Effect of process condition on the Mycoplasma retention of cell culture media filters

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Introduction

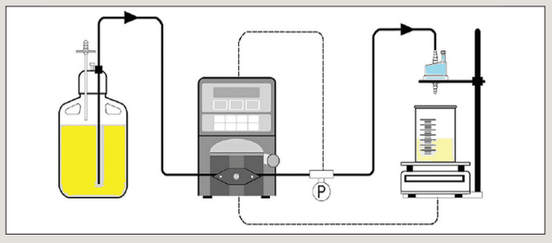
A Mycoplasma contamination event can have a major impact on a biopharmaceutical manufacturer. The loss of a cell culture due to a contamination incurs significant costs that can be attributed to both the initial bioreactor set-up and to the subsequent decontamination. Production facility throughput may be affected and in the worst cases the ability of the manufacturer to supply patients with medicines. Mycoplasma are extremely small in size and lack a cell wall giving the cells some flexibility that enables them to penetrate the 0.2 micron filters used to 'sterilize' cell culture media. The filtration of cell culture media through 0.1 micron filters alongside the effective screening of cell lines for infection and adherence to GMP principles of contamination control will minimize the risk of bioreactor contamination.

Typically the retention ability of filters to remove Mycoplasma is measured using the standard application outlined in PDA 26, replacing the standard test organism *Brevundimonas diminuta* with the Mycoplasma species *Acholeplasma laidlawii*. It is, however, important to understand how filters perform under typical process conditions in order to provide information that is valuable to the end user.

Methods for evaluating Mycoplasma retentiveness under process conditions

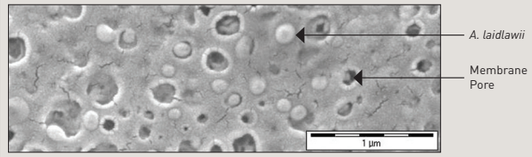
Two different types of typical cell culture media were used; Hyclone Dullbeccos Modified Eagle Media (DMEM) containing 10% Foetal bovine serum (FBS) and the Hyclone SFM4CHO™ media. The SFM4CHO™ media is chemically defined and free of animal proteins and serum. Each batch of media was sterile filtered before being inoculated with a known volume of high titre *Acholeplasma laidlawii (ATCC 23206)* to provide a minimum challenge level of >1 x 10^7 CFU/cm^2. The inoculated batches of media were sonicated prior to use to ensure mono-dispersed cultures. Filters were tested using 47mm discs run at a constant flow rate, with one litre of cell culture media containing a culture of *A. laidlawii*. The upstream pressure,flow rate, filtrate volume and time were recorded. During the process simulations the filters were operated at a range of pressures up to 1.9 bar (Figure 1).

Figure 1 - Normal flow filtration device - SciLog FilterTec™



Three samples of effluent were taken at low medium and high pressures throughout each run. Samples of filtrate were tested for the presence of *A. laidlawii* by culturing in a liquid detection media containing an indicator (Figure 3).

Figure 2 - SEM of membrane surface with *A. laidlawii* colonies



Quantification of Mycoplasma was performed by filtering the effluent of the Mycoplasma filter through a membrane filter and incubating the membrane filter on a selective Mycoplasma agar (Figure 3).

Figure 3 - Detection media for *A. laidlawii*

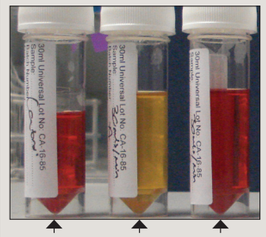
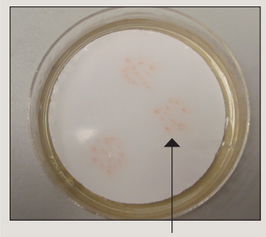


Figure 4 - Quantification of *A. laidlawii*



The total effluent for each run was also analyzed by membrane filtration. All samples for analysis were incubated at 37 °C (98.6 °F) for 10 days. If no *A. laidlawii* was identified on any of the samples the filter was deemed to be fully retentive. The Log Reduction Value (LRV) was calculated for each run based on the total volume processed.

