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ELIMINATION OF MYCOPLASMAS FROM CELL CULTURES BY A NOVEL SOFT AGAR TECHNIQUE

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SUMMARY

Mycoplasmal infection of cell cultures remains a significant threat to diagnostic and research procedures. In certain defined situations, curing of mycoplasmal infected cultures is a reasonable exercise. Four methods of curing were compared: treatment with BM-cycline, 5 bromouracil, use of specific antisera and treatment of infected cells suspended in soft agar with antibiotics. Antisera treatments were of low efficiency of curing: 50%. None of nine infected cell lines treated with 5-bromouracil were consistently cured of mycoplasmas. The use of BM-cycline was effective for some, but not all lines and required long periods of treatment, 12–21 days. 35 naturally or deliberately infected cultures were treated in soft agar a total of 119 times. This procedure which consisted of suspending infected cultures in soft agar containing appropriate antibiotics resulted in successful mycoplasmal elimination 118/119 times. This soft agar technique took 1–3 days. In separate studies, it was shown that certain *Mycoplasma fermentans* strains were resisted to this and other curing methods. This may be due to their intracellular location. Such strains may be more amenable to antibiotics that penetrate mammalian cells. It is concluded that the soft agar technique is a rapid, efficient and reliable method to eliminate cell culture mycoplasmas.

Key words: mycoplasma infection; mycoplasma elimination.

INTRODUCTION

The effects of mycoplasmal infection of cell cultures continues to invalidate results of diagnostic and research studies (13,16,21), and reviews have been published (11,12,13). Methods to prevent and detect mycoplasmal infection of cell cultures have also been published (12,14). Mycoplasmal infection of cell cultures often persists without apparent cell damage. We have generally recommended that mycoplasmal infected cell cultures be destroyed. Mycoplasmal infected cell cultures are themselves the major source of infection (9). In special cases, the infected cultures may not be replaceable, as with hybrids, hybridomas, cells transfected with foreign genes or cell cultures derived from rare diseases. Elimination of mycoplasmas from such cell cultures becomes a practicality.

Elimination of mycoplasmas from infected cell cultures is typically time consuming, often unsuccessful and poses risks of secondary infection to other cell cultures. Many reports have been published on various methods to cure mycoplasmas (15,17,20,22). Methods of elimination should be simple and easy, have minimal effect on the culture and result in no loss of specialized characteristics. Few comparative data are available. Unfortunately, most published reports have been based on small number of cell types and few mycoplasma species. More systematic studies have been reported by Jeansson and Bronson (2) and Gardella and Del Giudice (1). Other reports have described use of antibiotics, specific antiserum, macrophages, passage in athymic mice, combination of these techniques, 5-bromouracil (5BU), among others (8,18,19).

We have systematically examined various treatments to eliminate mycoplasmas from cell cultures using a variety of naturally and deliberately infected cell cultures with the mycoplasma species most commonly encountered in cell cultures. We report here the results of these studies as well as the development and evaluation of a relatively simple and reliable soft agar technique for curing mycoplasma infected cell cultures. This method is based on the survival of cell cultures, even non-tumor cell lines, in soft agar (7). Based on these results, it appears that the combination of appropriate antibiotics in soft agar constitutes a simple, rapid and effective treatment to eliminate different mycoplasma species from a variety of different cell cultures.

MATERIALS AND METHODS

Mycoplasmas. Mycoplasmas used for experimental infection of cell cultures in this study were from departmental stocks. Mycoplasma species and strains were: *Mycoplasma hyorhinis* strains GDL and CEM; *M. orale* strains A23 and JS; *M. arginini* strain VV; *M. fermentans* strains HK, 1095, 1092, 1062, 1582 and 572; *M. pneumoniae* strains FH and M-129-B16 and *Acholeplasma laidlawii* strain MG. *M. fermentans* strain sb_{51} was obtained from S. C. Lo of Armed Forces Institute of Pathology, Washington, D.C. *M. fermentans* type strain PG18 (ATCC 19989) was obtained from American Type Collection (Rockville, MD).

