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Seeking a Better Understanding of Bacterial Penetration of Sterilizing-Grade Filters by Complex Fluids

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INTRODUCTION AND PURPOSE

- *"A sterilizing-grade filter should be validated to reproducibly remove viable micro-organisms from the process stream, producing a sterile effluent."*[1]
- In the pursuit of improved drug efficacy and patient safety, use of nanoparticulate, liposome and emulsion-based drug delivery systems (complex fluids) are becoming increasingly common.
- In delivering drugs with these complex carriers, patient safety and pharmaceutical efficacy are maximized, however, sterile filtration of these complex fluids can be difficult and sometimes lead to initial filter validation failure.
- Our goal is to better understand what leads to filter validation failure when filtering these complex fluids so that successful sterilizing filtration can be achieved.

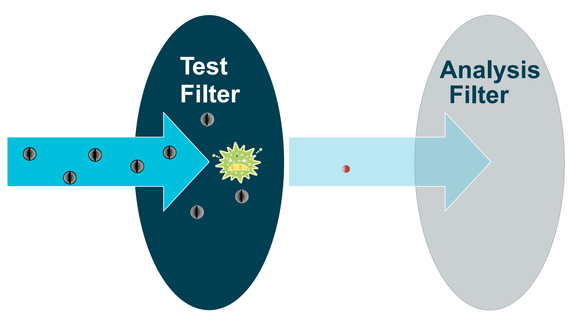
MATERIALS AND METHODS

- Test organism: *Brevundimonas diminuta*
- Minimum challenge level: 1 x 10^7 CFU/cm^2
- Test volume: 200 mL (various fluids)
- Test filter: 47 mm disc (a non-fully retentive filter to enhance the penetrative signal and allow smaller sample sizes)
- Test pressure: 2.1 bard (30 psid) (unless otherwise indicated)

BACKGROUND

- The definition of a sterilizing-grade filter is based on performance. Thus, it is a regulatory requirement to test under the conditions of actual use.
- **Definition: The filter completely retains 1 x 10^7 CFU/cm^2 of effective filter area of *Brevundimonas diminuta***
- Validation test conditions during process specific filter validation:
  - Test bacteria – industry standard is *Brevundimonas diminuta*
  - Test fluid (customer's fluid or surrogate)
  - Pressure (customer's process pressure)
  - Flow rate (customer's process flow rate)
  - Temperature (at the customer's temperature)
- **Bacteria that pass the test filter will be collected on the analysis filter (all the effluent is analyzed)**

Figure 1
Illustration of a validation test

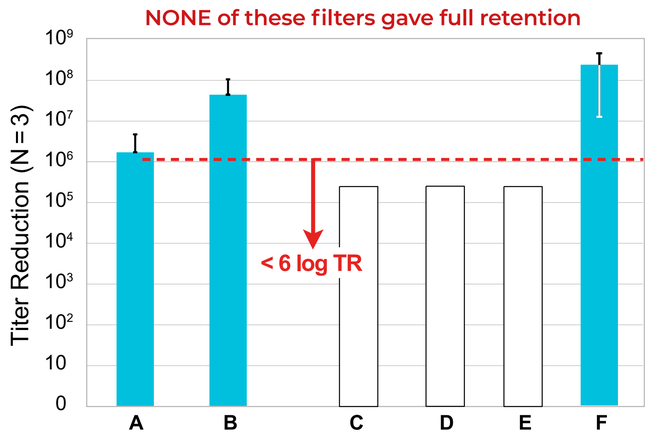


Important to note: To validate a sterilizing-grade filter in a manufacturing process (final sterile fill), we must show complete retention. The presence of even one CFU in the effluent will cause the validation to fail.

Industry-Wide Challenge

Bacterial penetration of sterilizing-grade filters by complex fluid is an industry-wide challenge (example shown here using a cholesterol-based liposome)

Figure 2
The titer reduction provided by commercial sterilizing-grade filters from 5 different manufacturers using a liposomal test fluid

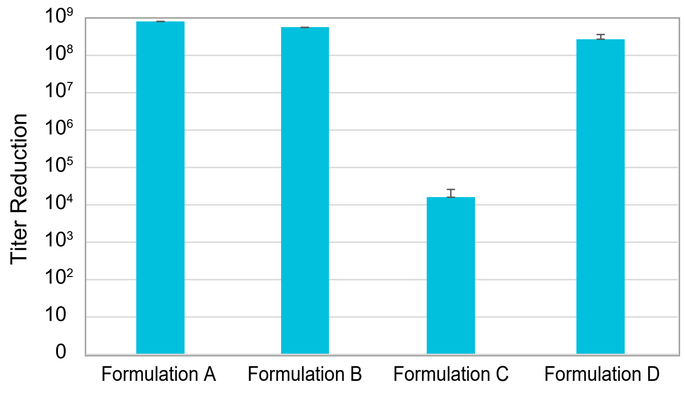


RESULTS

Formulations

Changes to the composition of the complex fluid can lead to changes in bacterial penetration risk. All but formulation B have a surface tension lower than water and the same concentration of the same lipid. Formulation B has no lipid.

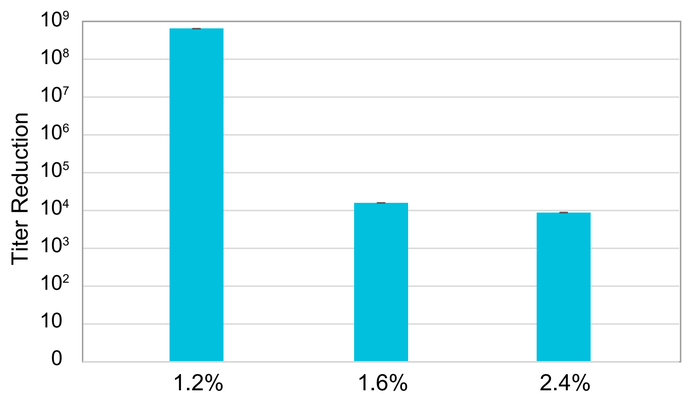
Figure 3
The titer reduction achieved in bacterial challenges using various formulations of a complex fluid



Concentration of Individual Components

Even slight changes to the concentration of a non-lipid component can lead to changes in bacterial penetration risk. All contain the same concentration of the same lipid.

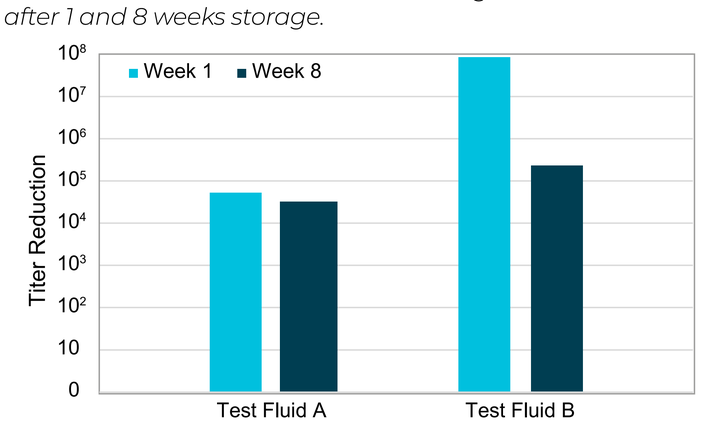
Figure 4
The titer reduction achieved in bacterial challenges using various formulations of a complex fluid (percentage of a non-lipid component)



Storage

Extended storage can change the bacterial penetration risk of some complex fluids. The two test fluids contain different lipids but at the same concentration.

