Millipore®
Preparation, Separation,
Filtration & Monitoring Products

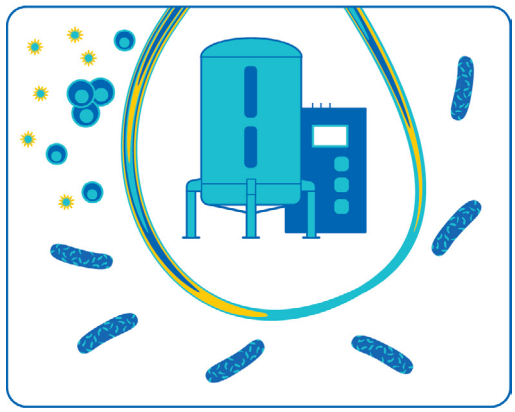
Technical Note

Mycoplasma clearance by Millipore Express® SHR filters at pilot and production scales

Summary

For biomanufacturers looking for a higher level of microbial risk reduction than sterilizing-grade filters, our Millipore Express® SHR filters are an attractive option. These filters contain a 0.1 µm polyethersulfone (PES) sterilizing membrane and are designed to efficiently remove mycoplasma contaminants from cell culture media.

Retention studies were performed with Millipore Express® SHR filters and *Acholeplasma laidlawii* to assess the filter's retention performance over extended filtration times. Results confirmed that both pilot and production-scale filters provide the expected level of mycoplasma retention under long duration, constant flow processing conditions.



Introduction

Our typical procedure for evaluating a filter's mycoplasma retention capabilities is based on the ASTM® F838 test for sterilizing-grade filters[1]. Similar to the ASTM® test protocol, our validated test method involves filter challenge studies at an *A. laidlawii* level of at least 10^7 CFU/cm^2 of effective filtration area (EFA) and a differential pressure of 30 psid. These conditions of high microorganism challenge under high pressure simulate 'worst-case' parameters and provide assurance that a filter that passes this challenge test will quantitatively retain large numbers of organisms [2].

While this testing regime is useful in providing quantitative assessment of sterilizing-grade filter performance, these conditions are not representative of typical bioprocessing conditions. The goal of this tech note is to summarize results of Millipore Express® SHR filter challenge studies with *A. laidlawi*i under long duration, constant flow operating conditions that are more representative of pilot and production-scale cell culture media processing.

Materials and Methods

Test System Configuration

Challenge testing was scaled up from throughput tests using 47 mm membrane disc filters (13.8 cm^2 EFA)[1].

Pilot-scale testing was performed with a Millipore Express® SHR with Prefilter, Opticap® XL 300 capsule filter (catalog number KHVEG003FF3) (290 cm^2 EFA). The standard challenge test was modified to accommodate the test stand setup, challenge and flush volumes[1], **Figure 1A**. Throughout the test, pressure was driven at 10 psid with a flow of 300 mL/min (~ 621 LMH) for eight hours.

To mimic a production-scale process test, 3 x 30 in. Millipore Express® SHR with Prefilter cartridges, (catalog number CHVE73TS3) with EFA of 44,100 cm^2, were challenged over five hours at a flow rate of 667 mL/min (9.1 LMH). A schematic of the test setup is shown in **Figure 1B.**

Mycoplasma Culture Medium

Oleic and palmitic acids were prepared in 100% ethanol to achieve a concentration of 10 mg/mL, and then sterile-filtered and stored.

Glucose Hydrolysate Broth (GHB): 4% w/v polypeptone, 0.5% w/v Trizma® base (2-amino-2-(hydroxymethyl)- 1,3-propanediol), 0.78% glucose, and 0.4% bovine serum albumin (BSA) in Milli-Q® water with 0.002% w/v of oleic and palmitic acid.

Glucose Mycoplasma Agar (GMA): 2% w/v Mycoplasma Broth Base, 0.5% w/v Trizma® base, 0.78% w/v glucose, 0.4% w/v BSA and ASTM® Type 1 water. The agar base was prepared by suspending 1.2% w/v agar in Milli-Q® water and sterilizing in an autoclave. The agar base was added to the GMA then oleic acid and palmitic acid were added to 0.002% w/v each. 2,3,4-triphenyltetrazolium chloride (TTC) was added for a final concentration of 0.05% (v/v).

