Technical Note

Demonstrating Millipore Express® SHR mycoplasma clearance capability at pilot and production scale over an extended filtration time

Introduction

Two separate *Acholeplasma laidlawii* retention studies were performed using sterilizing-grade Millipore Express® SHR with Prefilter (0.5/0.1 µm polyethersulfone) filters and *Acholeplasma laidlawii* ATCC® 23206™ to determine their mycoplasma retentive properties over an extended filtration time. In these experiments, the conditions of the test were modeled after typical processing parameters and were conducted using a low organism challenge level and typical operating pressures. The first experiment tested filters at production scale in a 3 x 30" cartridge configuration. The filters were fully retentive when challenged with 200 L of *A. laidlawii* at a level of 4.85 x 10^4 CFU/cm^2 over five hours. A second experiment tested the retention of *A. laidlawii* using Opticap® XL 300 pilot scale capsules. The Opticap® XL 300 capsules were fully retentive after an *A. laidlawii* challenge of 7.81 x 10^5 CFU/cm^2 for eight hours.

Background

Currently, no standard industry method exists for testing filters for mycoplasma retention; however, Merck Millipore has a validated procedure for mycoplasma testing that is modeled after the current ASTM® F838-05(2013) test for sterilizing-grade filters.

Similar to the ASTM® test protocol, Merck Millipore's validated test method involves using a high challenge level of *A. laidlawii* (≥ 10^7 CFU/cm^2 effective filtration area, EFA) and a high differential pressure (30 psid). These severe testing parameters are used to simulate worst case testing parameters and provide a high degree of assurance that a filter that passes this test will quantitatively retain large numbers of organisms[2].

While this testing regime is useful in providing a quantitative assessment of sterilizing-grade filter performance, these severe testing conditions are not typical of a pharmaceutical biomanufacturing process. As a result, the following two experiments were designed to examine filter performance at pilot and production scale under conditions which more closely models typical cell culture filtration operating parameters.

Procedure

Materials And Equipment

Production Scale Testing: 3 x 30" Configuration

The 3 x 30" filter extended challenge test was scaled from an established filtration process[1] using 47 mm membrane disc filters (13.8 cm^2 of total effective filtration area). For this test, three 30-inch Millipore Express® SHR with Prefilter cartridges (44,100 cm^2 of total effective filtration area) were challenged over five hours at a flow rate of 667 mL/min (flux = 9.1 LMH). Following the filter challenge, *A. laidlawii* culturing, recovery and enumeration, were performed.

Pilot Scale Testing: Opticap® XL 300 Configuration

The Opticap® XL 300 (290 cm^2 effective filtration area) small scale capsule (SSC) extended challenge test[1] was adapted with a few modifications including: test stand setup, challenge and flush volumes. *A. laidlawii* culturing, recovery and enumeration was also performed[3]. For the duration of the test, pressure was driven at 10 psid with a flow of 300 mL/min (flux = 620.7 LMH) for an extended challenge time of eight hours.

Membrane	Millipore Express® SHR with Prefilter 0.5/0.1 µm	
Test filter	3 x 30" code 7 cartridges	Opticap® XL 300 capsule
EFA (cm^2)	44,100	290
Equipment	• Mobius® MIX200 disposable mixing assembly • 3 x 30" multi-round code 7 cartridge housing • Sterifil® aseptic system • Aervent®-50 vent filter • Assay filter: Durapore® 0.22 µm PVDF 47 mm analytical membrane discs • Gauges: sanitized and calibrated • Pump: peristaltic • Tubing: silicone	• Pressure pots • Sterifil® aseptic system • Aervent®-50 vent filter • Assay filter: Durapore® 0.22 µm PVDF 47 mm analytical membrane discs • Gauges: sanitized and calibrated • Pump: peristaltic • Tubing: silicone

Fatty Acid Supplements

Oleic and palmitic acids were prepared separately in 100% ethanol to achieve a concentration of 10 mg/mL each. Each fatty acid was sterile filtered and stored separately.

Glucose Hydrolysate Broth (GHB)

Sterile GHB contains: 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. Oleic acid and palmitic acid were aseptically added to deliver a final concentration of 0.002% w/v each.

