Filtration for Protecting Cell Cultures

Strategies for Controlling Mycoplasma Infections

Paul Priebe and Holger Bromm

Substantial effort is expended to optimize the viability and productivity cell cultures. All this work is for naught, however, if a culture becomes contaminated with adventitious agents. Bacterial contaminations of animal cell cultures are serious, typically resulting in complete loss of an affected culture. For this reason, all reasonable means are taken to establish and maintain the sterility of the bioreactor. The bioreactor and piping are steamed to sterilize the vessel and all associated transfer lines. All incoming gases (e.g., oxygen and compressed air) are passed through sterilizing-grade gas filters. Off-gas venting is protected by both positive pressure and a sterilizing-grade vent filter as a barrier. All liquid additives are rendered sterile before addition to the bioreactor. Because some media components would be destroyed by heat sterilization, bacterial-retentive filtration is required.

But bacteria are not the only adventitious agents to be considered: Mycoplasma can contaminate cell cultures, with undesirable results. From a filtration standpoint, it is well known that 0.2-µm–rated sterilizing-grade filtration will not usually retain mycoplasma.

Product Focus: Products of animal cell culture

Process Focus: Production

Who Should Read: Process development, cell culture, production, filter purchasers and manufacturers

Keywords: Mycoplasma, cell culture, adventitious agents, contamination, sterility, viability, *Acholeplasma laidlawii*

Level: Introductory

Mycoplasma: Common Contaminants

Mycoplasma are the smallest free-living, self-replicating organisms currently identified. They lack a true cell wall and are therefore deformable under pressure. They contain protein, RNA, DNA, and enzymes.

Mycoplasma species often contaminate cell cultures, virus stocks, and other cell-derived biologicals. Because mycoplasma lack a cell wall, antibiotics (such as penicillin) that interfere with formation of cell walls are ineffective against them at the standard concentrations used. In cell cultures, mycoplasma are extracellular parasites, usually attached to the external surface of a cell membrane. They can contaminate a variety of eukaryotic cells in culture, leading to detrimental host effects that include changes in growth, morphology, metabolism, protein synthesis, and virus replication. They compete effectively with tissue-culture cells for medium nutrients, thus depriving those cells of essential nutrients, resulting in profound damage to cell metabolism and function. In the worst-case scenario, contamination leads to diminished cell growth and eventually to the loss of the culture. Mycoplasma from human, bovine, and porcine sources are the most prevalent groups, the most common isolates of which are *Acholeplasma laidlawii, Mycoplasma arginini, Mycoplasma fermentans, Mycoplasma hyorhinis*, and *Mycoplasma orale*.

Detecting Mycoplasma

Even at densities of 10^7–10^8 organisms/mL, mycoplasma infections do not affect turbidity and are not normally detectable with light microscopy. Many assays are commercially available for mycoplasma detection: direct DNA fluorescence staining, classical broth-agar microbiological colonization, RNA hybridization in solution, and PCR (in addition to other techniques).

Sources of Contamination

Virtually all mycoplasma infections can be traced to one of two sources: contaminated animal-derived materials and poor aseptic techniques. Animal-derived materials such as sera used in cell culture media always represent a risk for mycoplasma infection. People also are known to carry mycoplasma, making poor aseptic technique a primary cause of infection. (Improper handling can contaminate the cell line, but this infection is likely to be detected and controlled before inoculation of the bioreactor.)

Strategies for Control

Multiple strategies are used to reduce the incidence of mycoplasma infections in production-scale cell culture.

Careful Testing and Screening of Master Cell Banks: Cell lines used to inoculate large-scale bioreactors are thoroughly tested to identify mycoplasma contamination.

Sub-Sterile Filtration of Media Containing Serum: 0.1-µm–rated filtration of media that contains serum can help reduce the risk of infection from contaminated sera; however, all 0.1-µm filters are not created equal and offer varying levels of mycoplasma retention.

Serum-Free Media: The use of serum-free media can reduce the risk of infection.

Sub-Sterile Filtration of Serum-Free Cell Culture Media: So-called 0.1-µm filtration of the cell culture media can help reduce the risk of infection from improper techniques used during serum-free media preparation; however, once again, different 0.1-µm filters offer varying levels of mycoplasma retention.

Antibiotics: Mycoplasma-specific antibiotics can be added to the bioreactor.