Challenge diluent (mycoplasma phosphate buffer): 28 mM sodium phosphate monobasic, NaH_2PO_4 and 72 mM sodium phosphate di-basic, Na_2HPO_4, pH 7.1 (± 0.2).

Test Equipment

Test cartridge filters were installed into a filter housing and the system was steam sterilized. The Opticap® XL 300 capsule was attached to stainless steel fittings and the assembly was sterilized by autoclave at 121 °C[4]. Pressure was monitored throughout the tests.

For assay by membrane filtration, Durapore® 0.22 µm membrane filters were installed into Sterifil® aseptic systems and all associated equipment was sterilized by autoclave at a minimum of 121 °C.

Challenge Suspension

A frozen stock vial of *A. laidlawii* was thawed rapidly and transferred to GHB broth to achieve a final concentration of 4% (v/v) inoculum to broth. Cultures were incubated at 37 °C (± 2 °C), with 6% (± 1%) CO_2 for 22 (± 2) hours[4].

For production and pilot-scale tests, *A. laidlawii* from a 22 (± 2) hour culture in GHB was diluted into mycoplasma phosphate buffer to achieve a target of approximately 10^4 CFU/cm^2 EFA.

Sterility of the test systems was confirmed by the negative controls. These comprised sterile mycoplasma buffer that was flowed through the test systems, then evaluated for the presence of microorganisms using the membrane filtration method.

Challenge Tests

For the pilot-scale testing with the Opticap® XL 300 capsule, 2 L of challenge suspension was delivered at 300 mL/min (~ 621 LMH). After the dead-end challenge, 2 L of mycoplasma buffer in a separate sterile flask was recirculated through the capsule, under the same conditions, for a total test time of eight hours. The entire filtrate solution was then assayed by membrane filtration.

The production-scale tests were run under constant flow at 667 mL/min (9.1 LMH). Filtrate was collected in a 200 L Mobius® collection bag and 1 L in-process grab samples were collected hourly from a sample port installed before the collection bag and then assayed by membrane filtration. In these tests, the challenge solution was not recirculated.

Titer Determinations

Samples at high *A. laidlawii* concentration (culture and challenge suspensions) were serially diluted in mycoplasma buffer and enumerated using GMA agar plates following incubation at 37 °C (± 2 °C), with 6% (± 1%) CO_2 for four days[3].

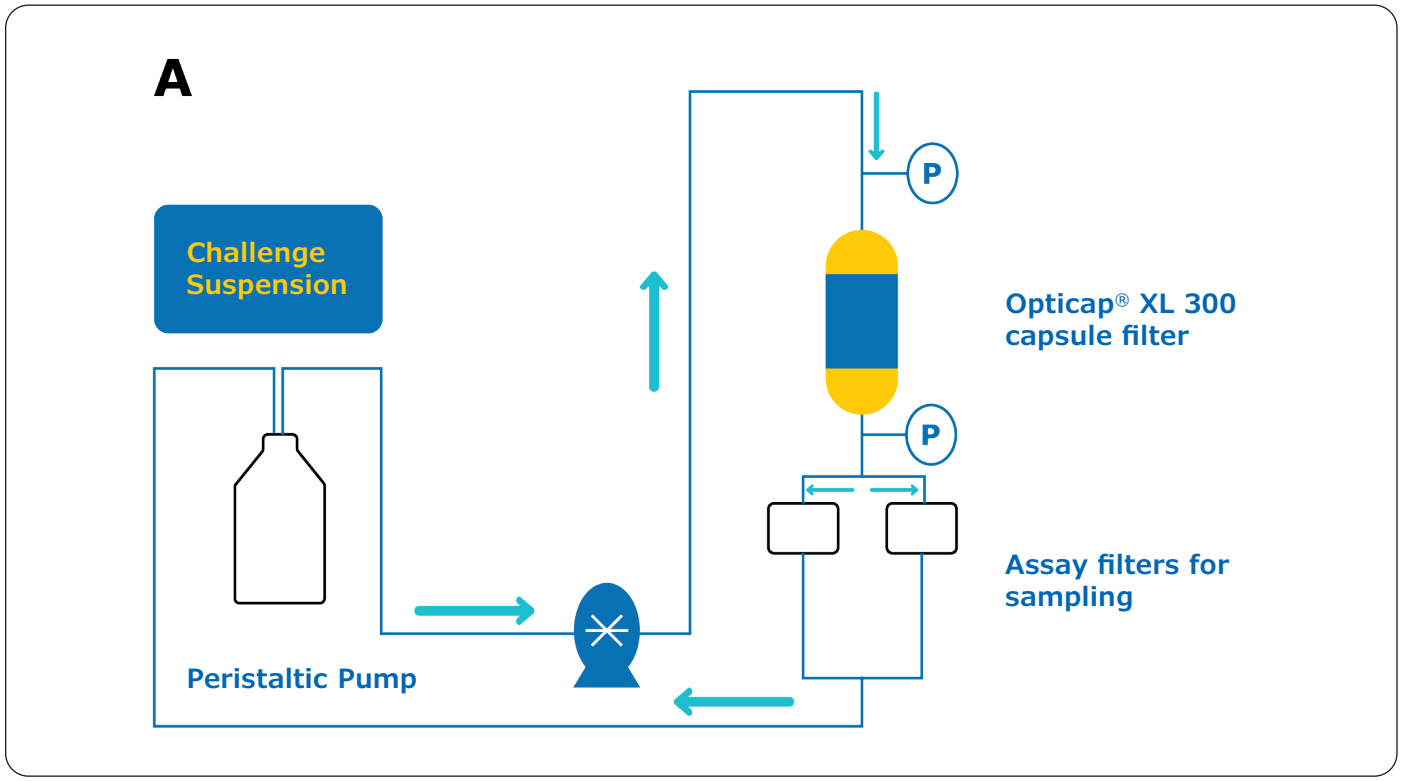
The presence of *A. laidlawii* in filtrate samples was determined using membrane filtration with Durapore® 0.22 µm assay filters which were transferred to GMA agar plates and incubated at 37 °C (± 2 °C), with 6% (± 1%) CO_2 for four days before enumeration.