Cell cultures. Cell cultures originally submitted to our labora-

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tory and found to contain mycoplasmas were used in these studies. Prior to treatment, all cultures were grown in appropriate media without antibiotics and passaged 3–5 times in our laboratory. Transfected CHO-B cells were an exception. For this culture, the medium contained 1 μM alprenolol and 3 μM methotrexate as selective agents. An additional 35 cell cultures were deliberately infected with the mycoplasma species most frequently encountered in cell cultures (13). Experimentally infected cell cultures were passaged at least five times and assayed for mycoplasmas to insure infection. All cell cultures were assayed for mycoplasmas with standard tests (10,12). Identification of mycoplasma species was performed by an immunobinding assay (3).

Human diploid fibroblasts 8936, 8937, 8983 and 8782 as well as glial cells 8074 were grown in Eagle's minimal essential medium (EMEM) containing 20% fetal bovine serum (FBS). Human lymphocytes (CEM, MOLT-3, MOLT-4 and H9), human monocytes (U937), mouse lymphoma cells (S49), melanoma cells (Du-Mel 13 and Du-Mel 17) and hybrid cells (m601 and m543) were grown in RPMI-1640 plus 10% FBS, glutamine, HEPES and/or other supplements. Mouse transformed 3T3 cells (KNIH), hybridoma cells (7-2C-3-30) and mouse fibroblast cells (3T6) and CHO transfected cells (CHO-B) were cultured in Dulbecco's minimal Eagles medium (DMEM) plus 10% FBS, glutamine and/or other supplements. Transfected CHO cells were maintained in DMEM with alprenolol and methotrexate as selective agents.

Mycoplasma assays. Mycoplasma infection of cell cultures were assayed by microbiological culture with broth and agar (12) and, DNA fluorochrome staining (10) before and after treatments. To be considered cured of mycoplasmas, treated cell cultures had to, by definition, be free of detectable mycoplasmas and were monitored by the above two methods at passages five and ten post treatment. An immunobinding assay (IBA) and/or epifluorescence assay on agar colonies was performed using specific anti-mycoplasma antisera (3).

Elimination of mycoplasma by BM-cycline treatment. BM-cycline (a combination of tiamulin and minocycline) treatment was carried out according to the manufacturer's instructions (Boehringer Mannheim Biochemicals, Indianapolis, IN). Each cell line was cultured in the appropriate medium containing BM-cycline-1 for three days, followed by four days in the medium containing BM-cycline-2. This treatment was performed a total of three times per culture.

Elimination of mycoplasmas by hyperimmune serum treatment. Hyperimmune serum prepared against membranes of *M. hyorhinis*, *M. orale* and *M. pneumoniae* were prepared in rabbits. Each cell culture was grown in medium containing 2% antiserum against homologous mycoplasma species. For those cultures which were infected with two mycoplasma species, two hyperimmune sera were added to the medium. The treatment was repeated three times.

Elimination of mycoplasmas by 5-bromouracil (5BU). Cell cultures were treated with 5BU, using a combination of 5BU, Hoechst 33258 fluorochrome and visible light treatment as described by Marcus et al. (8). The treatment was performed on five consecutive days.

Treatment with antibiotics and soft agar technique (SAT). The soft agar technique was originally described for cloning of tumor cells (7). A modification was made for elimination of mycoplasmas from cell cultures. The plates containing 0.5% Noble agar (Difco) in complete cell culture medium was prepared in 6 cm petri dishes. The top agar containing 0.3% Noble agar in complete medium was prepared and kept at 50° C. Five ml of top agar was added to the basal agar. Immediately, trypsinized single cells (100–200 cells for monolayer or suspension cultures) were inoculated into the molten top agar. The combination of antibiotics, 30 μg/ml of tetracycline hydrochloride (Sigma), 10 μg/ml of gentamycin sulfate, and 60 μg/ml of tylosine (Flow) were added to the top layer. The petri dish was gently rocked back and forth to spread the agar-cell suspension. The plate was incubated at 37° C in a humidified atmosphere of 5% CO_2/air. The length of incubation was dependent on cell type. Tumor and hybridoma cells were incubated for 1–2 days. Suspension and monolayer cells of non-tumor cells were incubated for 2–3 days. At that time, 20–50 single cells or cell clumps were removed using a Pasteur pipette under a dissecting microscope and transferred into a single well of a 96 well plate containing appropriate medium to prevent undesirable cloning. Plates were incubated until the cells reached 70–80% confluency. Cells were then transferred into 6 well plates. When these reached 70–80% confluency, the cells were transferred to 25 cm^2 flasks. Mycoplasma assays were performed five and ten passages after treatment.

