Comparison of Two Commercially Available Isolation Systems for Chlamydia trachomatis

VICKIE S. BASELSKI,1,2* MARY K. ROBISON,2 and B. R. JENNINGS1,2

Department of Pathology, University of Tennessee Center for the Health Sciences,1* and Clinical Microbiology Laboratory, The Regional Medical Center,2 Memphis, Tennessee 38163

Received 7 March 1983/Accepted 7 June 1983

In a comparison of two commercially available chlamydial isolation systems in which cycloheximide-treated McCoy cell monolayers are used, the system from Bartels Immunodiagnostic Supplies, Inc., Bellevue, Wash., was found to be superior to that from M. A. Bioproducts, Walkersville, Md. for the detection of Chlamydia trachomatis by iodine staining. Of 288 clinical specimens run in parallel, 47 (16.3%) were positive, with 16 of 47 positive results detected in the Bartels system only and 1 of 47 positive results detected in the M. A. Bioproducts system only (P < 0.001). A comparison of the number of inclusion-forming units per cover slip from clinical specimens and passaged isolates also showed that the Bartels cell system demonstrated higher inclusion counts than the M. A. Bioproducts system. In routine clinical use, overall isolation rates were higher (P < 0.001) and contamination rates were lower (P < 0.001) with the Bartels system as compared with results obtained in a previous time period in which the M. A. Bioproducts system was used.

Recent advances in chlamydial isolation procedures have made it technically feasible for clinical microbiology laboratories to offer isolation of Chlamydia trachomatis as a routine diagnostic procedure (15, 19). In addition, several commercial companies currently market materials for the isolation of C. trachomatis as complete systems. These systems use cycloheximide-treated McCoy cell monolayers, a method which has been shown in a number of studies to be optimal for C. trachomatis isolation (3, 6, 10, 12, 16). However, because it has been previously shown that McCoy cell lines from different sources may differ in their abilities to support chlamydial growth (19), this study was initiated to evaluate the performance characteristics of two of the commercially available cell culture systems for C. trachomatis detection in clinical specimens.


MATERIALS AND METHODS

Specimens. Specimens included in this study were submitted to the Clinical Microbiology Laboratory at the Memphis Regional Medical Center for routine culture of Chlamydia spp. Specimens from epithelial surfaces were collected on sterile cotton swabs and then suspended in Chlamydia Transport Medium (CTM) purchased from M. A. Bioproducts (MAB), Walkersville, Md. (0.2 M sucrose–0.02 M phosphate buffer with gentamicin and amphotericin B). Fluids and aspirates were mixed with CTM in a 1:1 ratio. Specimens in CTM were held at 4°C for up to 48 h after collection or at −70°C if more than 48 h were to elapse before inoculation onto monolayers.

Cell cultures. McCoy cell monolayers on cover slips in 1-dram shell vials overlaid with maintenance medium were obtained on a weekly basis from Bartels Immunodiagnostic Supplies, Inc., Bellevue, Wash., and MAB. The monolayers were held at room temperature for up to 1 week before use. Immediately before inoculation, the maintenance medium was removed aseptically.

Isolation procedures. The protocol for processing clinical specimens followed recommended procedures (4). After thorough shaking of the specimen in CTM containing sterile glass beads, 0.5 ml was added to each of two vials of the Bartels and MAB monolayers. A positive control culture was also inoculated to each cell type with each group of clinical specimens run. The vials were then spun for 1 h in a Sorvall GLC4 centrifuge with a free swinging head at 3,000 rpm. After centrifugation, the specimen was removed, and 1 ml of prewarmed overlay medium containing cycloheximide was added to each vial. The overlay media were purchased from the two commercial companies and used in the corresponding cell culture type. The Bartels medium was purchased ready for use and contained streptomycin (100 μg/ml), gentamicin (10 μg/ml), amphotericin B (4 μg/ml), and cycloheximide (1 μg/ml). The MAB medium contained gentamicin (10 μg/ml) and cycloheximide (0.5 μg/ml) and required the addition of 10% fetal calf serum (lacking antichlamydial antibodies) with amphotericin or nystatin (final concentration, 2 μg/ml).

