens have usually been reported to be considerably less sensitive than cell culture (1, 8). In the current study, 233 of the patients had blocking reagent-confirmed, Chlamydiazyme-positive results with *A*₄₉₂>S above the manufacturer’s suggested cutoff. An additional 34 (14.6%) *C. trachomatis*-infected patients were detected by retesting only those 5% of the specimens which gave borderline Chlamydiazyme results, ranging from 0.030 up to the cutoff. The DFA procedure did not confirm the presence *C. trachomatis* in any of the specimens with borderline ELISA results when those results could not be blocked with the blocking reagent. In contrast, the presence of chlamydial antigen was confirmed by DFA testing in 51% of those borderline specimens whose ELISA activity could be blocked by 50% or more.

It appears to be both cost-effective and clinically valuable to repeat the Chlamydiazyme test (with and without blocking reagent) on the relatively small number of endocervical specimens with initial *A*₄₉₂>S between 0.030 and the cutoff. Results of those tests which show blocking activity of greater than or equal to 50% could then be confirmed with the DFA procedure. Most (67.3%) of the specimens with borderline Chlamydiazyme results had blocking activity of less than 50%. These specimens could be considered negative for *C. trachomatis* without further testing by DFA.

Statistical analysis of the results was performed by Sally Cavanaugh.

### REFERENCES


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**TABLE 1. DFA detection of chlamydial elementary bodies from endocervical specimens which had borderline Chlamydiazyme results and blocking reagent activity of ≥50%**

<table>
<thead>
<tr>
<th>No. of elementary bodies found</th>
<th>0.030 to 50% of cutoff</th>
<th>51 to 79% of cutoff</th>
<th>80 to 99% of cutoff</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>15</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>1–2</td>
<td>2</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>3–10</td>
<td>4</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>11–100</td>
<td>2</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>&gt;100</td>
<td>0</td>
<td>4</td>
<td>6</td>
</tr>
</tbody>
</table>

* A borderline Chlamydiazyme result was an *A*₄₉₂>S ranging from 0.030 up to the manufacturer’s recommended cutoff.

* A DFA result of ≥3 elementary bodies per smear was considered positive.