Mycoplasmas are the smallest free-living organisms that, unlike other bacteria, lack a cell wall. The outer layer is instead, a three-layered membrane containing sterols. Diameters of these organisms may range from 0.2-0.3 μm and, due to their plasticity, are able to pass through the pores of a 0.2 micron filter with applied pressure. Because the morphology of Mycoplasma are pleomorphic, they occur as two different structural forms during a life cycle: coccoidal, a spherical or spheroidal shape, and filamentous, resembling rods.

Because Mycoplasmas lack a cell wall, the organisms are poorly stained, if at all, by bacterial stains. With the exception of *M. hyorhinis*, most Mycoplasma can be cultivated using standardized and Mycoplasma agar formulations, as well as in broth media, although growth is slow. When grown on agar, the colonies have a “fried-egg” appearance since the colony center grows into the agar and appears more dense than the rest of the colony.

Unless it is specifically tested for, cultures contaminated by Mycoplasma often remain undetected since there are no obvious signs of contamination, like the destruction of host cells. Chronic infections usually cause decreased cell proliferation, while acute infections result in total destruction of the cell culture. The following clues may help signal deterioration of a culture affected by Mycoplasma contamination:

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

Several methods exist to test for the presence of Mycoplasma. One is the Hoechst 33258 DNA staining method, which uses a fluorochrome dye that specifically binds to DNA. When viewed with fluorescent microscopy, uncontaminated cultures have a low cytoplasm/nucleus ratio, while the nuclei and extranuclear mycoplasmal DNA fluoresce in infected cells. The advantages to using the Hoechst 33258 DNA stain include rapid results and detection of the non-cultivable strain *M. hyorhinis*. However, this method has low sensitivity and fails to detect low titres. It may be difficult to differentiate Mycoplasma from disintegrating nuclei, or bacterial or fungal infections, if present. Other methods of detection include the culture method in which growth is observed on standardized agar or in broth media with the exception of *M. hyorhinis*. Mycoplasma detection kits and PCR may also be used to detect Mycoplasma contamination.

Some common organisms that cause cell culture contamination include *M. hyorhinis* from porcine, *M. arginini* from bovine, and *M. orales* and *M. fermentans* whose natural hosts are humans. Unfortunately, in the event of contaminated cultures, the best course of action is to discard the culture, as it can be difficult to completely eradicate the organism. If the cell line is irreplaceable, however, antibiotics such as ciprofloxacin may be used to eliminate the Mycoplasma. Prophylactic use of antibiotics as a preventative for any type of contamination is not recommended, as they may mask contamination and cause it to remain undetected, particularly if resistance is a problem or if the antibiotic is bacteriostatic instead of bacteriocidal. In addition, rigid aseptic technique is a must to prevent Mycoplasma infection since poor technique may become habit-forming. The use of antibiotics should be limited to contaminated cultures, and only when discarding is out of the question.

All Mediatech basal media and salt solutions are tested for the presence of Mycoplasma as an assurance that Mediatech products will not bring Mycoplasma into our customers’ labs.

References: