Detection of *Chlamydia trachomatis* Inclusions in McCoy Cell Cultures with Fluorescein-Conjugated Monoclonal Antibodies

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We compared two methods for identification of *Chlamydia trachomatis* inclusions in McCoy cell monolayers: conventional iodine staining and immunofluorescence staining with monoclonal antibodies against the species-specific major outer membrane protein antigen of *C. trachomatis*. Among 878 urethral and cervical specimens tested in parallel, the immunofluorescence method detected eightfold more inclusions per monolayer, identified a higher proportion of positive specimens on first passage (98 versus 62% by iodine staining; $P < 0.01$), and improved overall sensitivity (98% of total positive specimens detected versus 84% by iodine staining; $P < 0.01$). Improved sensitivity was most evident in specimens with low numbers of inclusions. Compared with conventional iodine staining, immunofluorescence staining with monoclonal antibodies improves sensitivity and offers more rapid detection of chlamydial inclusions in cell culture.

The lack of availability of chlamydial cultures to most clinicians has markedly impaired efforts to control these infections and has in part contributed to their increasing prevalence (2, 3, 6). Thus, laboratory techniques that simplify isolation methods for chlamydiae are urgently needed (6). Our laboratory previously reported that use of a microtiter system reduced the cost and technician time necessary to do chlamydial cultures with no apparent decrease in sensitivity or specificity (10), and others have reported success with a similar method (4). Using the microtiter system, we compared conventional iodine staining with immunofluorescence (IF) staining in which fluorescein-conjugated monoclonal antibodies that recognize a species-specific *Chlamydia trachomatis* outer membrane protein antigen are used (8). The IF staining method detected eightfold more inclusions per monolayer, exhibited greater sensitivity in tests on clinical specimens, and provided results more rapidly.

**MATERIALS AND METHODS**

**Source of specimens.** Patients attending the Harborview Medical Center Sexually Transmitted Disease Clinic (Seattle, Wash,) for evaluation of a new problem routinely have chlamydial cultures of the urethra (men) and cervix (women) obtained. Demographic characteristics of this population and prevalence of chlamydial infections in the clinic have been previously described (R. B. Brunham, B. Irwin, W. E. Stamm, and K. K. Holmes, Clin. Res. 29:47A; W. E. Stamm, L. Koutsy, J. Jourden, R. Brunham, and K. K. Holmes, Clin. Res. 29:51A). For men, specimens for culture were obtained by inserting a urethrogenital calcium-alginate swab (Inolex type 1) 2 to 3 cm into the urethra, rotating it, and withdrawing it. Specimens from women were obtained by inserting a plastic-shafted, calcium-alginate swab (Inolex type 3) into the cervical canal, rotating it for 5 s, and withdrawing it. Swabs from both men and women were immediately placed in 1.5 ml of a 0.2 M sucrose-phosphate transport medium containing gentamicin (10 μg/ml), vancomycin (25 μg/ml), and nystatin (25 U/ml) and were refrigerated until inoculation onto McCoy cell monolayers in the laboratory within 24 h.

**Microbiological techniques.** In the laboratory, specimens were inoculated onto McCoy cell monolayers in 96-well microtiter plates (Nunc, Inc.) as previously described (10).

In brief, each specimen was inoculated in parallel into four microtiter wells. After 48 h of initial incubation, one of the wells was stained with iodine and another with the conjugated fluorescent antibody (first passage). The remaining two monolayers were disrupted and passed onto fresh monolayers for another 48 h of incubation (second passage) and then were stained in the same manner as the first-passage specimens. Iodine-stained monolayers were examined on a specially prepared stage at ×250 magnification. Preliminary experiments with the fluorescein-conjugated monoclonal antibody staining procedure indicated that a dilution of 1:200 (6 μg of antibody per ml) provided excellent IF with minimal background fluorescence. Using the IF staining procedure, we found that inclusions could be identified as early as 18 h after inoculation but were easily recognized and exhibited the most characteristic morphology between 40 and 50 h.
Hence, cells inoculated with the clinical specimens reported here were fixed at 48 h with 50% acetone in methanol and were stained by flooding the microtiter well with 30 μl of a 1:200 dilution of the conjugate, followed by incubation at 37°C for 30 min. The conjugate was then removed by aspiration and Evans blue (0.02%) counter stain was applied and removed after 1 min. Specimens were then viewed by inverting the plate and reading it at ×160, using a Zeiss fluorescence microscope with vertical illumination. As with iodine, total inclusions per microtiter well were enumerated.

Statistics. We utilized Fisher’s exact test and McNemar's test of proportions to compare the results of iodine and IF staining.

RESULTS

Of 615 urethral specimens from males, 83 (13%) were positive by IF staining at the first passage compared with 47 (8%) by iodine staining (P < 0.01; Table 1). At the second passage, 26 new positives were identified in iodine-stained specimens, whereas only 1 new positive was observed by IF staining (P < 0.01). Thus, 83 of 84 positive specimens were recognized on first passage by IF staining, whereas only 47 of 73 were evident on first passage by iodine staining (P < 0.01). Overall, the isolation rate by IF staining (84 of 615, 14%) exceeded that by iodine staining (73 of 615, 12%), although this difference was not statistically significant (0.05 < P < 0.1). The mean inclusion count per well was 8.3-fold greater in the IF-stained specimens.

For 263 cervical specimens tested in duplicate, similar findings emerged (Table 1). IF staining detected significantly more positive specimens on first passage (46 versus 23; P < 0.01) and identified a higher proportion of the total positive specimens on first passage (46 of 47 by IF staining versus 23 of 39 by iodine staining; P < 0.01). The overall isolation rate by IF staining (47 of 263, 18%) exceeded the overall isolation rate by iodine staining (39 of 263, 15%), but this difference was not statistically significant (0.10 < P < 0.20). Mean inclusion counts per well were 7.2 times greater in IF-stained specimens.