Identification procedures. After 48 to 72 h of incuba-
tion at 35°C, the overlay medium was removed and the monolayers were fixed with absolute methanol for 15 min and then stained with Jones iodine (prepared by Edge Diagnostics, Memphis, Tenn.) for 15 min. The cover slips with the monolayers were then removed, mounted in a solution of 50% glycerol and 50% Jones iodine, and examined microscopically for typical, intracytoplasmic, glycogen-containing inclusion bodies.

Passage of isolates. Clinical isolates were passaged by suspending unfixed, infected monolayers in CTM and using the suspension to inoculate additional monolayers which were processed as described for clinical specimens. These monolayers were then used to prepare pooled suspensions in CTM. The pools were stored at −70°C and quick-thawed before use.

Statistical analysis. Statistical analyses of the data were performed according to standard methods for paired or unpaired data (2) as described in individual sections of this article.

RESULTS

Appearance of monolayers and inclusion bodies. The microscopic appearances of the two monolayer types were distinctly different, with the Bartels cells tending to be more elongated and fibroblast-like than epithelioid. However, the iodine-stained, chlamydial inclusion bodies were clearly distinct and easily discernible at a low-power magnification with both cell types, although the inclusion bodies in the MAB cells were generally larger, with a more distinct halo surrounding the glycogen mass.

Recovery from clinical specimens. The results from inoculation of clinical specimens to the two commercially available monolayers in parallel studies are shown in Table 1. The specimens included 270 endocervical, 4 male urethral, 5 conjunctival, 5 nasopharyngeal, 2 amniotic fluid, and 2 pelvic pus specimens. *C. trachomatis* was detected in 47 of 288 (16.3%) clinical specimens, including 2 nasopharyngeal, 1 eye, 1 urethral, and 43 endocervical specimens. The isolation rate in the Bartels system was 16.0% (46 of 288), as compared with 10.8% (31 of 288) in the MAB system. Sixteen positive specimens were detected in the Bartels system only, as compared with one detected in the MAB system only (P < 0.001 by the McNemar test).

Most of the specimens positive in both systems yielded high inclusion counts (22 of 30 or 73%). (On the basis of previous publications [3, 10, 16], high counts were defined in this study as ≥10 inclusions per cover slip and low counts as <10 inclusions per cover slip.) Conversely, most specimens positive in only one system yielded low inclusion counts (13 of 17 or 76%, 12 of which were positive in the Bartels system). However, >500 inclusions per cover slip were counted in one Bartels-positive, MAB-negative specimen. With both systems, the number of specimens uninterpretable owing to overgrowth of contaminants (primarily yeasts) was low, although greater with the MAB system (2.1%) than with the Bartels system (0.4%). In addition, some breakthrough growth of contaminants occasionally partially obscured observation of the monolayer in the MAB system. In three Bartels-positive, MAB-negative specimens, this contaminant growth may have contributed to the failure to detect inclusions.

A comparison of the inclusion counts for the 47 positive specimens also demonstrated that the Bartels system produced higher counts more frequently, with 34 (72%) counts greater in the Bartels system, 6 (13%) counts greater in the MAB system, and 7 (15%) counts being equivalent (P < 0.001 by the matched-pair sign test) in both systems. The Bartels monolayers generally yielded 2- to 10-fold-greater counts. Included in the group with greater counts in the MAB system were four low-titer specimens and both positive nasopharyngeal specimens. In the group showing equivalent counts, five were high-titer specimens with inclusions too numerous to count, and two had a single inclusion detected in each system.

Recovery from passaged clinical isolates. A comparison of the inclusion counts produced in the two systems, using inocula prepared from passaged clinical isolates, supported previous findings with clinical specimens that the Bartels system yielded higher counts (Table 2). In experiments in which three different pools of passaged endocervical isolates and different weekly cell shipments were used, significantly greater counts were noted in the Bartels system, although with pool 3 the difference was not evident initially but noted when the pool was diluted 1:100.