Assay for production of monoclonal antibody: Hybridoma cells 17-2C-3-30, which produces monoclonal antibody to *M. pneumoniae* were assayed by the IBA for monoclonal antibody production 10 passages after treatment with the SAT.

Results

Results of studies to eliminate cell culture mycoplasmas using antibiotics, 5BU and antiserum are listed in Table 1. Results with

TABLE 1

COMPARISON OF THREE METHODS TO ELIMINATE MYCOPLASMAS FROM CELL CULTURES

Cell Culture	Mycoplasma Species (Strain)	Treatment		
		BM-cycline	Antiserum (2%)	5BU
Human fibroblast				
8936	*M. hyorhinis*	1/4[a]	4/4	ND[b]
8937	*M. hyorhinis*	0/4	3/3	ND
8783	*M. hyorhinis*	0/2	0/2	ND
8782	*M. orale*	0/3	0/3	ND
Human B-Cell				
R8	*M. hyorhinis* and *M. fermentans*	3/3	ND	5/5[c]
Hybrid Cells				
m601	*M. hyorhinis* and *M. fermentans*	3/3	ND	10/10[c]
m543	*M. hyorhinis* and *M. fermentans*	3/3	ND	10/10[c]
Mouse fibroblast				
3T6[d]	*A. laidlawii* (MG)	ND	ND	7/7
	M. hyorhinis (GDL)	ND	ND	6/10
	M. fermentans (HK)	ND	ND	7/11
	M. fermentans (1095)	ND	ND	11/11
	M. fermentans (1062)	ND	ND	5/11
	M. fermentans (1092)	ND	ND	3/3

[a] No. of positive/No. of attempted cures.
[b] Not done.
[c] A combination of antibiotic and 5BU was used, *M. hyorhinis* was eliminated, but *M. fermentans* persisted.
[d] Deliberately infected.

5BU were disappointing. Two hybrid cells, m601 and m543 as well as human B lymphocyte R8 cells were treated with a combination of antibiotics and 5BU. These cultures were infected with both *M. hyorhinis* and *M. fermentans*. Neither of these cultures was cured by this method. *M. hyorhinis* was eliminated, but *M. fermentans* persisted. BM-cycline treatment was carried out on four human diploid fibroblasts cultures. Cultures 8937, 8783, and 8782 were all cured. However, 8936 remain infected with *M. hyorhinis*.

Results of treatment with hyperimmune serum were variable. Cultures 8783 and 8782 were cured. However, cultures 8936 and 8937 were not cured. This did not seem to be related to antibody titier, since the titier of the anti-*M. hyorhinis* antiserum was 1:100,000 in the IBA. This same antiserum preparation successfully cured culture 8783, also infected with *M. hyorhinis*.

In further studies, 3T6 cells were infected with *A. laidlawii* (MG), *M. hyorhinis* (GDL), and the following strains of *M. fermentans*: HK, 1095, 1062 and 1092. The efficiency of cure by 5BU in these cultures was only 26.4% (14/53).

Results of attempts to cure mycoplasma infected cell cultures using antibiotics, hyperimmune serum and 5BU were unsatisfactory. In addition, each method required repeated treatments. All three techniques were time consuming, 20–30 days, and the results, with the same infected cell culture were variable, as shown in Table 1.

TABLE 2

ELIMINATION OF MYCOPLASMAS FROM ARTIFICIALLY INFECTED CELL CULTURES BY SOFT AGAR TECHNIQUE (SAT)

Cell Culture	Mycoplasma Species (Strain)	MI After Treatment[a] No. Positive/ No. Attempted
Human leukemic T-cell		
CEM	*M. hyorhinis* (GDL)	0/2
	M. hyorhinis (CEM)	0/4
MOLT-4	*M. orale* (A23)	0/4
	M. pneumoniae (M123-B16)	0/4
Mouse transformed cell		
KNIH	*A. laidlawii* (MG)	0/3
	M. arginini (VV)	1/3
	M. fermentans (HK)	0/3
	M. orale (JS)	0/1
	M. orale (A23)	0/1
Hybridoma		
17-2C-3-30	*M. hyorhinis* (GDL)	0/3
	M. fermentans (HK)	0/3
	M. hyorhinis (GDL) and *M. fermentans* (HK)	0/3
Mouse thymic lymphoma		
S49	*A. laidlawii* (MG)	0/4
	M. hyorhinis (GDL)	0/4
	M. fermentans (HK)	0/4
	M. arginini (VV)	0/4
Mouse fibroblast		
3T6	*M. laidlawii* (MG)	0/4
	M. arginini (VV)	0/4
	M. hyorhinis (GDL)	0/4
	M. orale (A23)	0/4

[a] Detection of mycoplasma infection (MI) was carried out 2 times at 5 and 10 passages after treatment by microbiological culture and DNA staining.

