Improved Isolation of \textit{Chlamydia trachomatis} from a Low-Prevalence Population by Using Polyethylene Glycerol

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Received 11 August 1992/Accepted 11 November 1992

The effect of polyethylene glycerol (PEG) on the isolation of \textit{Chlamydia trachomatis} was evaluated in our laboratory. Initial range-finding experiments demonstrated that the number of chlamydial inclusion bodies increased with increasing PEG concentrations. However, PEG concentrations above 10.5% became progressively more toxic to the McCoy cell monolayers. When 50 frozen clinical \textit{Chlamydia} isolates were inoculated onto McCoy cell cultures with and without 7% PEG, the PEG-treated cultures produced three- to fivefold more chlamydial inclusions than cultures without PEG. This enhancement was also observed when 1,144 fresh clinical specimens from a low-prevalence population were tested. With fresh clinical specimens, PEG-treated cultures produced two- to sixfold more inclusions than standard cultures. The addition of 7% PEG to the chlamydial overlay medium significantly increased the number of inclusions in each culture, improved the sensitivity of the culture, and decreased the probability of missing a weakly positive specimen.

\textit{Chlamydia trachomatis} is the leading cause of sexually transmitted disease in the United States, with an estimated 3 to 5 million new infections occurring each year (3, 6). Detection and treatment of chlamydial infections present a formidable public health challenge because nearly 75% of all \textit{Chlamydia}-infected women are asymptomatic and do not seek medical treatment (2, 8, 12). Laboratory identification of \textit{C. trachomatis} infection is usually based upon identification of chlamydial elementary bodies by direct fluorescent-antibody (DFA) methods, detection of solubilized chlamydial antigens by enzyme immunoassays (EIA), or isolation and identification of \textit{C. trachomatis} in cell culture. However, DFA methods and EIA are generally not suitable for screening asymptomatic or low-prevalence populations because, for these patients, the positive predicative value of DFA and EIA tests can be 50% or less (4, 13, 14, 16). Cell culture amplification is usually necessary because these patients often have very low antigen loads (4). However, cell culture isolations are far from perfect, and cell culture isolations from a single endocervical specimen may be only 70 to 80% sensitive (1, 9, 15).

Since its introduction in 1965, the \textit{C. trachomatis} isolation procedure of Gordon and Quan (5) has been extensively modified to improve its sensitivity. In 1985, Mohammed and Hillary (11) described an improved overlay medium containing 35% polyethylene glycerol (PEG) in barbitone buffer which significantly improved \textit{Chlamydia} isolation rates in their moderate- to high-prevalence population. In this report, we describe a modification of the Mohammed and Hillary procedure that is suitable for use in low-prevalence patient populations. This procedure has been routinely used in our laboratory since 1986.

**MATERIALS AND METHODS**

**Study population.** William Beaumont Hospital is a 960-bed community hospital that serves a predominantly middle- and upper-middle-class patient population in the northern suburbs of Detroit. The vast majority of the chlamydial specimens submitted to our laboratory were collected from asymptomatic female patients during routine obstetric and gynecologic examinations. The overall prevalence of \textit{C. trachomatis} in our patient population was 4.7%, with 3.1% prevalence in males and 4.9% prevalence in females.

From October 1991 through March 1992, all specimens submitted to the virology laboratory for \textit{C. trachomatis} isolation were divided and cultured in the presence and absence of 7% (wt/vol) PEG. A total of 1,144 specimens were received during the study. Of these specimens, 1,016 (88.8%) were from females and 1,027 (89.8%) were collected from urogenital sites. Eighty-two percent (96 of 117) of the nonurogenital specimens consisted of ocular and nasopharyngeal swabs from neonates.

**Specimen collection.** Single swabs for \textit{C. trachomatis} isolation were collected by the medical or nursing staff and placed into transport vials containing 3 ml of 0.2 M sucrose phosphate transport medium containing 10% fetal bovine serum (HyClone Laboratories, Logan, Utah) (17). Gentamicin and amphotericin B were added in the laboratory to produce final concentrations of 66.7 and 2.5 \(\mu\)g/ml, respectively. Specimens were stored at 4°C until they were inoculated onto cell cultures.

