Protocol

Antibiotic Treatments of Microbe-Contaminated Cell Cultures

Adapted from an article by Rosalie Coté

Introduction

Cell culture contamination is a common and serious problem that adversely affects the quality of experimental results in many bioscience laboratories. The organisms responsible for this contamination are numerous, but the most common ones are bacteria, fungi and mycoplasma. In the cases of bacteria and fungus, the contamination of cell lines can have a human or environmental origin, while mycoplasma in cell culture laboratories usually originates from another contaminated cell line or the Bovine serum used in the culture media. Another complication that accompanies cell line contamination is that it can go undetected for long periods of time. Once contamination is detected and the causative organism is identified, laboratory personnel can attempt to salvage the cell line through the use of antibiotics. However, antibiotics should not be used routinely, as their overuse can result in antibiotic resistant microbes and cytotoxicity. Here, we outline a procedure on the use of different antibiotics to eliminate fungal, bacterial and mycoplasma contamination of cell lines.

Materials

- Contaminated cell culture
- Sterile antibiotic stock solution(s) (Refer to Table 1)
- DMSO (GoldBio Catalog # D-361)

Method

1. Quarantine the cell culture as soon as contamination has been detected. This step prevents the contamination of other cell cultures in the laboratory.

   Note: The laboratory space where the contaminated culture was grown must be cleaned and sterilized to prevent further contamination.

2. Identify the type of organism the contaminant is: bacteria, fungi or mycoplasmal (see step 7).

   Note: If contamination is due to bacteria, identification of the *genus* may help in choosing an effective antibiotic for treatment.

3. If the microbial contaminant is identified, select an appropriate antibiotic from Table 1.

4. Prepare the antibiotic at the appropriate concentration. Filter and sterilize the antibiotic after dissolving in appropriate solvent.

Note: Antibiotics dissolved in EtOH, DMF or DMSO do not need to be filter-sterilized.

Table 1. Antibiotics

Organism	Antibiotic	GoldBio Catalog #	Solvent	Stability (37°C)	Working Concentration
Bacteria (gram positive only)	Ampicillin	A-301	Water	3 days	100 mg/liter
	Erythromycin	E-350	2M HCl or EtOH[a]	3 days	100 mg/liter
	Gentamicin sulfate	G-400	Water	5 days	50 mg/liter
	Kanamycin sulfate	K-120	Water	5 days	100 mg/liter
	Neomycin sulfate	N-620	Water	5 days	50 mg/liter
	Penicillin-G (Potassium salt)	P-303	Water	3 days	100 mg/liter
	Streptomycin sulfate	S-150	Water	3 days	100 mg/liter
	Tetracycline HCl	T-101	Water	4 days	10 mg/liter
Fungi (molds and yeasts)	Amphotericin B	A-560	DMSO; DMF[2]	3 days	2.5 mg/liter
	Nystatin	N-750	DMF	3 days	50 mg/liter
Mycoplasmas	Gentamicin sulfate	G-400	Water	5 days	50 mg/liter

[a] EtOH stands for ethanol, DMSO stands for Dimethyl sulfoxide and DMF stands for Dimethylformamide.
[b] DMSO: dimethyl sulfoxide; DMF: dimethylformamide.

5. If the bacterial contaminant is unknown, prepare the following 10x antibiotic cocktail to treat the cell culture.
    a. 2500 U/ml Penicillin G Potassium
    b. 2.5 mg/ml Streptomycin
    c. 2.5 mg/ml Neomycin
    d. 25 U/ml Bacitracin (GoldBio Catalog # B-070)

Note: Be sure to filter-sterilize cocktail.

6. Add the antibiotic as follows:

a. For adherent or monolayer culture, suction out the medium and replace with medium containing antibiotics.
b. For cells grown in suspension, centrifuge the culture for 10 minutes at 125 x g. Remove the supernatant and resuspend cells in fresh medium containing antibiotics.

Note: For a known contaminant, add a specific antibiotic stock solution to fresh medium at a working concentration (Refer to Table 1).

Note: For unknown bacterial contaminant, add 1 volume of 10x antibiotic cocktail (described in step 4) to 9 volumes fresh medium (for example, add 1 ml of 10x antibiotic cocktail to 9 ml of fresh medium).

7. Add additional antibiotic solution every 3-5 days to maintain the working concentration. Continue treatment of cell lines for 14 days.

Note: Antibiotic potency decreases with time. Refer to Table 1 to determine how long a specific antibiotic is potent at 37°C.

Note: Change medium or passage cells during this time to maintain antibiotic potency.

8. Observe the contaminated culture microscopically during treatment. Examine for cytotoxic effects as well as elimination of the contaminant.

9. If after 14 days, contamination is still present, destroy the culture by autoclaving. If culture appears free of contaminant, inoculate into fresh antibiotic-free medium.

Note: Do not maintain cell cultures in medium containing antibiotics. Antibiotics can have cytotoxic effects, change cell characteristics (metabolism) and create antibiotic-resistant organisms.

10. Test for mycoplasma contamination.

Note: Mycoplasmas are very small organisms and thus are difficult to detect with microscopic inspection. To test for mycoplasma contamination, use specific identification methods (staining for DNA, PCR, direct culture). In general, mycoplasmas are not susceptible to common antibiotics. Thus, once identified, contaminated cell lines must be treated with a specific antibiotic (Refer to Table 1).

Tips

- Contaminated cell lines that have been treated with antibiotics and confirmed decontaminated must be retested periodically.
- Cell lines received from other sources must be tested, especially for mycoplasma (it is very common), before placing in common incubator with other cell lines. This testing will prevent use and further contamination of other cell lines in the laboratory.
- Other reagents in the lab must also be periodically tested for microbial contamination, including cell culture media and stock solutions.

Associated Products

- Ampicillin (GoldBio Catalog # A-301)
- Erythromycin (GoldBio Catalog # E-350)
- Gentamicin sulfate (GoldBio Catalog # G-400)
- Kanamycin sulfate (GoldBio Catalog # K-120)
- Neomycin sulfate (GoldBio Catalog # N-620)
- Penicillin-G, Potassium Salt (GoldBio Catalog # P-303)
- Streptomycin sulfate (GoldBio Catalog # S-150)
- Tetracycline HCl (GoldBio Catalog # T-101)
- Amphotericin B (GoldBio Catalog # A-560)
- Nystatin (GoldBio Catalog # N-750)
- DMSO (GoldBio Catalog # D-361)

References

Coté, R. (1999). Assessing and controlling microbial contamination in cell cultures. *Current Protocols in Cell Biology*, 1-5.

Uphoff, C. C. and Drexler, H. G. (2014). Detection of Mycoplasma Contamination in Cell Cultures. *Current Protocols in Molecular Biology*. Doi:10.1002/0471142727.mb2804s106.