Initial studies on the soft agar technique (SAT) were performed on cell cultures deliberately infected with various mycoplasma species. These cell cultures were: human T lymphocytes CEM and MOLT-4, transformed mouse 3T3 cells, mouse hybridoma cells 17-2C-3-30, mouse lymphoma cells S49 and mouse fibroblast cells 3T6. Results of these studies are presented in Table 2. In these studies, 20 of 21 cultures were cured of mycoplasmas. A total of 66 tests were performed in these 21 cell cultures. The SAT successfully eliminated mycoplasmas in 65 of these 66 tests (98.4%). The one failure in these studies occurred in KNIH cells infected with *M. arginini* strain VV. However, the SAT successfully eliminated this same strain of *M. arginini* from S49 and 3T6 cells. The SAT also eliminated the VV strain of *M. arginini* 2 of 3 times from KNIH cells. The above studies utilized one cycle of antibiotic treatment in soft agar for 1–2 days.

Subsequent studies were performed on cell cultures that were submitted to this laboratory and found to be infected with mycoplasmas. Results of these studies are presented in Table 3. The SAT technique successfully eliminated mycoplasmas in all mycoplasma infected cell cultures. The cell culture types included human diploid fibroblasts, glial cells, human T cell lymphoma, melanoma, other carcinomas and transfected CHO cells. Infecting mycoplasma species included: *M. hyorhinis*, *M. orale*, and *M. arginini*. One culture, 8074, was infected with both *M. orale* and *M. arginini*. Complete elimination was obtained after one treatment of 1–2 days in soft agar. The treated cell cultures were free of detectable mycoplasmas 5 and 10 passages after treatment when studies were terminated. Human diploid fibroblasts and glial cells remained free of mycoplasmas for the remainder of their in vitro lifespan.

TABLE 3

ELIMINATION OF MYCOPLASMAS FROM NATURALLY INFECTED CELL CULTURES BY SOFT AGAR TECHNIQUE (SAT)

Cell Culture	Mycoplasma Species	Mycoplasmas After Treatment[a] No. Positive/No. Attempted
Human fibroblast 8936	*M. hyorhinis*	0/2
Human fibroblast 8937	*M. hyorhinis*	0/2
Human fibroblast 8983	*M. hyorhinis*	0/2
Human fibroblast 8782	*M. orale*	0/2
Human glia 8074	*M. orale* and *M. arginini*	0/2
Human leukemic H9 T-cell	*M. arginini*	0/3
Hamster transfected cells CHO-B	*M. orale*	0/10
Human leukemic MOLT-3 T-cell	*M. arginini*	0/3
Human lymphoma VS 37	*M. hyorhinis*	0/10
Human carcinoma HeLa/D98	*M. hyorhinis*	0/5
Human melanoma DU-Mel 13	*M. hyorhinis*	0/3
Human melanoma DU-Mel 17	*M. hyorhinis*	0/5
Human/rodent hybrid	*M. arginini*	0/2
Human/rodent hybrid	*M. arginini*	0/2

[a] Detection of mycoplasmas was carried out 2 times at 5 and 10 passages after treatment by microbiological culture and DNA staining.

Results presented in Table 1 indicated that *M. fermentans* was difficult to eliminate from cell cultures with 5BU treatment, although strain HK was eliminated in studies presented in Table 2. Studies were performed to determine the efficiency of the SAT to eliminate various *M. fermentans* strains from deliberately infected 3T6 cells. Results of these studies are presented in Table 4. The SAT successfully eliminated control species *A. laidlawii* and *M. hyorhinis* from 3T6 cells. However, only 3 of 8 *M. fermentans* strains were reproducibly cured. One of these was type strain PG18 isolated from human urogenital tract. Strain HK was cured in 5 of 6 attempts. The other *M. fermentans* strains used in this study were all isolated from cell cultures. These 7 strains originated in 7 different institutions and 7 different cultures, minimizing the potential of strain selection. Preliminary results suggested that at least one of these strains, 1095, was intracellular in 3T6 cells by electron microscopic examination. If true, intracellular location of *M. fermentans* might render treatment more difficult. Tetracycline was effective against all these strains of *M. fermentans* when propagated in mycoplasma broth.