Glucose Mycoplasma Agar (GMA)

Sterile GMA contains: 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 resuspending 1.2 % w/v agar in Milli-Q® water and sterilizing in an autoclave with a validated slow exhaust cycle at a minimum of 121.1 °C. The agar base was tempered and aseptically added to the sterile GMA to arrive at a dispensing temperature of 43 to 45 °C. Oleic acid and palmitic acid were aseptically added to deliver a final concentration of 0.002% w/v each. Also, 2,3,4-triphenyltetrazolium chloride (TTC) was added aseptically for a final concentration of 0.05% v/v.

Diluent and Resuspension Buffer

Mycoplasma phosphate buffer: sodium phosphate monobasic, NaH_2PO_4, 28 mM and sodium phosphate dibasic, Na_2HPO_4 , 72 mM were dissolved in Type 1 water and adjusted to pH 7.1 (± 0.2). The buffer was sterilized by autoclaving in a validated slow exhaust cycle at a minimum of 121 °C.

Procedure

Test Equipment Preparation

Production Scale Testing: 3 x 30" Configuration

The test cartridges (three 30" cartridges) were installed into a multi-round filter housing and the cartridges and test system were steam sterilized. After steaming, the test system was cooled using filter sterilized compressed air.

Analytical membrane filters (Durapore® 0.22 µm) were installed into Sterifil® aseptic systems. The Sterifil® aseptic systems, challenge test manifolds, and other associated equipment were wrapped and sterilized in a validated autoclave at a minimum of 121 °C.

Pilot Scale Testing: Opticap® XL 300 Configuration

The Opticap® XL 300 capsule was attached to stainless steel fittings and the entire assembly was sterilized via autoclave, using a validated liquid cycle at 121 °C for 20 minutes[4].

Analytical membrane filters (Durapore® 0.22 µm) were installed into Sterifil® aseptic systems. The Sterifil® aseptic systems, challenge test manifolds, and other associated equipment were wrapped and sterilized in a validated autoclave cycle at a minimum of 121 °C.

Culture Preparation

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.

Challenge Suspension Preparation

3 x 30" Configuration

A. laidlawii from a 22 (± 2) hour culture in GHB was diluted into 200 L of mycoplasma phosphate buffer to deliver a final target concentration of 10^4 CFU/cm^2 of membrane effective frontal surface area. The cartridges provided an effective frontal area (EFA) of 44,100 cm^2.

Opticap® XL 300

A. laidlawii from a 22 (± 2) hour culture in GHB was diluted into 2 L of mycoplasma phosphate buffer to deliver a final target concentration of 10^4 CFU/cm^2 of membrane effective frontal surface area. The Opticap® XL 300 capsule provided an EFA of 290 cm^2.

Sterility Test

The test system sterility was assessed by flowing sterile mycoplasma phosphate buffer through the test system. For the 3 x 30" configuration, a 1 L filtrate sample was taken. For the Opticap® XL 300 capsule, a 2 L sample was taken.

Bacterial Challenge Test

The 3 x 30" cartridge retention test was a dead-end configuration. The *A. laidlawii* challenge suspension was delivered to the challenge filters by a peristaltic pump with a constant flow of 667 mL/min (flux = 9.1 LMH) and the filtrate was collected in a 200 L Mobius® collection bag. Once every hour, a 1 L sample was taken from a sample port installed before the filtrate collection bag, starting after one hour of test time, and ending after five hours for a total of five samples.

For the Opticap® XL 300 capsule experiment the 2 L challenge was delivered via peristaltic pump and the entire challenge was collected downstream and assayed by membrane filtration. After the dead-end challenge portion, the Opticap® XL 300 capsule experiment was conducted in a recirculation configuration, in which 2000 mL of mycoplasma buffer in a separate sterile flask was recirculated through the Opticap® XL 300 Capsule at a rate of 300 mL/min (flux = 620.7 LMH) for eight hours. After eight hours, the 2 L filtrate was then assayed by membrane filtration.

Challenge Titer

3 x 30" Cartridge Configuration

Samples of the *A. laidlawii* culture, initial *A. laidlawii* challenge (titer taken from the Mobius® mixing system), and intermittent samples (taken upstream of the test filters), were serially diluted in mycoplasma buffer and enumerated using the pour-plate method with GMA agar[3]. Plates were incubated at 37 °C (± 2 °C), with 6% (± 1%) CO_2 for four days. Post-incubation, the pour-plates were enumerated and the titer was determined.

