Add the following:

▲⟨1229.17⟩ MYCOPLASMA STERILIZATION

INTRODUCTION

Mycoplasmas, Acholeplasmas, and Ureaplasmas are small prokaryotes, all belonging to the Mollicutes class—a class of bacteria lacking a cell wall and surrounded by a deformable membrane. Mycoplasmas are contaminants found in biopharmaceutical, biologic, and mammalian cell culture processes that may infect eukaryotic cells, and, as such, they are less likely to contaminate small-molecule pharmaceutical processes and products. Mycoplasmas can be associated with microbiological growth media such as Soybean–Casein Digest Medium (SCDM). Mycoplasmas are easily inactivated by heat and radiation and are therefore not a concern where process streams are terminally sterilized. However, where sterilizing filtration is employed, the presence of mycoplasmas must be considered in designing filter validation studies. The small size (0.15–0.3 µm) and flexibility of the organism allows its passage through the 0.2-µm-rated microporous membrane filters commonly used to sterilize liquid pharmaceutical process streams.

INACTIVATION

Mycoplasmas are inactivated by sterilization processes such as autoclaving and irradiation.[1] Where mycoplasma contamination is a concern, raw materials and process streams can be subjected to lethal sterilization processes prior to use in biopharmaceutical product manufacture in order to minimize the presence of mycoplasmas prior to final sterilizing filtration, thereby reducing their population in the prefiltration bioburden and improving retention efficiency.

REMOVAL BY FILTRATION

The principles outlined in *Sterilizing Filtration of Liquids* ⟨1229.4⟩ are applicable to the removal of mycoplasmas from biopharmaceutical process streams. The small size and deformability of the organisms require the use of sterilizing filters with a pore-size rating finer than 0.2 µm, and mycoplasma-retentive filters having pore size ratings of 0.1 µm are available for this purpose.[2]

As with conventional sterilizing filtration, many factors influence the retention of mycoplasmas, including filter type and pore size rating; organism(s), concentration, and nutritive state; composition and viscosity of the filtered fluid; and filtration conditions, including differential pressure, contact time, and volume filtered. For example, nutritional conditions can affect the size of *Acholeplasma laidlawii*.[3] High differential pressure can force deformable mycoplasmas through microporous filter membranes.[4] These factors must be considered when designing and conducting validation studies for mycoplasma removal.

Parenteral Drug Association (PDA) Technical Report No. 75[5] is a consensus method that can be used as the basis for determining the retentivity of the chosen mycoplasma removal filter under controlled conditions comparable to those specified in American Society for Testing and Materials (ASTM) Standard F838 for bacterial retention.[6] Mycoplasma retentivity in the consensus method is determined using a challenge of *A. laidlawii* at a concentration of $\geq 1 \times 10^7$ cfu/cm^2. The chosen filter must then be validated for mycoplasma retention under actual conditions of use with *A. laidlawii* as the challenge organism in the process fluid or product, as appropriate. If complete retention is not achievable, a log reduction value (LRV) may be calculated and used with a safety factor based on prefiltration mycoplasma bioburden to ensure sufficient mycoplasma removal.▲ (USP 1-Aug-2020)

[1] Nikfarjam L, Farzaneh P. Prevention and detection of mycoplasma contamination in cell culture. *Cell J.* 2012;13(4):203–212.
[2] Priebe P, Bromm H. Filtration for protecting cell cultures: strategies for controlling mycoplasma infections. *BioProcess International.* 2005;October:72–74.
[3] Folmsbee M, Howard G, McAlister M. Nutritional effects of culture media on mycoplasma cell size and removal by filtration. *Biologicals.* 2010;38(2): 214–217.
[4] Roche K, Levy R. Methods used to validate microporous membranes for the removal of mycoplasma. *Bio Pharm.* 1992;5(3):22–33.
[5] Parenteral Drug Association. PDA Technical Report No. 75. Consensus Method for Rating 0.1 µm Mycoplasma Reduction Filters; 2016.
[6] American Society for Testing and Materials. Standard Test Method for Determining Bacterial Retention of Membrane Filters Utilized for Liquid Filtration; 2015.