

## NOTES

# Comparison of Buffalo Green Monkey Cells and McCoy Cells for Isolation of *Chlamydia trachomatis* in a Microtiter System

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**A total of 1,229 urogenital samples of patients with sexually transmitted diseases and prostitutes were inoculated simultaneously in McCoy cells and Buffalo green monkey (BGM) cells by using a microtitration technique. BGM cells proved to be slightly more sensitive than McCoy cells, and they usually showed more and larger inclusions and were more resistant to cytotoxic substances in samples. The decrease in sensitivity as a result of mycoplasmal contamination was much more prominent in McCoy cells than in BGM cells.**

*Chlamydia trachomatis* is a significant cause of blindness and sexually transmitted urogenital infections, as well as conjunctivitis in neonates and adults. Pneumonias in newborns are also often caused by this pathogen (7, 9). Traditional methods of chlamydial isolation involve the use of cell monolayers in vials containing glass cover slips. McCoy cells are widely used for this purpose. Recently, the isolation of *Chlamydia psittaci* in Buffalo green monkey (BGM) cells was described (1). Subsequently, it was shown that these cells are also well suited for cultivation of *C. trachomatis* (2, 10). The most striking features of BGM cells infected by *C. trachomatis* were the more numerous and larger inclusions compared with those of McCoy cells. These observations were made on monolayers grown on conventional vial-cover slips. However, the increasing demand for chlamydial diagnosis favors the isolation procedure that uses a microtitration system, a method which is less time-consuming and less labor-intensive (3, 5, 8, 11, 12). It was the aim of this study to answer the question of whether the use of BGM cells reveals more positive samples in patients with sexually transmitted diseases than does the use of McCoy cells when a microtiter system is used. Furthermore, we also wanted to see how often larger and more numerous inclusions can be observed in BGM cells grown in microtitration plates compared with McCoy cells, because this facilitates the screening of samples.

A total of 1,229 urogenital samples from males with urethritis and females with cervicitis or urethritis and from prostitutes were collected in 2SP transport medium. Samples were inoculated usually on the day of collection simultaneously on both cell lines by following a modification of the procedure of Ripa and Mårdh (6). In brief, mycoplasma-free BGM and McCoy cells were grown on the same microtitration plate (Nunc, Wiesbaden, Federal Republic of Germany) in parallel rows. The tube containing the swab in transport medium was vortexed after glass beads were added. Transport medium (100  $\mu$ l) was inoculated after the cell culture medium had been exchanged for Eagle minimal essential medium with 2% fetal bovine serum containing 2  $\mu$ g of

cycloheximide per ml. The plates were then centrifuged at  $1,500 \times g$  at 37°C for 1 h. After incubation at 37°C in a 5% CO<sub>2</sub> atmosphere for 2 to 3 days, the cells were fixed and stained with fluorescein isothiocyanate-labeled monoclonal antibody (MicroTrak; Syva Merck, Darmstadt, Federal Republic of Germany).

The stained monolayers were read under an inverse microscope (Zeiss IM) at a magnification of  $\times 400$ . The number of inclusions per monolayer was determined. Furthermore, the cell line in which the inclusions appeared larger was recorded. A cytotoxic effect of the sample was registered if destruction of the cell layer was more than 50%. A sample was evaluated for comparison only if the two cell lines were in an optimal condition, e.g., if an almost confluent monolayer was retained in the uninoculated control wells. The cells were checked at intervals for mycoplasmal contamination under a fluorescence microscope after DNA was stained with Hoechst 33258 reagent. The cells remained free of mycoplasmas over the study period.

In addition, 1,203 samples were inoculated in artificially cross-contaminated McCoy and BGM cells to see whether the presence of mycoplasmas influences the sensitivity of tissue culture isolation.

The source for the mycoplasmas was a contaminated McCoy cell line. Mycoplasmas grew to similar extents on both artificially contaminated cell lines as judged from microscopic examination of DNA-stained monolayers.

