A. laidlawii Filtration Through a 0.1 µm Rated Filter at Elevated Pressures

Martha Folmsbee, Ph.D. Scientific and Laboratory Services, Pall Corporation, Port Washington, NY.

ABSTRACT

Nine 47 mm 0.1 µm rated filter discs (Pall Fluorodyne® EX Grade EDT) were each challenged with ≥ 10⁸ CFU/cm² of Acholeplasma laidlawii (ATCC 23206) in 1 L of Mycoplasma Broth Base at 30 and 45 psi. An additional set of nine filters were again challenged at 45 psi with a challenge > 10⁸ CFU/cm², allowing for the demonstration of a high titer reduction even at elevated pressure. No penetration was observed at ≥ 10⁸ CFU/cm²/30 psi and only 1 cell penetrated 3 out of 9 filters tested at ≥ 10⁸ CFU/cm²/45 psi. At the excessive > 10⁸ CFU/cm² challenge level, only 2 to 7 cells penetrated 4 out of 9 filters. This resulted in a minimum titer reduction of 10⁸ (log reduction value, LRV > 8) in all cases. These results along with Fluorodyne EX Grade EDT filters higher filtration capacity and good scale-up provide for an improvement in process economics for filtering a cell culture media batch.

INTRODUCTION

Due to their unique characteristics, mycoplasmas are particularly adept at penetrating 0.2 µm rated sterilizing grade filters. As a result, protection from mycoplasma contamination requires the use of 0.1 µm rated filters. We previously reported on the effect of culture media on the generation of cells with an enhanced ability to penetrate 0.2 µm rated filters. We used these cells to challenge 0.1 µm rated filters at elevated pressures (30 and 45 psi).

Mycoplasma

- Bacteria that have no cell wall.
- Cells that are pleomorphic (assume various shapes from cocci to rods to filaments)
- Cells vary in size from <0.2 to >0.5 µm
- Consistently capable of penetrating 0.2 µm rated filters, but are retained by 0.1 µm rated filters.
- The extent of the retention by 0.1 rated filters varies with different filters and depends on the challenge conditions.

Why are Mycoplasma a challenge for filtration?

- Very small procaryotes (bacteria)
- Compare to Brevundimonas diminuta at 0.3 – 0.4 µm x 0.6 – 1.0 µm
- Potentially flexible due to absence of rigid cell wall

Figure 1

Acholeplasma laidlawii (mycoplasma)

METHODS AND MATERIALS

Test Conditions

- Test organism: Acholeplasma laidlawii ATCC 23206 (A. laidlawii)
- Culture media: Mycoplasma broth with 10% horse serum, incubated at 37 °C for 72 hours
- Minimum challenge level: ≥ 10⁸ CFU/cm²
- Challenge volume: 1 L
- Test Filters: 47 mm discs (13.8 cm² EFA) Fluorodyne EX (P/N FTK ED T)
- Test set up: Pressure vessel at 30 and 45 psi connected to sterile, stainless steel, 47 mm disc holders
- All test filters were bubble point tested (T) in 60/40 IPA/ DI water before and after bacterial challenge testing
- All filters passed pre and post T.

Test Culture Evaluation

- Measurement of penetrative ability of the test mycoplasma culture = degree to which the test culture penetrates a 0.2 µm rated filter.
- Test organism is cultivated in test broth
- The titer reduction through a 0.2 µm rated filter is measured
- Relative penetration = T/Titer Reduction (T).

Equation 1

Definition of Titer Reduction

TR = Upstream Titer x Volume = Total Influent
Downstream Titer x Volume = Total Effluent

Figure 3

Relative Penetrative Ability of the Culture Through a 0.2 µm Rated Filters used for Testing of 0.1 µm Rated Filters at Elevated Pressures

Table 1

<table>
<thead>
<tr>
<th>Filter Rating</th>
<th>Lot Number</th>
<th>Average Flow Rate (mL/min)</th>
<th>Challenge Level/cm² (CFU)</th>
<th>Total Recovery (CFU)</th>
<th>Titer Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>FTK EX (1)</td>
<td>00788-030</td>
<td>114.3</td>
<td>3.2E+09</td>
<td>0</td>
<td>7.1E-08</td>
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RESULTS

Figure 1

- Nine filters were again challenged at 45 psi with a challenge > 10⁸ CFU/cm², allowing for the demonstration of a high titer reduction even at elevated pressure. No penetration was observed at ≥ 10⁸ CFU/cm²/30 psi and only 1 cell penetrated 3 out of 9 filters tested at ≥ 10⁸ CFU/cm²/45 psi. At the excessive > 10⁸ CFU/cm² challenge level, only 2 to 7 cells penetrated 4 out of 9 filters. This resulted in a minimum titer reduction of 10⁸ (log reduction value, LRV > 8) in all cases. These results along with Fluorodyne EX Grade EDT filters higher filtration capacity and good scale-up provide for an improvement in process economics for filtering a cell culture media batch.

CONCLUSIONS

- A. laidlawii mycoplasma challenges of 47 mm discs of 0.1 µm rated Fluorodyne EX Grade EDT membrane at 30 and 45 psi resulted in titer reductions of > 10⁸ (log reduction value, LRV > 8)
- Mycoplasma with an enhanced penetrative ability can be effectively retained by 0.1 µm rated Fluorodyne EX Grade EDT filters at elevated pressures.

REFERENCES

3. Evaluating the performance of 0.1 µm rated filters for mammalian cell culture media filtration, BioPharm International, 20, April 2009