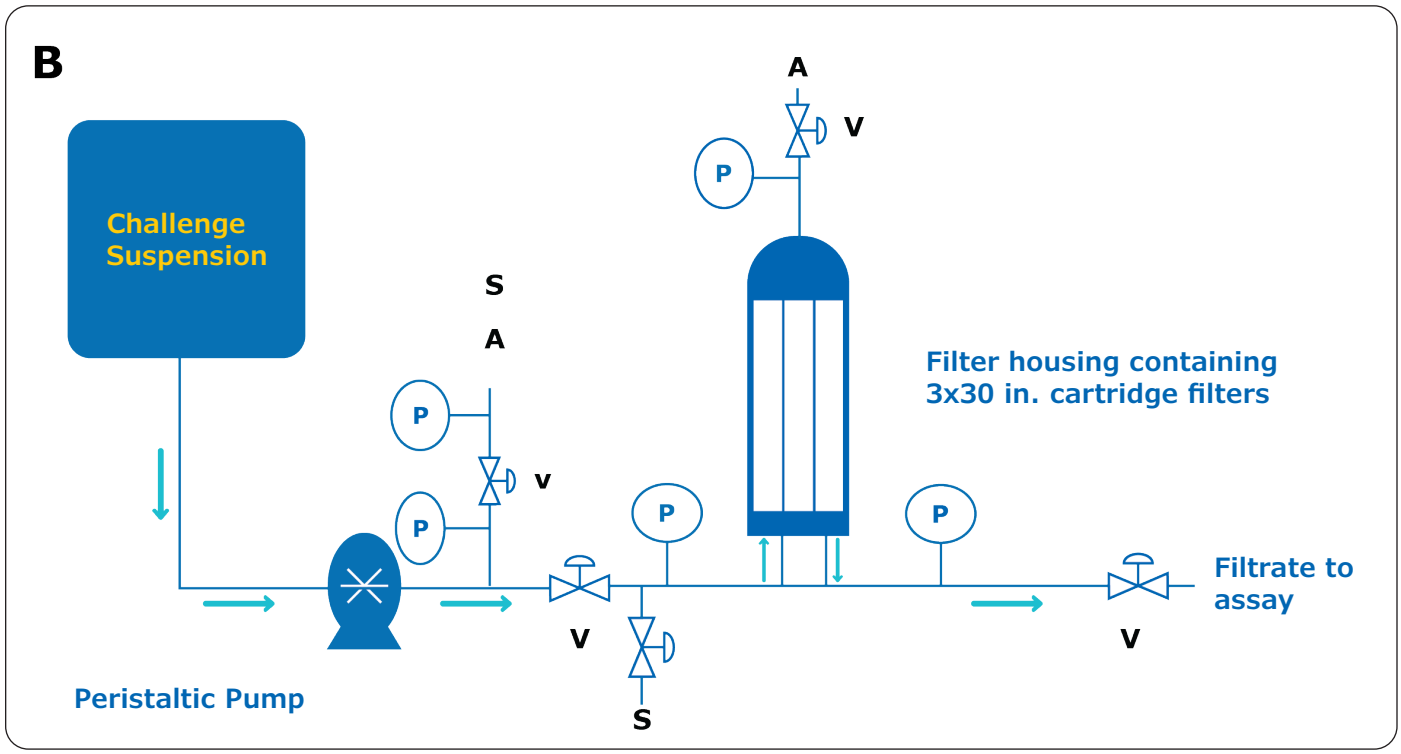
Titers of the challenge suspensions were used to calculate the bacterial challenge. The presence of organisms recovered by membrane filtration was used to determine microbial retention performance of Millipore Express® SHR test filters and, in the case of the negative control, the sterility of the system.

