

General Mycoplasma Information

Mycoplasma are the smallest free-living organisms that, unlike other bacteria, lack a cell wall. The outer layer is a three-layered membrane containing sterols. Diameters of mycoplasma may range from 0.2-0.3 μm and, due to their plasticity, they may squeeze through the pores of a 0.22 micron filter with applied pressure. Pleomorphic in morphology, some may appear coccoidal and other filamentous.

Because mycoplasma lack a cell wall, the organisms are poorly stained, if at all, by bacterial stains. With the exception of *M. hyorhinis*, most are able to be cultivated with use of standardized agar and broth media. When grown on the agar, the colonies have a "fried-egg" appearance due to the colony center growing into the agar and appearing more dense than the rest of the colony.

Cell cultures contaminated with mycoplasma may go undetected due to slow growth of the organism without the destruction of the host cell to make it noticeable. Contamination is undetected with the naked eye except for signs of deterioration of the culture. The following are effects mycoplasma may have on a culture:

- Interference with the rate of cell growth
- Changes in cell morphology
- Aberrations in chromosomes
- Altered DNA, RNA, and protein synthesis
- Induced cell transformation

Decreased cell proliferation may occur in chronic infections and total deterioration in acute infections. As a result, it is extremely important that cell lines are tested for the presence of mycoplasma.

Several methods exist to test for the presence of mycoplasma. One is the Hoechst 33258 DNA staining method utilizing a

fluorochrome dye that specifically binds to DNA. When viewed with fluorescent microscopy, uncontaminated cultures have a low cytoplasm/nucleus ratio; the nuclei and extranuclear mycoplasma DNA fluoresce in infected cells. Advantages to this method are rapidity and the ability to detect the non-cultivable strain *M. hyorhinis*. The disadvantages are that this test does not detect low titres of mycoplasma and it lacks sensitivity. It may be difficult to differentiate mycoplasma from disintegrating nuclei or DNA from bacteria and fungi if they are also contaminating the culture. Other methods of detection include the culture method in which growth is observed on standardized agar and in broth media (with the exception of *M. hyorhinis*). Mycoplasma detection kits and PCR may also be used.

Mycoplasma that commonly contaminate a culture include *M. hyorhinis* (natural host is porcine), *M. arginini* and *Acholeplasma laidlawii* (natural hosts are bovine), and *M. orale* and *M. fermentans* (natural hosts are humans). If a contaminated culture exists, the best action is to discard the culture as it can be difficult to completely eradicate the organism. Discarding contaminated cultures should always be done unless the culture is irreplaceable and cannot be lost. If the cell line is irreplaceable, antibiotics, particularly ciprofloxacin (61-277), may be used to eradicate the mycoplasma. Prophylactic use of antibiotics as a preventative for any type of contamination is not recommended. Use of antibiotics in this way may mask contamination and cause it to go undetected, particularly if resistance is a problem or if the antibiotic is bacteriostatic instead of bacteriocidal. In addition, poor technique and the use of antibiotics only when the culture cannot be discarded is ideal.

All cellgro[®] basal media and salt solutions are tested for the presence of mycoplasma using the Kern and Barile method as per "Isolation of Mycoplasmas".

References:

1. Davis, J. M. *Basic Cell Culture: A Practical Approach*. New York: Oxford University Press, 1994: 251-254.
2. Doyle, A. And J. Bryan Griffiths, eds. *Mammalian Cell Culture: Essential Techniques*. New York: John Wiley and Sons, 1997: 125, 132-133, 135, 137.
3. Freshney, R. Ian. *Culture of Animal Cells: A Manual of Basic Technique*. 3rd ed. Wiley-Liss: New York, 1994: 245-250.
4. Champoux, et. al. *Sherris Medical Microbiology: An Introduction to Infectious Diseases*. 3rd ed. Ed. Kenneth J. Ryan. Stamford: Appleton and Lange, 1994: 373