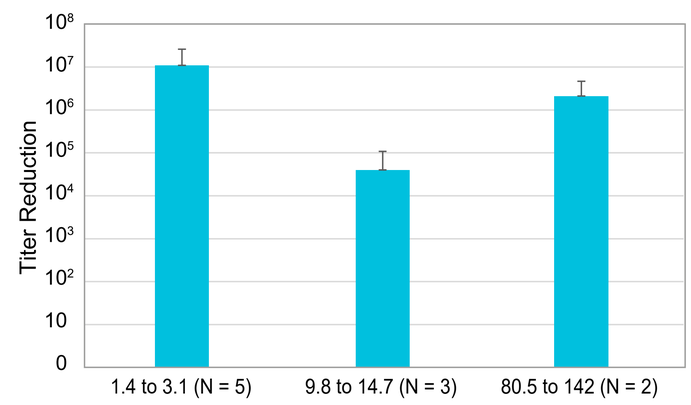
Figure 5
The titer reduction achieved when testing two different formulations after 1 and 8 weeks storage.



Flow Rate and Pressure

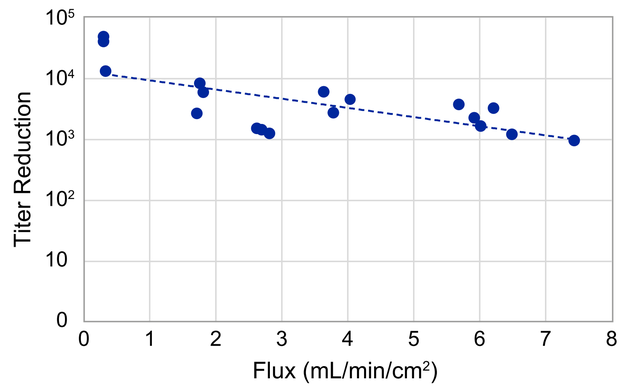
There may be an optimal flow rate for a given complex fluid that will reduce the risk of bacterial penetration and filter validation failure. This should be determined prior to filter validation. All flow rate tests for Figure 5 were performed with a single formulation.

Figure 6
The titer reductions achieved with a complex formulation when tested at various flow rates (mL/min)



With some complex fluids, decreasing flux may lead to a better validation outcome.

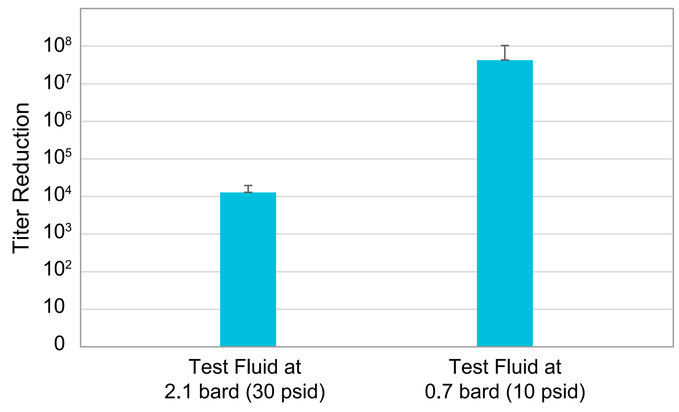
Figure 7
The plot of titer reduction vs. flux for various formulations



- Broader data analysis seemed to suggest a "sweet spot" with high risk for penetration at low flow rates
- Validation specialists have found that decreasing flow rate (thus flux) can help improve validation testing outcome
- Low flux may improve the outcome

Filtration at a lower differential pressure reduces the risk of bacterial penetration when filtering some complex fluids. Both are the same test fluid.

Figure 8
The titer reduction achieved when testing the same fluid at two different differential pressures



CONCLUSIONS

- **Sterile filter validation is an industry-wide challenge when filtering complex fluids.**
- **Although high lipid concentration and low surface tension is a potential risk-factor, it is not the whole story and some of those fluids will not pose a challenge to sterile filtration.**
- **Some specific components are much more likely to contribute to filter validation failure than others.**
- **Some slight changes in concentration can have a large impact on bacterial retention risk.**
- **Extended storage of some of these complex fluids can lead to increases in bacterial penetration risk.**
- **Optimization of a complex formulation for bacterial retention when working with complex fluids may improve outcomes and lower risk.**
- **Optimization of process parameters for bacterial retention when working with complex fluids may improve outcomes and lower risk.**

References

1. *FDA Guidance for Industry, Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice" (PDF). U.S. Department of Health and Human Services. 2004.*
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