Infectivity of *Chlamydia trachomatis* in Tissue Culture with Newborn Calf Serum

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By using representative strains of *Chlamydia trachomatis* serotypes, the efficacy of substituting fetal bovine serum with newborn calf serum for the isolation of this organism in tissue culture was examined. The criteria used were the quality and quantity of the iodine-stained cytoplasmic inclusions and the characteristics of the McCoy cells. Complete substitution of fetal bovine serum with newborn calf serum produced a detrimental change in the quality of the cytoplasmic inclusions and a dramatic decline in the inclusion count (*P < 0.001*) with all of the chlamydial strains tested. There appeared to be no significant alteration in the characteristics of the McCoy cells. It is recommended from this preliminary investigation that newborn calf serum should not be used for the isolation of *C. trachomatis* in tissue culture.

*Chlamydia trachomatis* has been recognized as the etiological agent of a wide spectrum of adult and pediatric infections (3, 5, 7, 8, 13–15, 20). The diagnosis to ensure adequate treatment of these infections is primarily dependent upon the isolation of this organism by tissue culture procedures. McCoy cells, which are a mouse fibroblast cell line, are commonly used with media containing fetal bovine serum (FBS) as a critical component for the adequate replication of the host cells and the chlamydia. The use of FBS has become somewhat difficult because of the increasing expense, and at times the unavailability, of this product. Newborn calf serum (NCS) is inexpensive, readily available, and has been used previously to support the replication of various cell lines (10, 11). Information is unavailable as to the effect on the quality and quantity of the *C. trachomatis* cytoplasmic inclusions produced when FBS is substituted with NCS. The aim of the present investigation, using representative strains of *C. trachomatis* serotypes, was to obtain preliminary data on the efficacy of substituting FBS with NCS for the isolation of this organism in tissue culture. Three criteria were used: (i) the characteristics of the McCoy cells, (ii) the characteristics of the iodine-stained cytoplasmic inclusions, and (iii) the number of inclusions per milliliter of inoculum.

McCoy cells were maintained routinely in our laboratory for no more than 35 passes after their removal from liquid nitrogen storage.

Growth medium used for the replication of the McCoy cells was previously described (9). This medium routinely contained 10% heat-inactivated FBS. The medium used for the growth of the chlamydia was termed maintenance medium and contained components identical to those in the growth medium, but with 5% instead of 10% heat-inactivated FBS and 0.5% glucose (9). For this study, three types of growth and maintenance media were used, each with different proportions of FBS to NCS. The three ratios were: 100% FBS:0% NCS, 50% FBS:50% NCS, and 0% FBS:100% NCS. The FBS and NCS were obtained from GIBCO Laboratories, Grand Island, New York. The batch numbers for the FBS and NCS used in this study were 27-D0202 and 21-P4100, respectively. The NCS was obtained from a pool of several calves whose ages were 10 days old or less.

The laboratory strains of *C. trachomatis* serotypes used in this study were provided as previously described (9). The organisms were passed in McCoy cells treated with 1 μg of cycloheximide per ml and adjusted to a final concentration of 104 to 108 inclusion-forming units per ml.

Standard pools were made of each strain and stored at −70°C. Before inoculation of the McCoy cells, the pool was diluted with maintenance medium containing the appropriate FBS/NCS ratio. Each pool was set up in duplicate and tested concurrently in the different cell cultures on 2 separate days. Preparation, maintenance, and treatment of the McCoy cells with cycloheximide have been previously described (9).

Monolayers of McCoy cells with growth and maintenance media containing FBS/NCS in each of the three ratios were inoculated with 0.1 ml of
each stock chlamydial strain. The tubes were centrifuged at 2,000 \times g at 30°C for 1 h and then incubated for 48 h at 37°C in an atmosphere of 5 to 10% CO₂. Positive and negative controls, consisting of a pool of a stock chlamydial strain and transport medium, respectively, were included in all experiments. At 48 h post-inoculation, the monolayers were fixed in methanol and stained with iodine for the detection of the carbohydrate matrix of the chlamydial inclusions. The number of inclusions per milliliter was calculated by scanning the entire monolayer at a \times 100 magnification with a light microscope. Inclusions were examined at a \times 430 magnification for characteristic morphology. All data obtained were statistically examined by analysis of variance (16).

Representative strains of C. trachomatis serotypes D through K were used to determine infectivity in McCoy cells in which FBS was substituted with NCS. Three criteria were used: (i) the characteristics of the McCoy cells, (ii) the characteristics of the iodine-stained cyttoplasmic inclusions, and (iii) the number of inclusions detected per milliliter of inoculum.

A comparison of the morphology of the McCoy cells grown under the three culture conditions indicated that the cell morphology remained essentially the same. However, the characteristics of the chlamydial inclusions varied appreciably when grown in McCoy cells with media containing the different FBS/NCS concentrations. The inclusions produced in medium containing solely FBS were large, brightly stained, and easily detectable. When half of the FBS was replaced with NCS, the inclusions were appreciably smaller, darker, and occasionally difficult to detect. In medium containing NCS only, there was a dramatic change in the characteristics of the chlamydial inclusions for all of the strains tested. The inclusions were pinpoint in size, almost black, and very difficult to detect.