Routine screening of obstetric patients. A comparison of isolation rates for *C. trachomatis* from endocervical specimens submitted for routine screening of obstetric patients during differ-
TABLE 2. Inclusion counts in two commercially available cell lines inoculated with three different clinical isolates

<table>
<thead>
<tr>
<th>Expt no.</th>
<th>Isolate no.</th>
<th>Mean ± SE IFU of following cell line per cover slip$^a$</th>
<th>$p^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bartels</td>
<td>MAB</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1.341 ± 86</td>
<td>208 ± 21</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>1.090 ± 125</td>
<td>212 ± 18</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>3.814 ± 356</td>
<td>3.193 ± 89</td>
</tr>
<tr>
<td>4</td>
<td>3$^c$</td>
<td>3.136 ± 320</td>
<td>422 ± 67</td>
</tr>
</tbody>
</table>

$^a$ Mean ± standard error from 10 determinations. IFU: Inclusion-forming units.
$^b$ Determined by the two-group, two-tailed $t$ test.
NS: Not significant.
$^c$ Pool from isolate 3 diluted 1:100.

ent time periods in which one system was used exclusively is shown in Table 3. A significantly greater isolation rate was seen when the Bartels system was used ($P < 0.001$ by standardized normal deviate analysis for unpaired data). In addition, the number of specimens which were uninterpretable owing to overgrowth of genital microflora was significantly lower in the Bartels system ($P < 0.001$). Although one cannot exclude the possibility that factors other than isolation system used contributed to these differences, the results nevertheless parallel the findings in the matched-pair studies.

DISCUSSION

Previous studies have demonstrated that differences in detection of *C. trachomatis* in cycloheximide-treated McCoy cell monolayers related to differences in isolation systems. Smith et al. (19) noted that McCoy cell lines from different sources differed in their abilities to support chlamydial growth from clinical specimens and stock cultures, with a University of Washington line superior to the MAB or Flow lines. They suggested that these differences might be related to specific differences in cell culture handling procedures which could affect chlamydial susceptibility. It is of note that the Bartels system uses a University of Washington-derived line which has been selected on the basis of optimal recovery of chlamydia (P. Bartels, personal communication). The results of this study similarly indicate that the cell cultures in the two commercial systems evaluated may not be equivalent for the recovery of *C. trachomatis*.

Differences in the composition of the overlay media in the two systems may also have contributed to the differences observed in chlamydial recovery. Serum source is known to influence chlamydial development (1, 11), and some lots of fetal calf serum have been found to have a profoundly suppressive effect on isolation in cycloheximide-treated McCoy cell monolayers (5). The difference in cycloheximide concentration (1.0 μg/ml in Bartels; 0.5 μg/ml in MAB) may have also been a contributing factor (16).

Although the contamination rates were low with both systems compared with previously reported values, particularly for cervical specimens (15), the data here indicate that the Bartels system better suppressed overgrowth by other microorganisms. This difference is probably related to differences in the antimicrobial composition of the overlay media in the two systems.

Of the specimens detected in only one system, 76% were low-yield specimens (<10 average inclusions per cover slip), and a similar percentage of specimens detected in both systems were high-yield specimens (>10 inclusions per cover slip). This finding is consistent with previous observations in comparisons of isolation methods that high-yield specimens will generally be detected by any method used and that critical differences in recovery should be particularly noted in low-yield specimens (3, 10, 16). We have found that 40 to 50% of positive clinical specimens which originate from routine endocervical screening of obstetric patients are low-yield specimens. In view of the reported maternal and neonatal morbidity associated with *C. trachomatis* infection (7–9, 13, 14, 17, 18, 20, 21), it is particularly important in this population to use procedures for optimal detection of all positive specimens. This study did, in fact, demonstrate a significant increase in isolation rate from an obstetric population upon changing from the MAB to the Bartels system.

Although both systems were technically similar, the Bartels system was found in a comparative evaluation of two commercially available *C. trachomatis* isolation systems to be superior to the MAB system in terms of increased number of positive specimens detected, increased number of inclusions detected per sample, and decreased number of specimens noninterpretable owing to overgrowth with other microorganisms. These findings are probably related to specific differences in the system components

TABLE 3. Recovery of *C. trachomatis* from routine screening of obstetric patients with two commercially available cell lines in different time periods

<table>
<thead>
<tr>
<th>Time period (mo/day/yr)</th>
<th>Cell line</th>
<th>Total no. of specimens inoculated</th>
<th>No. (%) positive</th>
<th>No. (%) contaminated</th>
</tr>
</thead>
<tbody>
<tr>
<td>5/1/81 to 5/1/82</td>
<td>MAB</td>
<td>741</td>
<td>45 (6.3)</td>
<td>60 (8.1)</td>
</tr>
<tr>
<td>9/6/82 to 11/25/82</td>
<td>Bartels</td>
<td>885</td>
<td>153 (17.3)</td>
<td>22 (2.5)</td>
</tr>
</tbody>
</table>
LITERATURE CITED