For the production-scale test, 1 L samples were collected hourly and organisms in the filtrate were recovered by membrane filtration. Retention by the test filter from these in-process grab samples was calculated as a ratio of organisms in the challenge and filtrate samples, assuming a 1 L volume for each.

Retention performance of the pilot and production-scale runs were calculated as the ratio of organisms in the entire challenge solution to those recovered from the filtrate. For the pilot run, the entire filtrate was collected and assayed. For the production-scale run, 1 L samples, but not the entire filtrate volume, were assayed. In these tests, the organism load in the filtrate was calculated from the filtrate volume (mL) and titer (cfu/mL) of the last sample collected.

Figure 1. Process flow diagram for the tests with filters containing Millipore Express® SHR with Prefilter membrane. P: pressure transponder, V: valve, S: steam, A: compressed air. Figure 1A, pilot-scale test with Opticap® XL 300 capsule filter. Figure 1B, process scale test with 3x30 inch cartridge filters.





Equation 1: Total challenge in CFU/filter

Total challenge = challenge concentration (CFU/mL) x challenge volume (mL/filter)

Equation 2: EFA (effective frontal area challenge in CFU/cm²)

$$\text{EFA challenge} = \frac{\text{total challenge (CFU/filter)}}{\text{filter surface area (cm}^2\text{/filter)}}$$

Equation 3: Total passage in CFU/filter*

Total passage = total CFU on the analytical membrane

Equation 4: Log Reduction Value

$$\text{LRV} = \log_{10}\left(\frac{\text{total challenge (CFU/filter)}}{\text{total passage (CFU/filter)}}\right)$$

Equation 5: Filtrate flux (LMH)

Flux = filtrate flow rate (mL/min)/membrane area (cm²) x 600

Equation 6: LRV of in-process grab samples (production-scale runs)

Challenge/ 1000 mL sample (CFU) = challenge concentration (CFU/mL) x challenge volume (1000 mL)

Total passage (CFU/1000 mL sample) = total CFU on the assay filter

LRV = challenge/1000 mL sample (CFU) / total passage (CFU/1000 mL sample)

*For the production-scale run, this was determined from sample titer (CFU/mL) and filtrate volume (mL)

Figure 2. Representative equations

Results and Discussion

Millipore Express® SHR filters are designed to reduce contamination risk of upstream cell culture processes. Under the conditions of our validated mycoplasma test, these filters are challenged with A. *laidlawii* to at least 10^7 CFU/cm² effective filtration area, under constant pressure of 30 psid. Filters that pass this test typically provide LRV ≥7.

However, cell culture media is generally processed under constant flow operations. This study was designed to evaluate retention performance of Millipore Express® SHR filters over an extended duration test under constant flow operating conditions that might be more representative for processing cell culture media in pilot and production-scale manufacturing. In these studies, pilot-scale tests included an Opticap® XL 300 capsule filter challenged for eight hours and the production-scale test was represented represented by 3 x 30 in. cartridge elements challenged for five hours.

To minimize the amount of the microbial challenge required for these extended duration tests, the *A. laidlawii* challenge level was reduced from the standard 10^7 CFU/cm² to 7.8 x 10^5 CFU/cm² and 4.8 x 10^4 CFU/cm² for the pilot and production-scale tests respectively. To minimize any potential impact of filter fouling and premature termination of the runs, all tests were run in the same buffer as the challenge suspension diluent.