When the results for male and female patients were combined, IF staining detected 131 positive specimens, whereas iodine staining detected 112 (Table 1; P < 0.05). Three specimens were positive by iodine staining only, 22 were positive by IF staining only, and 109 were positive by both staining procedures.

Sensitivity of the iodine staining method was lowest in specimens containing fewer than 20 inclusions per microtiter well by IF staining (Table 2). In these specimens, only 22 (or 61%) of the 35 specimens positive by IF were also positive by iodine. In contrast, 90 (or 94%) of the 96 high-titer IF-positive specimens were also positive by iodine staining (Table 2; P < 0.01).

DISCUSSION

We have successfully used iodine staining of cycloheximide-treated McCoy cells grown in microtiter wells as our primary method of chlamydial isolation for over 4 years (10). The sensitivity and specificity of this method compares favorably with the more traditional system of growing monolayers on cover slips in 1-dram vials, particularly for overtly symptomatic patients, among whom inclusion counts nearly always exceed 20 per well (4, 10). Among patients with inclusion counts under 20 per well, many of whom have asymptomatic infections, the sensitivity of the microtiter method declines as compared with that of cover slips in vials, probably owing to the smaller inoculum (0.1 versus 0.2 to 0.5 ml) and lesser centrifugal force (1,200 to 1,500 x g versus 1,800 to 2,000 x g) usually used (10; W. E. Stamm, unpublished data). Nevertheless, the ability to process large numbers of specimens more quickly and economically than in vials (4, 10) makes the microtiter method an excellent screening technique and hence a logical system in which to assess the value of IF staining with monoclonal antibodies.

In this study, IF staining with fluorescein-conjugated monoclonal antibodies to a species-specific C. trachomatis surface antigen improved recognition of infected McCoy cell monolayers as compared with iodine staining. In addition, the IF method identified nearly all positive specimens at the first passage, whereas

### Table 1. Number of Chlamydia-positive specimens detected by iodine staining and by IF staining with monoclonal antibodies

<table>
<thead>
<tr>
<th>Stage</th>
<th>No. of positive specimens</th>
<th>Men (n = 615)</th>
<th>Women (n = 263)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IF</td>
<td>Iodine</td>
<td>IF</td>
</tr>
<tr>
<td>First passage</td>
<td>83</td>
<td>47</td>
<td>46</td>
</tr>
<tr>
<td>Second passagea</td>
<td>1</td>
<td>26</td>
<td>1</td>
</tr>
</tbody>
</table>

*a Numbers indicate newly positive specimens only.

### Table 2. Comparison of iodine and IF staining in specimens with high (≥20) and low (<20) numbers of inclusion-forming units per monolayer

<table>
<thead>
<tr>
<th>Passage</th>
<th>High-inoculum specimens</th>
<th>Low-inoculum specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IF</td>
<td>Iodine</td>
</tr>
<tr>
<td>First</td>
<td>96</td>
<td>58</td>
</tr>
<tr>
<td>Second</td>
<td>0</td>
<td>32</td>
</tr>
</tbody>
</table>

*a Specimens with ≥20 inclusion-forming units per well on IF staining.
with iodine staining, 38% of infections were not recognized until the second passage. Since in excess of 95% of the positive results were detected upon the initial inoculation, the IF staining method could probably be used as a single-passage technique in laboratories handling routine clinical specimens for chlamydial isolation. Elimination of the second passage would halve the laboratory workload and reduce reporting time from 7 to 8 days to 3 to 4 days. As the IF plates can also be scanned more quickly than an iodine-stained plate (≈30% faster, on the average, in our laboratory), the actual time in technician time with this method probably exceeds that attributable solely to elimination of the second passage.

The improved sensitivity of the IF method as compared with iodine staining probably results in part from the fact that iodine stains only those inclusions containing glycogen, whereas the IF technique presumably recognizes all antigenically intact inclusions. However, Stephens et al. (7) have compared a monoclonal IF technique with Giemsa staining and detected both a fourfold increase in inclusion counts and improved sensitivity, particularly in low-inclusion-count specimens, with the IF technique. Thus, nonstaining of inclusions that lack glycogen probably does not entirely explain the superiority of the IF method as compared with iodine staining. In a previously reported study by Thomas and colleagues (9), IF staining with antibodies obtained from a patient with lymphogranuloma venereum infection also demonstrated more inclusions per specimen as compared with Giemsa staining. Darougar and co-workers (1) found little difference between these two methods of staining but did not study many patients whose specimens gave low inclusion counts, the most stringent assessment of a chlamydial culture test. Overall, these studies suggest that IF staining is a more sensitive method of detecting inclusions than either iodine or Giemsa staining.

A more readily available routine diagnostic test for *C. trachomatis* genital infections is urgently needed. Although lower-genital-tract infection with chlamydiae may eventually progress to endometritis, salpingitis, or infertility in women and epididymitis or Reiter’s syndrome in men (2, 3), early uncomplicated genital infection due to chlamydiae often escapes detection (3, 6; Stamm et al., Clin. Res. 29:51A). Nonspecific or mild symptoms and the absence of specific signs upon examination characterize most cases and make diagnosis on clinical grounds difficult (3; Stamm et al., Clin. Res. 29:51A). Serology has not proven useful as a diagnostic test in uncomplicated lower-genital-tract infections (5). Thus, an improved culture method would have considerable value in the diagnosis, management, and prevention of chlamydial infections. The method reported here deserves further evaluation as a potential means of making chlamydial cultures more widely available.

LITERATURE CITED