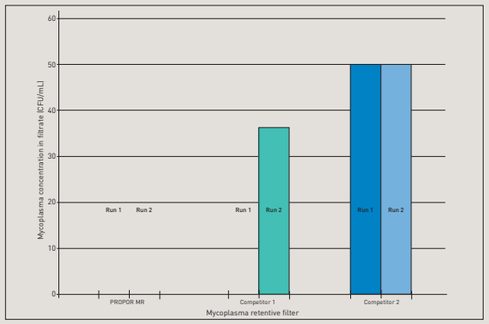
The LRV is calculated by:

$$\text{Log}^{10} \frac{\text{Number of bacteria in the total influent (CFU/cm}^2\text{)}}{\text{Number of bacteria in the total effluent (CFU/cm}^2\text{)}}$$

Comparison of Mycoplasma retention performance of three different filters under process conditions

Mycoplasma retentive filters from two leading competitors were compared with the Parker domnick hunter PROPOR MR filter currently in development. A culture of *A. laidlawii* grown in the Hyclone SFM4CHO™ media was filtered through the three Mycoplasma retentive filters at 1.9 bar. During the course of the experiment Mycoplasma was detected in the filtrate of both competitor filters (Figure 5). The loss of Mycoplasma retentiveness of these filters could be linked to the increase in pressure drop across the filter as the filters plug or it could be a function of the cumulative volume that has been filtered eventually leading to Mycoplasma breakthrough.

Figure 5 - Comparison of retention performance of three different Mycoplasma filters under process conditions.



The Mycoplasma retentiveness of PROPOR MR under process conditions with two different cell culture medias

Figure 6(a) shows the pressure and flow rate profiles for the filtration of the Hyclone DMEM containing fetal bovine serum using the Parker domnick hunter's PROPOR MR filter. Three samples of the filtrate were taken during the course of the filtration as indicated and analyzed for the presence of Mycoplasma. Figure 6(b) shows that no Mycoplasma was detected in the filtrate from the PROPOR MR at any stage of the filtration showing that the PROPOR MR gives a sterile filtrate under process conditions when challenged with a Mycoplasma-contaminated, serum-containing, cell culture medium.

Figure 6a - Pressure and flow rate profiles of Hyclone DMEM containing Fetal Bovine Serum with Mycoplasma challenge being filtered through a PROPOR MR showing when filtrate samples were taken and analyzed for the presence of Mycoplasma.

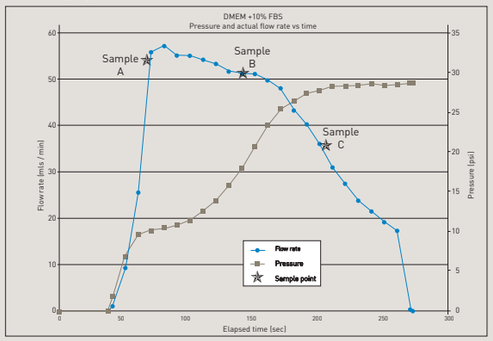


Figure 6b - Results of Mycoplasma testing on filtrate samples taken over the course of the filtration demonstrating the absence of Mycoplasma in the filtrate.

DMEM	Sample A (cfu)	Sample B (cfu)	Sample C (cfu)	Total recovery (cfu)	LRV
Run 1	0	0	0	0	>7
Run 2	0	0	0	0	>7
Run 3	0	0	0	0	>7

Figure 7(a) shows the pressure and flow rate profiles for the filtration of the Hyclone SFM4CHO™ media using the Parker domnick hunter's PROPOR MR filter. Figure 7(b) shows that no Mycoplasma could be detected in the filtrate.

Figure 7a - Pressure and flow rate profiles of Hyclone SFM4CHO™ media with Mycoplasma challenge being filtered through a PROPOR MR showing when filtrate samples were taken and analyzed for the pressure of Mycoplasma.

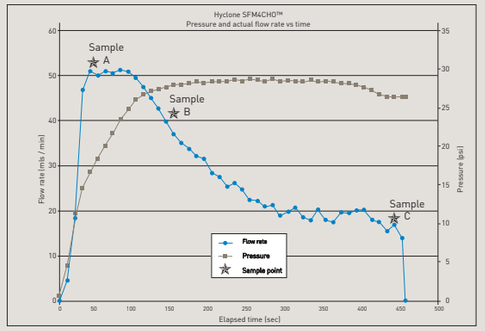


Figure 7b - Results of Mycoplasma testing on filtrate samples taken over the course of the filtration demonstrating the absence of Mycoplasma in the filtrate.

SFM4CHO	Sample A (cfu)	Sample B (cfu)	Sample C (cfu)	Total recovery (cfu)	LRV
Run 1	0	0	0	0	>7
Run 2	0	0	0	0	>7
Run 3	0	0	0	0	>7

Conclusion

- A Mycoplasma contamination event can have a major impact on a biopharmaceutical manufacturer.
- Biomanufacturers need to know that Mycoplasma retentive filters will retain Mycoplasma under process as well as standard conditions.
- It has been demonstrated that competitor Mycoplasma filters do not always retain Mycoplasma under process conditions.
- Experiments conducted with two commonly used cell culture medias spiked with Mycoplasma have shown that the PROPOR MR from Parker domnick hunter retains Mycoplasma under process conditions.

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