In BGM cells, 90 of the 1,229 samples were positive for *C. trachomatis*, whereas only 87 samples were positive in McCoy cells (Table 1). Thus, McCoy cells had a sensitivity of 96.6% in relation to BGM cells. Table 2 shows that in McCoy cells samples with low infectivity usually produced lower numbers of inclusions per monolayer than did BGM cells. Of the 87 samples positive in both cell lines, 50 produced more than 50 inclusions per monolayer in either cell line. Of the remaining 37 positive samples, 31 showed more inclusions in BGM cells, 2 displayed equal numbers of inclusions, and 4 had fewer inclusions in BGM cells than in McCoy cells. In only two of the paired cultures were the inclusions clearly smaller in BGM cells. Cytotoxic effects were seen in McCoy cells in 4.9% of all samples tested compared with only 0.5% in BGM cells. Furthermore, even in impaired BGM monolayers, there were in any case enough intact cells left to recognize a

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TABLE 1. Effect of cell line and artificial cross-contamination with mycoplasmas on sensitivity

Cell line comparison	No. of samples tested <sup>a</sup>	No of positive cultures	Decrease in sensitivity (%)
BGM vs McCoy, both not cont <sup>b</sup>	1,229	90 vs 87	3.3
BGM not cont vs BGM cont	1,203 <sup>c</sup>	90 vs 87	3.3
McCoy not cont vs McCoy cont	949 <sup>c</sup>	59 vs 42	28.8

<sup>a</sup> The same sample obtained from one swab was inoculated on both cell lines.

<sup>b</sup> cont, Contaminated with mycoplasmas.

<sup>c</sup> Lower numbers were tested because mycoplasma contamination sometimes resulted in monolayers of poor quality which were excluded from comparison.

positive result. However, samples with only very few infectious organisms might have been missed.

Mycoplasma contamination substantially reduced the sensitivity of intact McCoy cell monolayers (28.8%), whereas the influence on BGM cells was minimal (Table 1). Compared with optimal, mycoplasma-free BGM cultures, the contaminated McCoy cells gave a sensitivity of only 67%.

Our results show a slightly higher sensitivity of mycoplasma-free BGM cells compared with McCoy cells for the isolation of *C. trachomatis* from urogenital specimens by using microtitration plates. The rather low detection rates (7.3 and 7.1%, respectively) were the result of a sharp decrease in the number of positive samples during an anti-acquired immunodeficiency syndrome campaign. Thus, the number of positive patients was too low to calculate exact figures for sensitivity and the difference is not statistically significant ( $\chi^2 > 0.05$  in the sign test). However, since the inclusions also were usually more numerous and larger in BGM cells, it is very likely that these cells more readily detect samples with low numbers of infectious particles.

The difference in sensitivity was much more pronounced if the two cell lines were artificially cross-contaminated with

TABLE 2. Numbers of inclusions in BGM and McCoy cells resulting from samples with low infectivity (fewer than 11 inclusions in either cell line)

Sample no.	No. of inclusions <sup>a</sup>	
	BGM	McCoy
1	3	9
2	3	0
3	12	3
4	4	2
5	60	8
6	16	7
7	10	7
8	1	1
9	3	0
10	16	7
11	35	8
12	10	5
13	6	2
14	8	4
15	1	0
16	3	29
17	7	13
18	4	5
19	17	6
20	14	2
Mean <sup>b</sup>	11.65	5.90

<sup>a</sup> The same sample obtained from one swab was inoculated on both cell lines.

<sup>b</sup> Difference is significant with the Wilcoxon test for paired differences ( $P < 0.005$ ).

mycoplasmas. We could not identify the species of contaminating mycoplasmas or other mollicutes, since cultivation of mycoplasmas from cell cultures is sometimes difficult (4). Further experiments are required to establish whether analogous results are obtained with contamination of cells by known mycoplasma species.

We are well aware of the fact that microscopic detection of mycoplasmas in cell culture is not very sensitive (4). However, we were able to subculture our cell lines over the study period of more than 1 year without noting the appearance of mycoplasmas. Thus, we assume that the cultures remained free of mycoplasmas throughout the study period.

We are also aware that different cell clones might differ in their susceptibilities to *C. trachomatis* and that our McCoy cells might be less sensitive for isolation than McCoy cells used in other laboratories. However, the advantages of BGM cells in our microtiter system were in agreement with previous findings for vial cultures (2, 10). The inclusions were usually larger and more numerous than in McCoy cells, which allows a more efficient reading. Furthermore, BGM cells were easier to maintain and optimal monolayers could be prepared at any time. In addition, we observed about 10 times fewer cytotoxic samples in BGM cells. In conclusion, BGM cells were easier to read and slightly (statistically not significant) more sensitive than McCoy cells. They were also less affected by cytotoxic substances in the sample and by mycoplasma contamination.

We are grateful to M. F. Paccaud for providing the mycoplasma-free cell lines.

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