Opticap® XL 300

A sample from the challenge flask was taken immediately prior to testing and assayed by the pour plate method with GMA agar[3]. Plates were incubated at 37 °C (± 2 °C), with 6% (± 1%) CO_2 for four days. Post-incubation the pour-plates were enumerated and the titer was determined.

Legend	
V-1 through V-11	Valve
Luer	Female Luer-Lok™ fitting
FH-1	Filter Housing, 316SS, 3x30" for test membrane filters
P-1 through P-5	Pressure Gauge or Transmitter
FC-1 and FC-2	Millipak®200 Filter, 0.22 µm membrane filter
T-1 through T-3	Sample Ports

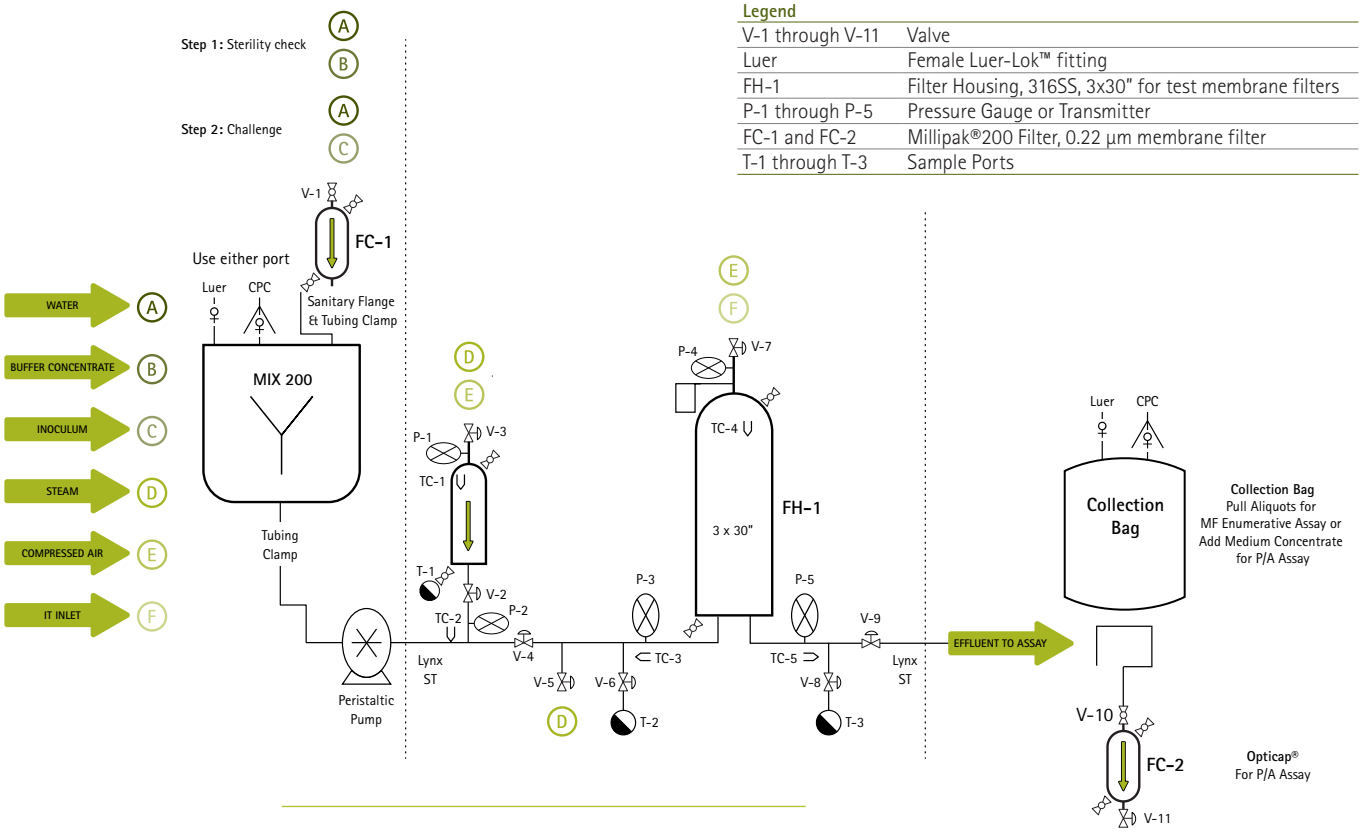

Figure 1. Process flow diagram for the 3 x 30 inch challenge.