To determine the effect of treatment on differentiation characteristics, the secretion of monoclonal antibody by hybridoma cells, 17-2C-30 was measured. Cells cured by the SAT all produced monoclonal antibodies, and no difference in quantity of IgG by cured and non-cured cells was noted.

Discussion

Many reports have been published on methods to eliminate mycoplasmas from infected cell cultures. Unfortunately, many of these studies utilized a small number of cell types and/or mycoplasma species. This has rendered interpretation of relative efficiencies difficult. Combination of treatments have, as expected, often increased efficiency. Ideally, the treatment method should be simple, efficient and not have adverse effects on the cell culture. We believe the soft agar technique (SAT) described in this report fulfills these criteria, based on studies in 35 different cell cultures using six different mycoplasma species and many strains.

The advantage of this method lies in its speed and simplicity. One cycle of treatment is adequate. This takes 1–3 days. Soft agar in complete medium can be readily prepared. The soft agar technique was originally used for cloning of tumor cells (7). While non-tumor cells do not grow in soft agar, they do survive for several days so they can be readily treated by this method. Suspension in soft agar may facilitate exposure of mycoplasma infected cells to the antibiotics because the rounded cells are in suspension. Also, the preparation of the soft agar overlay exposes mycoplasma to temperatures of approximately 50° C for a brief period. Therefore, some species of mycoplasmas might be heat damaged during this period. Perhaps both mechanisms are operative.

TABLE 4

RESULT OF SOFT AGAR TECHNIQUE ON 3T6 CELLS INFECTED WITH DIFFERENT STRAINS OF *M. FERMENTANS*

Mycoplasma Species (Strain)	Result: No. of Positive/No. of Attempted
A. laidlawii (MG)	0/6
M. hyorhinis (GDL)	0/6
M. fermentans (HK)	1/6
M. fermentans (1095)	5/6
M. fermentans (1092)	6/6
M. fermentans (1062)	3/3
M. fermentans (1582)	0/2
M. fermentans (572)	2/2
M. fermentans (sb51)	0/3
M. fermentans (PG18)	0/2

Accidental clonal selection of treated cells can be a difficulty. In SAT, cloning of cells is typically avoided. Depending on the cell type and its respective differentiation characteristic, treated cells should be plated at greater than clonal densities, and be transferred in high numbers.

The antibiotics used in this study did not exhibit significant toxicity to any of the cell cultures treated. The antibiotics used in these studies have been used extensively in cell cultures. It would be preferable to perform appropriate antibiotic sensitivity tests to maximize success. We performed susceptivity tests in the past, but have found them to be unnecessary on a routine basis except for unusual mycoplasmas, or mycoplasma in selective medium for gene expression. The effects of antibiotics on cell cultures should be considered, especially when these are used for prolonged periods as in some protocols. Deoxycoformycin which has been used in some studies has several effects on mammalian cells, including the inhibition of deoxyadenosine deamination (16).

The difficulty of successfully curing *M. fermentans* infected cultures is intriguing. We do not believe this failure is due to resistance of the strains used in this study to antibiotics. In fact, the antibiotics used in this study killed all the broth propagated *M. fermentans* strains. If mycoplasma killing is also affected by heat, then increased heat resistance of this organism is also a possibility. However, we have preliminary electron microscopic evidence that suggests that *M. fermentans* can be found intracellularly in cell cultures. If this is true, appropriate concentrations of antibiotics might not be able to have access to these intracellular organisms. Ciprofloxacin may be useful because of its hydrophobicity. There seems to be precedence for this. Lo et al. have shown that *M. fermentans* are intracellular in vitro and in vivo (6, in press). On the other hand, we successfully cured another intracellular mycoplasma, *Spiroplasma mirum*, from infected 3T3 cells using a regimen of gentamycin and tylocine (4). This area needs further study.

Based on the results presented here, the combination of antibiotics and soft agar treatment, can be of significant value for elimination of mycoplasmas from cell cultures.

