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Letter to the Editor

TREATMENT OF MYCOPLASMA CONTAMINATION

Dear Editor:

Two papers (2,3) concerning treatment of mycoplasma-positive cell cultures were published in your journal during this year. Drexler et al. (2) recommend BM-Cyclin (BMC) but Hlubinova et al. (3) propagate a more convenient method. This letter describes our experience with BMC (eight cell lines) and presents two representative examples.

Cell cultures: MDCK/H (Mardine-Darby canine kidney) and NB-E (human newborn kidney) cells were cultivated in Eagle s minimal essential medium (MEM) or Dulbecco s MEM containing 10% fetal bovine serum and standard antibiotics (penicillin, streptomycin). During the BMC treatment, medium without antibiotics was used. All cells were dispersed by 0.125% trypsin and 0.05% EDTA.

Mycoplasma elimination: BMC treatment was carried out with 2.5-fold increase over the manufacturer s instructions (Boehringer Mannheim, Germany).

Detection of mycoplasma: Detection was performed by DNA fluorochrome Bisbenzimide (1) or by enzyme-linked immunoassay (ELISA, Boehringer Mannheim).

In addition to the discussion of the above-mentioned papers, the following points should be taken into consideration:

1. The success of mycoplasma elimination depends on the degree of contamination and concentration of antibiotics. Low doses of BMC as recommended by the manufacturer did not produce a stable mycoplasma inhibition. The 2.5-fold increase of BMC concentration prolonged the effect of this mixture of antibiotics. After three treatment cycles the growth rate increased (Table 1, split ratio).

2. The contaminating mycoplasma species *M. hyorhinis* and *A. laidlawii* were more difficult to eliminate than the others. This does not agree with already described data (2,5).

3. The suggested antibiotic combination enables only a decrease but not a complete elimination of mycoplasma. Each concentration for an individual cell line should be tested. Concentrations that produce more than 60% cell growth inhibition are successful.

4. Mycoplasma detection kits are useful to monitor and differentiate the mycoplasma infection or elimination.

5. BMC-treated cells have a higher quality with regard to cell proliferation. This is an advantage for a virus assay with a culture period longer than 1 wk. With BMC-treated NB-E cells virus titration was possible and an increase of virus propagation was achieved (4).

6. Periodic monitoring of the cured cells should be continued.

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TABLE 1

EXAMPLE OF SUCCESSFUL AND LESS SUCCESSFUL BM-CYCLIN TREATMENT

Experimental Group	Optical Density 405 nm				Percent DNA Staining	Split Ratio
	A	H	L	O		
Positive control	2.330	2.036	2.150	2.202	—	—
Negative control	0.309	0.282	0.277	0.249	—	—
MDCK/H						
Before	2.825	2.277	0.363	0.673	27.7	1:8
After	0.319	0.306	0.368	0.313	2.8	1:15
NB-E						
Before	0.321	2.348	0.596	0.369	69.3	1:4
After	0.297	2.330	0.638	0.246	30.4	1:12

Key: Solution 1: 50 μg/ml; solution 2: 25 μg/ml; absorbance: 405 nm; A: *M. arginini*; H: *M. hyorhinis*; L: *A. laidlawii*; O: *M. orale*; DNA staining: % of cells with fluorochromestained plasma membrane; mean cell growth of treated cells: 24.3% ± 1.4