Recovery Assay

The Membrane Filtration (MF) method was employed for enumeration of colonies in the filtrate. For both experiments, the effluent was filtered through a Sterifil® system containing a Durapore® 0.22 µm analytical membrane. Each analytical membrane was then aseptically transferred to a separate GMA agar plate and incubated at 37 °C (± 2 °C), with 6% (± 1%) CO_2 for four days, to test mycoplasma retention.

Calculations

The pour-plates were used to calculate the bacterial challenge concentration in CFU/filter and the EFA challenge in CFU/cm^2. The MF-Millipore™ filter plates were used to determine system sterility and test filter passage.

Equation 1 Total challenge in CFU/filter

$$\text{Total challenge} = \text{challenge concentration (CFU/mL)} \times \text{challenge volume (mL/filter)}$$

Equation 2 EFA (effective frontal area) challenge in CFU/cm^2

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

Equation 3 Total passage in CFU/filter

$$\text{Total passage} = \text{total CFU on the analytical membrane}$$

Equation 4 Log Reduction Value

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

If the total passage for a sample is zero (absence of growth), the raw data is recorded as 0 CFU. However, to calculate LRV a "1" is placed in the denominator to represent the Total Passage and the LRV is reported with a "greater than" symbol.

Equation 5 Filtrate flux (LMH)

$$\text{Flux} = \text{filtrate flow rate (mL/min)/membrane area (cm}^2\text{)} \times 600$$

Figure 2. Representative equations

Acceptance Criteria

System Sterility: Absence of microbial growth.

Results

For all experiments, the system sterility met the acceptance criteria demonstrating the absence of microbial growth and indicating that all equipment was properly sterilized. The average delivered challenge level was 4.85 x 10^4 CFU/cm^2 for the 3 x 30" configuration and 7.81 x 10^5 CFU/cm^2 for the Opticap® XL 300 capsules. There was zero passage of challenge organism detected downstream of the 3 x 30" cartridge filters (LRV ≥ 4.0). The limit of detection for the 3 x 30" configuration experiment was 40 CFU. There was zero passage of challenge organism detected downstream of the Opticap® XL 300 capsules (LRV ≥ 8.35); the limit of detection for these experiments was 1 CFU.

Conclusion

Challenge testing performed at pilot and production scale using *A. laidlawii* demonstrated that Millipore Express® SHR with Prefilter cartridge filters in a 3 x 30" configuration were fully retentive when challenged with 200 liters of *A. laidlawii* at a concentration of 4.85 x 10^4 CFU/cm^2 over five hours. The Opticap® XL 300 small scale capsules were also fully retentive when challenged at an average *A. laidlawii* level of 7.81 x 10^5 CFU/cm^2 over eight hours. These pilot and production scale filtration tests demonstrate that Millipore Express® SHR filter mycoplasma retention capability at typical cell culture filtration operating parameters and extended processing times. Under these conditions, Millipore Express® SHR filters are retentive for relative concentrations and volumes.

References

1. *Acholeplasma laidlawii* Retention Test of Flatstock Membrane (Merck Millipore Document #0003007TM)
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. Merck Millipore document #00081823SO "Culturing, Enumeration and Challenge Preparation of *Acholeplasma laidlawii*."
4. Merck Millipore document #065473SO ("Getinge Autoclave Model 91415 Validated Load Profile & Operator").

To Place an Order or Receive Technical Assistance

In Europe, please call Customer Service:

France: 0825 045 645
Germany: 01805 045 645
Italy: 848 845 645
Spain: 901 516 645 Option 1
Switzerland: 0848 645 645
United Kingdom: 0870 900 4645

For other countries across Europe, please call: +44 (0) 115 943 0840

Or visit: **www.merckmillipore.com/offices**

For Technical Service visit: **www.merckmillipore.com/techservice**

MERCK MILLIPORE

www.merckmillipore.com/offices

Lit No. TN1209EN00 Rev. B PS-14-09843 3/2014 Printed in the USA.