Failure To Detect *Chlamydia trachomatis* in Cell Culture by Using a Monoclonal Antibody Directed against the Major Outer Membrane Protein

JENS BOMAN,1* CHARLOTTE GAYDOS,2 PER JUTO,1 GÖRAN WADELL,1 AND THOMAS C. QUINN2

Department of Clinical Virology, The University Hospital of Umeå, Umeå University, Umeå, Sweden,1 and Department of Medicine, The Johns Hopkins University, Baltimore, Maryland2

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Two commercially available monoclonal antibodies for cell culture confirmation of *Chlamydia trachomatis* were compared in two prospective studies and one large retrospective study. In total, more than 33,000 genital specimens were cultured in parallel and stained with both antibodies, one of which was directed against the major outer membrane protein (MOMP) and one of which was directed against the lipopolysaccharide (LPS). We found the anti-LPS-based assay to be more sensitive and as specific as the anti-MOMP-based assay for *C. trachomatis* cell culture confirmation of genital specimens.

*Chlamydia trachomatis* is one of the most common sexually transmitted bacterial infections in the world with an estimated number of 50 million new cases each year (11). Since genital chlamydial infections can cause severe clinical diseases, especially in women (2), correct diagnosis and treatment are imperative. Several diagnostic methods are used including cell culture, enzyme immunoassay, and direct fluorescent assay, and recently, different nucleic acid amplification methods have been introduced. Agreement between different methods is not always very good (7, 9).

In order to investigate whether the Swedish results could be confirmed by another laboratory, and to establish whether LPS-positive–MOMP-negative specimens were true *C. trachomatis* culture positives, all genital samples cultured at the Johns Hopkins University Chlamydia Research Laboratory from January 1991 through December 1996 were reviewed. A total of 31,266 genital specimens were cultured for *C. trachomatis* in cycloheximide-treated McCoy cells in duplicate wells in 96-well microtiter plates. After 72 h of incubation, each of the two wells was fixed and stained with the above-described commercially available monoclonal antibodies; for all specimens the first well was stained with the anti-LPS monoclonal antibody (Kallestad) and the second well was stained with the anti-MOMP monoclonal antibody (Syva). There were 277 (0.89%) specimens toxic to cell culture, and these were not included as part of the study. Of the remaining 30,989 cultures, 2,519 were culture positive (prevalence, 8.13%). A total of 2,484 cultures were positive by both monoclonal antibodies, whereas 35 were positive only by the anti-LPS monoclonal antibody. None of these isolates were positive for *Chlamydia pneumoniae* by using a species-specific monoclonal antibody. Thus, 1.4% of the *C. trachomatis*-positive samples were negative by the anti-MOMP antibody (Table 2). In addition, 301 (12.1%) of the culture-positive samples stained lightly (+, approximately 60%; 1, 40%) with the anti-MOMP monoclonal antibody and had normal staining (2 and 3) with the anti-LPS monoclonal antibody.

Due to the discrepancy between the results of the two tests we decided to repeat the study with a protocol that was accepted by both companies. Therefore, all genital samples sent to the Department of Clinical Virology, University Hospital of Northern Sweden, Umeå, Sweden, from 28 August to 29 September 1995 for *C. trachomatis* analysis were included. For positive samples the fluorescence was graded from 0 to 3 (+, organisms just visible; 1, light-green staining; 2, moderate fluorescent-green staining; and 3, intense fluorescent-green staining) according to Montalban et al. (6). A total of 969 samples were analyzed, 58 of which were toxic to the cell culture and, therefore, were not included in this study. Of the remaining 911 samples, 32 (3.5%) were culture positive by the anti-LPS antibody, whereas 28 (3.1%) were positive by the anti-MOMP antibody. All anti-MOMP-positive samples were positive by the anti-LPS antibody. The fluorescence intensity was equal for 22 samples; however, in 6 samples (21%) the fluorescence was stronger with more numerous and larger inclusion bodies by the anti-LPS assay (Table 1).
due to its broader range and ability to detect chlamydial spe-
MOMP antibody. It may be argued that the anti-LPS antibody,
reacting anti-LPS antibody is more sensitive than the anti-
another diagnosis, the results thus indicate that the broadly
were 6.5, 22.7, 23.7, and 18.7%, respectively, and in Europe
C. trachomatis
isolates (2,795 from the United States and 2,394 from Europe)
prevalent in both the United States and Europe. In total, 5,189
from 14 published studies (6 from the United States and 8 from
question, van de Laar et al. (10) compiled data on the distri-
and F) is dependent on their frequency. To answer such a
included serovars D (17%), E (15%), and F (5%). The clinical
positive cultures were predominantly serovar J (63%) but also
infect the genital tract should be treated. Furthermore,
there is very little evidence of genital infections in humans with
other species of Chlamydia. For direct fluorescent assay stain-
ing of elementary bodies, Cles et al. (3) have shown in in vitro
experiments that monoclonal antibodies against the MOMP of
C. trachomatis may produce a brighter and more consistent
fluorescence than monoclonal antibodies against the LPS.
However, in the study by Cles et al. (3), the anti-LPS antibody
from Kallestad was not used.
The explanation as to why the anti-LPS assay had a higher
sensitivity in culture confirmation in our studies might be due to
a more pronounced capability of detecting both forms of the
organism (elementary bodies and reticulate bodies) (8) or to
the presence of new MOMP variants of C. trachomatis, which
have been shown to be able to escape neutralization by both
monoclonal antibodies and human immune sera (5). In con-
clusion, we found the anti-LPS antibody to be more sensitive
than the anti-MOMP antibody and as specific as the anti-
MOMP-based assay for C. trachomatis cell culture confirma-
tion of genital specimens.