References

1. Gardella, R. S.; Del Giudice, R. A. Antibiotic sensitivities and elimination of mycoplasmas from infected cell cultures. Isr. J. Med. Sci. 20:931–934; 1984.
2. Jeansson S.; Bronson, J. E. Elimination of mycoplasmas from cell cultures utilizing hyperimmune sera. Exp. Cell Res. 161:181–188; 1985.
3. Kotani, H.; McGarrity, G. J. Rapid and simple identification of mycoplasmas by immunobinding. J. Immunol. Methods 85:257–267; 1985.
4. Kotani, H.; Phillips, D. M.; McGarrity, G. J. Malignant transformation of NIH-3T3 cells and CV-1 cells by a helical mycoplasma, *Spiroplasma mirum* SMCA. In Vitro 22:756–762; 1986.
5. Kotani H.; Butler, G. H.; Tallarida, D., et al. Microbiological cultivation of *Mycoplasma hyorhinis* from cell cultures. In Vitro Cell. Dev. Biol. 26:91–96; 1990.
6. Lo, S.; Dawson, M. S.; Wong, D. M., et al. Identification of *Mycoplasma incognitus* infection in patients with AIDS: an immunohistochemical, *in situ* hybridization and ultrastructural study. Amer. J. Trop. Med. Hyg. 41:601–611; 1989.
7. MacPherson, I.; Montagnier, L. Agar suspension culture for the selec-

tive assay of cells transformed by polyoma virus. Virology 23:291–294; 1964.
8. Marcus, M.; Lavi, U.; Nattenberg, A., et al. Selective killing of mycoplasmas from contaminated cells in cell cultures. Nature 285:659–661; 1980.
9. McGarrity, G. J. Spread and control of mycoplasmal infection of cell cultures. In Vitro 12:643–648; 1976.
10. McGarrity, G. J.; Steiner, T.; Vanaman, V. Detection of mycoplasmal infection of cell cultures by the DNA fluorochrome staining procedure. In: Rayin, S.; Tully, J. G., eds. Methods in mycoplasmology vol. II. New York: Academic Press; 1983:183–190.
11. McGarrity, G. J.; Vanaman, V.; Sarama, J. Cytogenetic effects of mycoplasma infection of cell cultures: a review. In Vitro 20:1–18; 1984.
12. McGarrity, G. J.; Steiner, T.; Gamon, L. Prevention, detection and control of mycoplasmal infection of cell cultures. In: Kilbey, B. J.; Legator, M.; Nichols, W., et al., eds. Handbook of mutagenicity test procedures; 2nd edition. New York: Elsevier; 1984:823–839.
13. McGarrity, G. J.; Kotani, H. Cell culture mycoplasmas. In: Razin, S.; Barile, M. F., eds. The mycoplasmas. Vol. IV. New York: Academic Press; 1985:353–590.
14. McGarrity, G. J.; Kotani, H. Detection of cell culture mycoplasmas by a genetic probe. Exp. Cell Res. 163:273–278; 1986.
15. Mowles, J. M. The use of ciprofloxacin for the elimination of mycoplasma from naturally infected cell lines. Cytotechnology 1:355–358; 1988.
16. Plagemann, P. G. W.; Woffendin, C. Mycoplasma contamination alters 2′-deoxyadenosine metabolism in deoxycoformycin treated mouse leukemia cells. J. Cell. Biochem. 43:161–172; 1990.
17. Ravaoarinoro, M.; Leconte, J. Evaluation of three methods for curing hybridomas from mycoplasma contamination. Hybridoma 7:79–86; 1988.
18. Schimmelpfeng, L.; Langenberg, U.; Peters, J. H. Macrophages overcome mycoplasma in infections of cells in vitro. Nature 285:661–662; 1980.
19. Schmitt, K.; Daubner, W.; Bitter-Suermann, D., et al. A safe and efficient method for elimination of cell culture mycoplasmas using ciprofloxacin. J. Immunol. Methods 109:17–25; 1988.
20. Van Diggelen, O. P.; Shin, S.; Phillips, D. M. Reduction in cellular tumorgenicity after mycoplasma infection and elimination of mycoplasma from cultures by passage in nude mice. Cancer Res. 37:2680–2687; 1977.
21. White, G. R. M.; Ockey, C. H. SCE induction and harlequin staining in mycoplasma contaminated Chinese hamster cells. Chromosoma 93:165–168; 1985.
22. Ziegler-Heitbrick, H. W. L.; Burger, R. Rapid removal of mycoplasma from cell lines mediated by a direct effect of complement. Exp. Cell Res. 173:388–394; 1987.

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