**Cell culture.** McCoy cell monolayers were established on 12-mm coverslips in flat-bottom glass shell vials (Baxter, McGaw Park, Ill.) by seeding the vials with \(2 \times 10^6\) to \(4 \times 10^6\) cells in 1 ml of Leibovitz's L-15 medium (GIBCO Laboratories, Grand Island, N.Y.), pH 7.3, containing 10% fetal bovine serum, 0.44% glucose, 50 \(\mu\)g of gentamicin (Eklins-Sinn, Inc., Cherry Hill, N.J.) per ml, and 1.0 \(\mu\)g of amphotericin B (E.R. Squibb & Sons, Inc., Princeton, N.J.) per ml. All McCoy cell monolayers were used for chlamydial isolations within 72 h of seeding. Chlamydial overlay medium consisted of culture medium (described above) containing 2% fetal bovine serum and 0.75 \(\mu\)g of cycloheximide (Sigma Chemical Co., St. Louis, Mo.) per ml. The PEG overlay medium was identical to the chlamydial overlay medium except that autoclaved, 35% polyethylene glycerol (hybridoma grade; average molecular weight, 3,350; product P3640; Sigma Chemical Co.) in Hanks balanced salt solution,
RESULTS

Initial range-finding experiments were done to determine the effect of increasing PEG concentrations on the number of chlamydial inclusions. For these experiments, a *C. trachomatis* isolate was diluted in phosphate-buffered saline to produce 10 (experiment 1) or 5 (experiment 2) inclusions per coverslip culture. Duplicate cultures were inoculated for each experiment. In both experiments, the number of chlamydial inclusions increased as the PEG concentration increased (Fig. 1). The number of inclusions increased 3-fold with 3.5% PEG, 4-fold with 7% PEG, 8-fold with 10.5% PEG, 15-fold with 14% PEG, and 16-fold with 17.5% PEG. However, at PEG concentrations at or above 10.5%, the PEG was progressively more toxic and increasing numbers of syncytia were observed in infected and uninfected McCoy cell monolayers. All subsequent evaluations were done with 7% PEG concentrations.

To determine the effect of PEG on *C. trachomatis* isolations, 50 frozen isolates were thawed and 0.2-ml aliquots were inoculated onto duplicate McCoy shell vial cultures containing chlamydial overlay medium or PEG overlay medium. Eleven isolates (22%) did not produce inclusions in either culture system, and two isolates (4%) produced inclusions only in the PEG-treated cultures. The remaining data were divided into groups on the basis of the number of inclusions per coverslip in the untreated vials (Table 1). In these experiments, the PEG overlay medium produced 3- to 5-fold increases in the number of inclusions, with a 3.6-fold mean increase (*P* = 0.01).

The effect of PEG on the isolation of *C. trachomatis* from a low-prevalence population was determined by culturing all specimens received from October 1991 through March 1992 in the presence and absence of 7% PEG. A total of 1,144 specimens were received during this period, and *C. trachomatis* was isolated from 54 (4.7%) specimens. Inclusions were counted for all positive cultures except one. One coverslip culture produced 18 inclusions in the PEG-treated culture and none in the standard culture system. The remaining data were divided into groups on the basis of the number of inclusions per coverslip in the untreated vials (Table 2). In these cultures, the PEG treatment produced a 2- to 6-fold increase (mean = 3.7-fold) in the number of inclusions on the coverslip (*P* = 0.001).

DISCUSSION

Detection of *C. trachomatis* in asymptomatic and low-prevalence patient populations is a special challenge for the clinical laboratory. In these populations, cell culture isola-

### TABLE 1. Effect of 7% PEG on the recovery of frozen *C. trachomatis* isolates

<table>
<thead>
<tr>
<th>No. of inclusions/ coverslip</th>
<th>No. of specimens</th>
<th>Mean no. of inclusions ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Without PEG</td>
</tr>
<tr>
<td>------------------------------</td>
<td>------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>1-10</td>
<td>4</td>
<td>2.8 ± 1.1</td>
</tr>
<tr>
<td>11-100</td>
<td>14</td>
<td>35 ± 5</td>
</tr>
<tr>
<td>101-1,000</td>
<td>11</td>
<td>267 ± 39</td>
</tr>
<tr>
<td>&gt;1,000</td>
<td>8</td>
<td>4,637 ± 1,135</td>
</tr>
</tbody>
</table>

* Fifty *C. trachomatis* isolates were thawed, and 0.2-ml volumes were inoculated onto standard and PEG-treated shell vial cultures. Eleven isolates (22%) did not produce inclusions in either culture system, and two isolates (4%) produced inclusions only in the PEG-treated cultures. The data for the remaining 37 isolates are summarized here.