Data on the effects of the three culture conditions on the number of cytoplasmic inclusions observed with the stock strains are shown in Table 1. Medium containing solely FBS consistently yielded the highest inclusion counts. Comparative analysis with medium containing a 1:1 ratio of the two sera indicated that there was a substantial decline in the inclusion-forming units per milliliter with one exception. However, this decline was not statistically significant. The most dramatic decrease in the inclusion count was when FBS was completely substituted with NCS (Table 1). A statistical analysis of the data determined that this reduction in inclusion-forming units per milliliter was significant (P < 0.001) with all of the chlamydial strains tested. The mean determinations indicated that the use of NCS in complete substitution of FBS resulted in a sevenfold reduction in the inclusion-forming units per milliliter for all strains (Table 1).

Most cultured cells, especially fibroblasts, require the presence of animal serum to proliferate (2). The mouse fibroblast cell line, termed McCoy cells, is commonly used with media containing FBS as a critical component for the growth of the host cells and the chlamydia. It has been previously reported that host cell susceptibility to Chlamydia psittaci infection is altered by sera from different animal species (1). Since NCS is inexpensive and readily available in contrast to FBS and has been used previously to support the replication of various cell lines (10, 11), the aim of this investigation, using representative strains of C. trachomatis serotypes, was to obtain preliminary data on the efficacy of substituting FBS with NCS for the isolation of this organism in tissue culture. The criteria used in this study were the characteristics of the McCoy cells and the quality and quantity of the cytoplasmic inclusions.

Under the conditions of this study, the results indicated that the complete substitution of FBS with NCS produced a detrimental change in the quality of the cytoplasmic inclusions and a dramatic decline in the inclusion count. There appeared to be no significant alteration in the characteristics of the McCoy cells. The adverse effect of NCS on the quality and quantity of the chlamydial cytoplasmic inclusions may be the result of various factors, in particular, the presence of specific chlamydial antibodies, the lack of significant growth-promoting substances, or both (4, 12, 17–19). In relation to the former possibility, C. psittaci is a recognized etiological

<table>
<thead>
<tr>
<th>Serotype/strain</th>
<th>IFU/ml in the following culture condition:</th>
<th>100% FBS:</th>
<th>50% FBS:</th>
<th>0% FBS:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0% NCS</td>
<td>50% NCS</td>
<td>100% NCS</td>
<td></td>
</tr>
<tr>
<td>D/IC-CAL-3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E/UW-5</td>
<td>3,980</td>
<td>2,910</td>
<td>350</td>
<td></td>
</tr>
<tr>
<td>F/IC-CAL-3</td>
<td>5,810</td>
<td>4,050</td>
<td>1,030</td>
<td></td>
</tr>
<tr>
<td>G/392-F</td>
<td>2,720</td>
<td>1,650</td>
<td>460</td>
<td></td>
</tr>
<tr>
<td>H/580</td>
<td>8,660</td>
<td>6,590</td>
<td>270</td>
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</tr>
<tr>
<td>I/870</td>
<td>2,070</td>
<td>1,330</td>
<td>470</td>
<td></td>
</tr>
<tr>
<td>J/UW-36</td>
<td>1,730</td>
<td>1,700</td>
<td>860</td>
<td></td>
</tr>
<tr>
<td>K/UW-31</td>
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<td>10,950</td>
<td>1,560</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>4,604</td>
<td>3,828</td>
<td>659</td>
<td></td>
</tr>
</tbody>
</table>

a Values represent mean of four determinations performed on 2 separate days.
b IFU, Inclusion-forming units.
agent of cattle involved in a wide spectrum of infections such as encephalitis, pneumonia, polyarthritis, gastroenteritis, conjunctivitis, abortion, and orchitis (18). Since commercial NCS is harvested from the calf within the first 10 days after birth, maternal chlamydial antibodies may be transferred to the calf in the colostrum and milk. Support for this thesis comes from a report by Storz (17) in which bovine abortion by chlamydia was studied with the use of experimental in utero infection of bovine fetuses. Specific chlamydial antibodies could not be detected in the infected fetuses, but antibody levels were demonstrated in a surviving calf after the first nursing.

It is important to note that these investigations were performed with one batch each of FBS and NCS. Evans (6) previously reported that different batches of FBS significantly affected the number of cytoplasmic inclusions detected with C. trachomatis in cycloheximide-treated McCoy cells. Further studies remain to be done with more C. trachomatis stock strains and unselected clinical specimens with several batches of FBS and NCS. In addition, studies could be performed with McCoy cells from various sources to determine whether there would be any difference in the results presented in this report.

It is recommended from this preliminary investigation that NCS should not be used for the isolation of C. trachomatis in tissue culture because of the detrimental effect on the quality and quantity of the cytoplasmic inclusions.

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LITERATURE CITED