Confusing Data

Filter users familiar with sterilizing-grade filters may mistakenly believe that a standard exists for 0.1-µm filters and, further, that all 0.1-µm–rated filters will equally or fully retain mycoplasma. All 0.2-µm sterilizing-grade filters must demonstrate reliable retention of *Brevundimonas diminuta* at 10^7 cfu/cm^2 under standard conditions. This allows users to select filters based on easily demonstrated performance characteristics: frequently, flow rate and total throughput with actual filtrate. But no standards currently exist for filters beyond 0.2-µm sterilizing grade. Those must, of course, meet the definition of a sterilizing-grade filter, but no strict retention requirement exists beyond that, including no requirement at all to retain mycoplasma. In fact, a filter manufacturer could label a 0.2-µm filter as 0.1-µm strictly for marketing reasons.

Each of the major filter manufacturers, aware that mycoplasma retention is of interest to its customers, provides retention data. In fact, all give retention data for *Acholeplasma laidlawii*. This, however, can confuse end users because each filter manufacturer probably chose *Acholeplasma laidlawii* for the same reasons: It is known to be responsible for more than 50% of all cell culture mycoplasma contaminations. Additionally, it is also the easiest mycoplasma to cultivate to the appropriate challenge concentrations. But this choice of challenge organism is where comparability of filtration data ends: No consistent standards guide the choice of challenge conditions or the format for reporting the results (Figures 1 and 2). Results, for example, can be reported as log reduction values or as titer reduction — another possible source of confusion.

The following challenge conditions can affect retention or subsequent recovery and detection of penetrating organisms: the nutritive state of the organism, the minimum challenge concentration, the suspension fluid, the differential pressure, the filter format, and the presence of an appropriate control for viability.

Table 1: Comparative mycoplasma retention

Filter	PES 0.2/0.1 µm	PVDF 0.2/0.1 µm	N66 0.1/0.1 µm	PVDF 0.1 µm
Challenge	1.9×10^7	1.3×10^7	7.0×10^7	6.0×10^7
Detected colonies	0	(too numerous to count)	22	1000
LRV[1]	7	3–4	6	4
Plated capture membrane				

[1]LRV = log reduction value

Challenge Testing

A study was designed to challenge 0.1-rated filter cartridges under identically stringent conditions. The conditions specified use of high differential pressure, both to demonstrate the differences between filters and because mycoplasma are deformable under pressure. The study additionally considered flow rate and total throughput of the cartridges — relevant performance characteristics. Only by examining these characteristics in combination can a filter user gain a complete view of comparative filter performance.

Challenge Conditions: Each 10-in filter element was challenged with a suspension of *Acholeplasma laidlawii* at a challenge concentration of 10^7 cfu/cm^2. The challenge organisms were suspended in 40 L of Difco PPLO broth, filtered in a single pass. The cartridges were challenged at a differential pressure of 2 bar with a positive control for viability and recovery. The recovery filter membranes were then plated, incubated, and counted.

Results

All filters tested are marketed as mycoplasma-rated filters. The language used to describe them varies, but it is clear that the manufacturers intend to market them to remove mycoplasma from cell culture media. However, the filters demonstrate significant differences in retentivity when subjected to identical challenge conditions and reporting format. Additionally, total throughput and flow rate vary widely among the available filters, with a demonstrated lack of correlation between flow rate, total throughput, and retention.

Reducing the Risk

The risk from the two primary causes of mycoplasma infections in bioreactors — unintentional contamination of cell culture media by poor technique and contaminated sera — can be significantly reduced by mycoplasma-retentive filtration of cell culture media. Selecting a filter to protect against mycoplasma infections is not nearly as straightforward as the choice of a sterilizing-grade filter. The true retentivity of the filter in question must be compared with other 0.1-µm–rated filters — using the same criteria. This information, combined with a comparison of the total throughput and flow rate of filters using the actual culture media formulation, will allow users to make informed filter selections.

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Figure 1: Comparative flow rate, 0.1-µm–rated sterilizing-grade filters

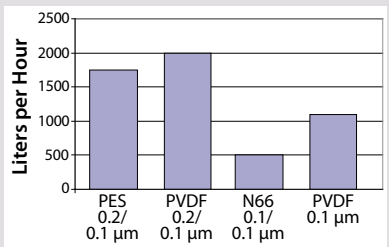

Figure 2: Comparative total throughput performance, 0.1-µm–rated sterilizing-grade filters