* Fold increases were calculated by dividing the mean number of inclusions obtained with PEG by the mean number of inclusions obtained with overlay medium without PEG.

* *P* values were calculated by Student's *t* test.
tion is the method of choice because EIA and DFA methods are relatively insensitive and produce more false-positive reactions than true-positive reactions (4, 9, 10, 13). However, cell culture is far from perfect (9, 13, 15) and a number of procedural modifications have been made to improve the sensitivity of cell culture isolations. In 1985, Mohammed and Hillary (11) described an improved overlay medium (1.5% PEG in barbitone buffer) that produced a fivefold increase in the number of chlamydial inclusions. Our modified procedure yielded similar results (three- to fivefold increase) with significantly lower PEG concentrations. In our hands, PEG concentrations of 10.5% or higher were toxic, and treated cells exhibited increased levels of cellular granularity and syncytium formation as the PEG concentration increased. This result was not unexpected, because 35 to 50% PEG concentrations are routinely used for fusing lymphocytes and plasmacytomas to produce hybridomas (7). Laboratories that are not as concerned about changes in cell morphology could achieve an 8- to 10-fold increase in the number of inclusions by using 10% PEG in the overlay medium. It must be noted, however, that the PEG quality and molecular weight should be closely monitored. Only hybridoma-grade PEG should be used in this procedure, and PEG lots must be screened for toxicity before routine use.

The 7% PEG overlay medium produced similar results with frozen clinical isolates (2.9- to 5.0-fold increase) and with fresh clinical specimens (2.4- to 5.6-fold increase). Using 350 frozen specimens, Mohammed and Hillary observed a significant increase in the number of positive specimens (11 versus 16%) with a 35% PEG overlay medium. Our limited testing of 50 frozen isolates using 7% PEG produced a similar (4%) increase in chlamydial recovery. These data also indicate that PEG overlay medium is likely to improve the sensitivity of blind passages as well as primary isolations.

Our testing of 1,144 fresh specimens revealed one specimen that was positive (18 inclusions) with the PEG overlay medium and negative with the standard overlay medium. While this increase is not statistically significant, any improvement in assay methods for asymptomatic and low-prevalence patient populations can have a significant impact on patient care and decrease the transmission of Chlamydia trachomatis in the population. In addition, the more serious complications of untreated Chlamydia trachomatis infections, such as salpingitis, pelvic inflammatory disease, and infertility, could be avoided if highly sensitive detection systems were available. We anticipate that, like the Mohammed and Hillary procedure, the use of 7% PEG in the overlay medium will also improve the isolation rates in higher-prevalence patient populations.

In addition to improving the sensitivity of the procedure, increased numbers of inclusions can also make it easier to find inclusions on the coverslip. Our data indicate that the most dramatic increases in the number of inclusions occurred in specimens producing fewer than 1,000 inclusions per coverslip under standard conditions. This is an important consideration because, without PEG, 83% of our positive specimens produce fewer than 1,000 inclusions per coverslip. Fifty percent of our positive specimens produce 100 or fewer inclusions and 25% of our positive specimens produce 10 or fewer inclusions per coverslip. Thus, a practical benefit of increasing the number of inclusions is the improved ability to detect weakly positive specimens that would normally produce very few inclusions.

Cell culture isolation is the preferred method for detecting Chlamydia trachomatis in asymptomatic and low-prevalence populations, and any convenient method that improves the sensitivity of cell culture procedures can have a significant impact on patient care. In our study, the addition of 7% PEG to the chlamydial overlay medium significantly increased the number of inclusions in each positive shell vial culture. The increased number of inclusions can improve the sensitivity of cell culture isolations, make culture results easier to interpret, and decrease the possibility of reporting false-negative results. The PEG overlay medium may also increase the number of inclusions seen after blind passages. Although this study was done in a low-prevalence population, we anticipate that the PEG overlay medium will also produce significantly more chlamydial inclusions in cultures from higher-prevalence populations.

REFERENCES