TABLE 1. Grading of fluorescence intensity in 6 of 28 positive
samples in which the intensities were different between the
anti-LPS and anti-MOMP antibodies

<table>
<thead>
<tr>
<th>Sample</th>
<th>Anti-LPS antibody</th>
<th>Anti-MOMP antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>±</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>±</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>±</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>±</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>±</td>
</tr>
</tbody>
</table>

* Shown as ±, 1, and 2 as defined in the text.

typed as C. trachomatis serovar J. In addition, the light-staining
positive cultures were predominantly serovar J (63%) but also
included serovars D (17%), E (15%), and F (5%). The clinical
impact of not diagnosing the J serovar and the others (D, E,
and F) is dependent on their frequency. To answer such a
question, van de Laar et al. (10) compiled data on the distribu-
tion of serovars of genital infections due to C. trachomatis
from 14 published studies (6 from the United States and 8 from
Europe) and found that the J, D, E, and F serovars are rather
prevalent in both the United States and Europe. In total, 5,189
isolates (2,795 from the United States and 2,394 from Europe)
were included in the compilation. The average percentages of
C. trachomatis serotypes J, D, E, and F in the United States
were 6.5, 22.7, 23.7, and 18.7%, respectively, and in Europe
they were 6.2, 13.2, 38.8, and 19.3%, respectively.

Since all patients in our studies of whom the results of the
two tests differed had signs and symptoms compatible with a
genital chlamydial infection, and there was nothing to suggest
another diagnosis, the results thus indicate that the broadly
reacting anti-LPS antibody is more sensitive than the anti-
MOMP antibody. It may be argued that the anti-LPS antibody,
due to its broader range and ability to detect chlamydial spe-
cies other than C. trachomatis (6), yields a lower specificity in
the detection of C. trachomatis. Nonetheless, from a clinical
point of view this is not a problem since all chlamydial species
infecting the genital tract should be treated. Furthermore,
there is very little evidence of genital infections in humans with
other species of Chlamydia. For direct fluorescent assay stain-
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than the anti-MOMP antibody and as specific as the anti-
MOMP-based assay for C. trachomatis cell culture confirma-
tion of genital specimens.

TABLE 2. Summary of two Swedish prospective and one U.S.
retrospective study comparing the sensitivities of anti-LPS and
anti-MOMP antibodies for culture confirmation of C. trachomatis in genital specimens

<table>
<thead>
<tr>
<th>Type of study (location)</th>
<th>No. of samples</th>
<th>No. of positive samples (%)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Both antibodies</td>
<td>Anti-LPS antibody only</td>
<td></td>
<td></td>
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<tr>
<td>Prospective (Sweden)</td>
<td>1,456</td>
<td>43 (81)</td>
<td>10 (19)</td>
<td></td>
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<tr>
<td>Prospective (Sweden)</td>
<td>911</td>
<td>28 (88)</td>
<td>4 (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retrospective (United States)</td>
<td>30,989</td>
<td>2,484 (98.6)</td>
<td>35 (1.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>33,356</td>
<td>2,555 (98.1)</td>
<td>49 (1.9)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Pathfinder Chlamydia Culture Confirmation Assay, Sanofi Diagnostics Pasteur/Kallestad.

* MicroTrak Chlamydia trachomatis Culture Confirmation test, Behring Diagnostics/Syva Company.

REFERENCES