Sensitivity of Immunofluorescence with Monoclonal Antibodies for Detection of *Chlamydia trachomatis* Inclusions in Cell Culture

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Monoclonal antibodies which recognize the species-specific major outer membrane protein antigen of *Chlamydia trachomatis* were used for immunofluorescence staining of chlamydial inclusions in cell culture. A total of 115 clinical specimens were inoculated onto replicate HeLa 229 cell monolayers and assayed for chlamydial inclusions by immunofluorescence staining and Giemsa staining. Of the isolates, 38 were detected by immunofluorescence staining on passage 1 and 1 was detected on passage 2; 23 isolates on passage 1 and 13 isolates on passage 2 were detected by Giemsa staining. Immunofluorescence staining was significantly more sensitive than Giemsa staining for detecting chlamydial inclusions, particularly from specimens containing low titers of *Chlamydia*.

Chlamydial inclusions in smears or tissue from clinical specimens or in cell culture have conventionally been identified by Giemsa staining, immunofluorescence (IF) staining, or iodine staining methods. Iodine staining has been employed only for *Chlamydia trachomatis* biotypes because *C. psittaci* biotypes do not produce sufficient quantities of glycogen. Unfortunately, iodine-detectable glycogen may only be demonstrated during part of the chlamydial growth cycle (2). Earlier reports have shown that IF staining is more sensitive and specific than Giemsa staining for the direct detection of *C. psittaci* inclusions in clinical specimens and cell culture (12). For the detection of *C. trachomatis* inclusions in conjunctival scrapings, IF staining has also been shown to be superior to Giemsa staining (7). However, comparisons of the sensitivity of these two methods for the detection of *C. trachomatis* inclusions in cell culture have been inconclusive. Thomas et al. demonstrated enhanced sensitivity of IF staining in terms of inclusion counts, but this did not result in a greater rate of chlamydial isolation from clinical specimens (11). In a similar comparison, Darougar et al. observed no marked differences in the number of *C. trachomatis* inclusions identified in cell cultures or in the rates of chlamydial isolation (3).

In this study, we utilized monoclonal antibodies which recognize a species-specific surface antigen of *C. trachomatis* (10) for IF staining of chlamydial inclusions in cell culture. IF staining was shown to be significantly more sensitive and rapid than Giemsa staining for the detection of *C. trachomatis* inclusions which were produced from clinical specimens inoculated into cell culture.

MATERIALS AND METHODS

Specimens. A laboratory strain of *C. trachomatis* F/UW-6/Cx (1) or clinical specimens were used in this study. Specimens for isolation of *C. trachomatis* were collected from the urethra and cervix of women attending the Sexually Transmitted Disease Clinic at Harborview Medical Center, Seattle, Wash., or from pregnant women enrolled in a prospective study of chlamydial neonatal infection at the University Hospital, University of Washington, Seattle. Specimens were collected with calcium alginate urethrogenital swabs, placed in 1 ml of sucrose-phosphate-glutamate medium (8), and frozen at −70°C until tested.

Inoculation of cell monolayers. Clinical specimens were inoculated onto DEAE-dextran-pretreated HeLa 229 cell monolayers as previously described (8), except that 0.5 μg of cycloheximide (Sigma Chemical Co., St. Louis, Mo.) per ml was added to the culture medium (9). Each specimen was inoculated onto four separate HeLa cell monolayers and incubated for 48 or 72 h (passage 1). After incubation, two inoculated monolayers on 12-mm cover slips were fixed with methanol and stained with either Giemsa stain or fluorescent antibody. At 72 h the two remaining inoculated monolayers were passaged onto fresh monolayers and reincubated (passage 2). Specimens which demonstrated inclusions on passage 1 by both staining methods were not passaged a second time.

Monoclonal antibodies. Hybrid cell lines (*C. trachomatis* 2C1 and 1H8) which secrete monoclonal antibodies that recognize the species-specific major outer membrane protein of *C. trachomatis* were used to produce ascitic fluids in mice as previously described.
(10). For the direct IF staining technique, immuno-
globulins from ascitic fluid 2C1 were affinity purified
on a protein A- sepharose column (Pharmacia Fine
Chemicals, Uppsala, Sweden) (4). Fluorescein isothio-
cyanate-conjugated monoclonal antibodies (2C1) were
prepared by the method described by Goding (6). A
1:50 dilution of ascitic fluid (1H8) or fluorescein iso-
thiocyanate-conjugated monoclonal antibody (20 µg/ml)
was used for the indirect and direct IF staining tech-
niques, respectively.

