MICROBIAL CONTAMINATION

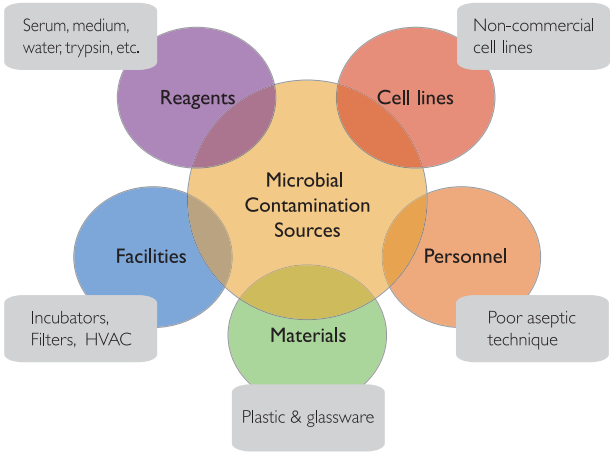
Microbial contamination of cell cultures is easily the most common problem encountered in cell culture laboratories, sometimes with very serious consequences. The use of infected cell lines can lead to unreliable experiments and unsafe biologicals and biopharmaceutical drugs, and is costly in time and materials. Microbial contamination falls into two groups: those that can be easily detected (e.g. bacteria, yeast and fungi) and those that are more difficult to detect (e.g. mycoplasma). While it is impossible to eliminate contamination entirely, it is possible to reduce its frequency and seriousness by gaining a thorough understanding of it sources and by following good aseptic technique.

Mycoplasma Contamination

Mycoplasmas are the smallest and simplest self-replicating organisms. They lack a rigid cell wall and grow mostly associated with the mammalian cell membranes. In most cases, there are no signs of mycoplasma contamination. They cannot be detected by visual inspection and do not cause consistent perceptible changes in a cell culture, such as rapid pH change and medium turbidity. Thus, mycoplasmas commonly remain undetected in the cell cultures for long periods. Mycoplasmas can cause disastrous effects on eukaryotic cells, as they can alter every cellular parameter, from proliferation to virus susceptibility and production, leading to unreliable experimental results and potentially unsafe biological products[1, 2]. This is a serious problem, as 5 to 35% of cell-lines worldwide are infected with mycoplasmas[2-4].

Bacterial Contamination

Although bacterial contamination can be detected using a light microscope, it can easily be mistaken for cellular debris, especially when the baterial contamination is in the early stages of infection. Signs of bacterial contamination include signs of mobility and a sudden decrease in pH with the culture media changing to a yellowish color. Bacteria are a large and ubiquitous group of unicellular microorganisms. They are typically a few micrometers in diameter, and can have a variety of shapes, ranging from spheres to rods and spirals. Because of their ubiquity, size, and fast growth rates, bacteria are the most commonly encountered biological contaminants in cell culture[5, 6].



Fungal Contamination

With yeast, molds and fungi, the pH of the culture remains stable in the initial stages of contamination, then rapidly increases as the culture becomes more heavily infected and gets turbid. By microscopy fungi usually appear as long thin filaments, while yeast are round or oval bodies that can form chains or clusters[7]. In the advanced stages of contamination, fuzzy patches can be easily seen in the culture[8]. Fungal contamination presents difficulties for eradication, as it can spread via spore mobility in air. Spores of many fungal species can survive in extremely harsh and inhospitable environments in their dormant stage, only to become activated when they encounter suitable growth conditions. Cell cultures can often be cured of fungal contamination when detected early and treated with certain antibiotics.

Endotoxin Contamination

Endotoxin, also known as lipopolysaccharide (LPS), is the major cell wall component of Gram-negative bacteria. Endotoxin is a potent stimulator of the humoral and cellular response *in vivo*. *In vitro*, endotoxins can introduce a bias in experiments involving cells sensitive to endotoxins[9, 10]. Thus, monitoring the presence of endotoxins in cell culture reagents is crucial. Sources of endotoxins include media, sera, water, buffers and other cell culture reagents, such as trypsin. Other biologically active organic contaminants that can induce significant experimental variabilityinclude flagellin and lipoproteins. Care needs to be taken with solutions that are sterile but may still contain bacterial components, such as endotoxins that could interfere with cell cultures.

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HEK-Blue™ LPS Detection Kit 2- Detection of endotoxins

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Plasmocin™ Treatment - Treatment for mycoplasma contamination
Plasmocure™ - Alternative treatment for mycoplasma contamination
Fungin™ - Elimination and prevention of yeast and fungal infections
Normocure™ - Elimination of bacterial infections

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Normocin™ - Prevention of mycoplasmal, bacterial and fungal infections
Primocin™ - Prevention of mycoplasmal, bacterial and fungal infections in primary cells