Negative controls comprised buffer processed through the test systems, which was then evaluated for the presence of microorganisms. No microorganisms were recovered from the control tests, confirming system sterility and acceptable aseptic test conditions.

Figure 3 shows retention results from 1 L samples collected hourly during processing of *A. laidlawii* spiked buffer solution through 3 x 30 in. cartridge filters. The LRVs reflect the log ratio of organisms in the challenge and filtrate samples, assuming a 1 L volume for each. No *A. laidlawii* was detected downstream in any samples resulting in calculated LRVs of at least 7 logs through the duration of the test. These results confirm no change in retention in Millipore Express® SHR cartridge filters for up to 5 hours of processing under constant flow conditions.

Table 1 summarizes the test parameters and retention results of the pilot and production-scale testing with Opticap® XL 300 capsule and 3 x 30 in. cartridge filters respectively. Analysis of the entire filtrate from the pilot test confirmed no mycoplasma was detected downstream of the filter. For the production-scale test 1 L samples were collected throughout the test and assayed for the presence of mycoplasma. No organisms were detected in any of the samples collected. The titers of these in-process grab samples were used to calculate retention of the cartridge filters.

At the end of the tests, no mycoplasma was detected downstream of the Millipore Express® SHR capsule or cartridge filters confirming retention performance of the filters was maintained under long duration, constant flow processing conditions.

Conclusions

In summary, challenge testing using *A. laidlawii* demonstrated that Millipore Express® SHR filters provide high mycoplasma retention performance over extended durations with both pilot and production-scale filters.

This sustained performance confirms these filters can be used to mitigate the risk of mycoplasma contamination in upstream cell culture processes.

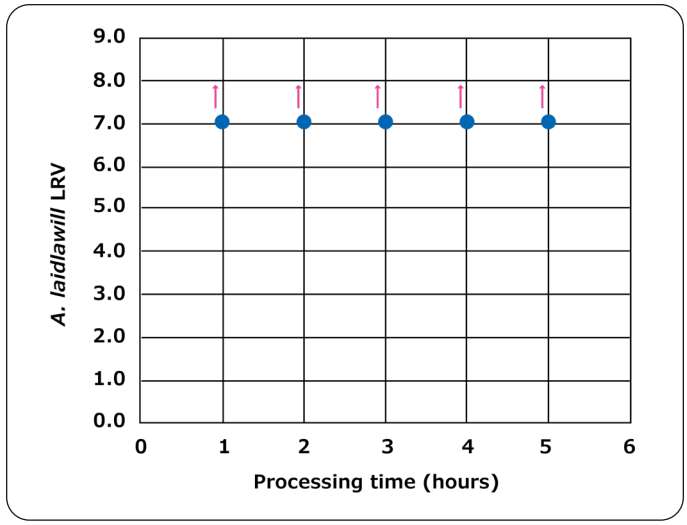


Figure 3. *A. laidlawii* retention results from samples collected during production-scale testing of Millipore Express® SHR cartridge filters. Arrows indicate filtrate samples were at assay limit with no microorganisms present in the filtrate samples.

Table 1: *A. laidlawii* retention of Millipore Express® SHR filters under constant flow for extended duration

Filter (Test)	Total EFA (cm^2)	Challenge Concentration (CFU/mL)	Challenge Volume (mL)	Total Challenge (CFU/filter)	Challenge Level (CFU/cm^2)	Total Passage (CFU)	LRV
Opticap® XL 300 (pilot-scale)	290	1.10E+05	2000	2.20E+08	7.59E+05	0	≥ 8.3
3 x 30 in. cartridge filters (production-scale)	44,100	1.10E+04	200,000	2.20E+09	4.99E+04	0	≥ 7.0

References

1. *Acholeplasma laidlawii* Retention Test of Flatstock Membrane (Internal Document).
2. American Society for Testing and Materials. Standard Method for Determining Bacterial Retention of Membrane Filters Utilized for Liquid Filtration. 2005 ASTM® Standards on Materials and Environmental Microbiology, Second Edition, Designation F838-05(2013).
3. "Culturing, Enumeration and Challenge Preparation of *Acholeplasma laidlawii*." (Internal Document).
4. "Getinge Autoclave Model 91415 Validated Load Profile & Operator." (Internal Document).

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