Giemsa staining and IF staining. May-Grunwald-
Giemsa staining (5) was performed as follows. A
saturated solution of May-Grunwald stain (Hopking &
Williams, Chadwell Heath, Essex, England) in metha-
ol was applied to cover slips for 5 min, washed with
water, and stained with 0.08% Giemsa stain (Fisher
Scientific Co., Pittsburgh, Pa.) for 10 min. Cover slips
were then washed with water, dehydrated sequentially
with acetone, acetone-xylene (1:1), and xylene, and
mounted with Permount (Fisher Scientific Co.).

Both indirect and direct IF staining techniques were
assessed. Indirect IF staining was performed with an
anti-mouse immunoglobulin G fluorescein conjugate
(Hyland Diagnostics, Deerfield, Ill.). The direct stain-
ing procedure was performed with fluorescein isothio-
cyanate-conjugated monoclonal antibodies. Evans
blue (0.2%) counterstain was applied in the final step.

Examination of cell cultures. Stained cell monolayers
were examined for chlamydial inclusions by bright-
field or fluorescent microscopy (fluorescent micro-
scope from Wild, Heerbrugg, Switzerland) at a 100×
magnification. Inclusions observed at a 100× magnifi-
cation were verified for typical morphology at a 400×
magnification. The total number of inclusions per
cover slip was recorded. Inclusion counts were per-
formed independently by two experienced observers;
the results obtained by one observer were not revealed
until those of the other were recorded.

Statistics. The paired-sample t test and McNemar’s
test of proportions were used to determine statistical
significance.

RESULTS

Standard control cultures. Replicate monolay-
ers infected with serial dilutions of a laboratory
strain of C. trachomatis (UW-6) were examined for
inclusions at 18, 24, 48, and 72 h. Although
Giemsa-stained inclusions were recognizable at
48 h, the limited development of these inclusions
made examination difficult and imprecise com-
pared with the inclusions observed at 72 h.

IF staining revealed small inclusions in infect-
ed cultures after 18 and 24 h. Within 48 h,
fluorescent chlamydial inclusions displayed a
sharply defined mass within the cytoplasm of
HeLa 229 cells. Although IF was evident as
early as 18 h, typical inclusion morphology was
not definitively displayed until 48 h. The number
and morphology of inclusions observed at 48 h
by IF staining were not significantly different
from those observed at 72 h. Nonspecific IF
staining was not observed with uninoculated
HeLa cell monolayers. Thus, for the subsequent
comparison of IF staining and Giemsa staining
for the identification of chlamydial inclusions
from clinical specimens in cell culture, we evalu-
ated cultures at 72 h with Giemsa staining. IF-
stained cultures were evaluated at 48 or 72 h,
depending on a previously defined work sched-
ule for these clinical specimens.

Evaluation of clinical specimens. A total of 53
specimens were studied by indirect IF staining,
and 62 specimens were studied by direct IF
staining. No significant differences were ob-
erved between the sensitivity of the two IF
staining techniques; thus, these data were
poored for analysis.

Inclusions were detected by either IF staining
or Giemsa staining in 39 of the 115 specimens
after passage 2. Inclusions from every specimen
in which Giemsa staining revealed inclusions
were detected by IF staining. Of the 39 isolates,
38 (97%) on passage 1 and 1 on passage 2 were
detected by IF staining. However, only 23 (59%)
isolates on passage 1 and 13 (33%) on passage 2
were detected by Giemsa staining. (The three
remaining samples in which inclusions had been
detected by IF staining but not by Giemsa
staining on passage 2 were reexamined. One and
two inclusions per cover slip were detected in
two of these specimens, but none was detected
in the third specimen. After passage 3, inclu-
sions were detected in the third sample.) The
difference in sensitivity between IF staining and
Giemsa staining for the detection of inclusions
on passage 1 was significant (P < 0.0005).

The 15 specimens which revealed inclusions
on passage 1 by IF staining but not by Giemsa
staining all contained fewer than 20 inclusions
per cover slip. A comparison of the isolation
rates on passage 1 between the IF and Giemsa
methods of those specimens which contained
fewer than 20 inclusions per cover slip showed
22 positive specimens by IF staining and 7
positive specimens by Giemsa staining (Table 1).

Thus, in 68% of these low-titer specimens, inclusions
were not detected by Giemsa staining on
passage 1.

The number of inclusions per specimen de-
tected by IF staining was significantly greater
than that detected by Giemsa staining (IF/
Giemsa = 2.3; P < 0.05). The enhanced sensitiv-
ity of IF staining was most evident in those
passage 1 samples which contained fewer than
200 inclusions per cover slip (IF/Giemsa = 3.0;
P < 0.001). In contrast, samples containing
more than 200 inclusions per cover slip showed
no significant differences in counts between the
two staining methods (IF/Giemsa = 1.1).

DISCUSSION

For the detection of C. trachomatis inclusions
from clinical specimens we have routinely used
May-Grunwald–Giemsa staining of HeLa 229
cell monolayers at 72 h after inoculation, followed by a second passage of negative specimens onto fresh monolayers. For the past 10 years this procedure has been effectively utilized for the isolation of *C. trachomatis*. We tried fluorescent-antibody techniques previously (8), but the poor growth of *C. trachomatis* biotypes in the available culture systems seriously limited practical attempts to grow enough antigen for the adequate immunization of large laboratory animals to obtain antiserum for use in IF staining. The limitations inherent in obtaining conventional antiserum are avoided by utilizing monoclonal antibodies which are available in potentially unlimited quantities and which recognize a *C. trachomatis* species-specific major outer membrane surface antigen.

The threefold increase in sensitivity of IF staining over Giemsa staining for the number of inclusions counted in cell culture agrees with the results obtained by Thomas et al. (11). The fact that another study (3) found no marked differences in sensitivity between IF staining and Giemsa staining can probably be understood in terms of a high multiplicity of inclusions per sample. In our study and in that of Thomas et al., as the number of inclusions per sample increased, the disparity between the IF and Giemsa methods rapidly diminished. The most stringent comparison of these methods should thus be made with samples containing only a few organisms per sample, which tests the ability of these methods to demonstrate a greater rate of chlamydial isolation. Such a comparison has not been reported. This information can be derived from our study by comparing passage 1 samples producing fewer than 10 inclusions per cover slip by IF staining. In only three of these samples were inclusions detected by Giemsa staining (Table 1; *P* < 0.005). Furthermore, after passage 2, inclusions in three of the IF-positive specimens were not detected by the initial Giemsa reading.

The diagnosis of *C. trachomatis* infection is currently based on cell culture isolation. The isolation results are usually assessed by two cell culture passages (6 days) with either Giemsa or iodine staining for the detection of chlamydial inclusions. IF staining with the reagent used in this study is now being compared with iodine staining for the isolation of *C. trachomatis* in cycloheximide-treated McCoy cells at another laboratory. The preliminary results are similar; they show a higher sensitivity for IF staining than for iodine staining, both in the rate of isolation and in the number of inclusions counted (Walter Stamm, personal communication). The IF staining method did not significantly enhance the sensitivity of the isolation procedure if negative cultures were passaged a second time. However, IF staining with monoclonal antibodies that recognize species-specific antigens provides a reproducibly sensitive and specific assay for detecting *C. trachomatis* inclusions in cell culture after only one passage. Furthermore, the time required to scan cover slips stained with fluorescent antibody (average time, 5 min) was less than half the time required to scan Giemsa-stained cover slips (average time, 12 min). These savings in time and material can substantially lower the cost of isolation and provide for earlier diagnoses.

### Table 1. Number of specimens from which *C. trachomatis* inclusions were detected by IF staining or Giemsa staining

<table>
<thead>
<tr>
<th>Cell culture passage</th>
<th>All positive specimens</th>
<th>Specimens with &lt;20 inclusions per cover slip</th>
<th>Specimens with &lt;10 inclusions per cover slip</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>IF</td>
<td>Giemsa</td>
<td>IF</td>
</tr>
<tr>
<td>1</td>
<td>38</td>
<td>23</td>
<td>22</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>13</td>
<td>1</td>
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</table>

* The difference in total positive specimens between the IF and Giemsa methods reflects three specimens for which either reexamination or a 3rd passage was required to detect inclusions by Giemsa staining.

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### LITERATURE CITED


