BIOTECH

Technical Regulatory Topic

USTR 3658

Selection of a Surrogate Solution for Bactericidal Process Fluids

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Author: Morven McAlister

Contents

1 Objective

The purpose of this paper is to outline the approach for performing bacterial retention testing on bactericidal fluids, and to provide the scientific rationale for selection of a surrogate solution when the process fluid is determined to be bactericidal.

2 Summary

Although bacterial penetration through sterilizing grade filters is not a frequent occurrence, the probability of a failed bacterial retention test is increased if the process fluid has a surface tension lower than water, but more specifically, if the lower surface tension is due to the presence of liposomes, lipids, or surfactants [1, 2, 3].

If a process fluid is bactericidal, and direct inoculation of *Brevundimonas diminuta (B. diminuta)* American Type Culture Collection (ATCC®) 19146 (or other relevant bioburden) is not possible, Pall's approach for selection of a carrier fluid for the bacterial challenge is either:

- To use a placebo with the bactericidal agent removed (e.g., placebo contains no active product ingredient or pH is neutralized), which must be provided by the customer, or
- To select a surrogate solution based on an approximately equivalent surface tension value to the process fluid.

For bactericidal fluids, the challenge duration is typically 120 minutes (if viability data supports this duration), which constitutes a worst-case test based on analysis of bacterial penetration by 'high risk' fluids.

3 Background

For sterile fill applications, it is a regulatory requirement to perform filter validation under process specific conditions [4, 5, 6, 7]. Performing a bacterial retention test is critical and is required to show that the process fluid and/or processing parameters will not impact the ability of the selected sterilizing grade filter to fully retain either a standard test bacterium (*B. diminuta* ATCC 19146) or other relevant bioburden indigenous to the manufacturing process.

A viability test is performed first, to assess the ability of *B. diminuta* to be inoculated directly into the process fluid. This test involves inoculating *B. diminuta* directly into the process fluid and removing samples to determine the titers at various time-points (typically 1, 60, 120 minutes, and the full process time). The titers are compared to those from a control fluid (e.g., phosphate buffered saline, water, or equivalent). From this data, the process fluid can be defined as bactericidal, moderately bactericidal, or non-bactericidal in accordance with the definitions below:

- **Non-bactericidal:** A decline in viability of less than one log over the process time,
- **Moderately bactericidal:** A decline in viability of less than 1 log over 60 minutes, in conjunction with a decline in viability of 1 log or more over the process time,
- **Bactericidal:** A decline in viability of greater than 1 log over 60 minutes.

If the product is non-bactericidal, then the fluid can be used as the carrier vehicle for challenging the filters with *B. diminuta*, with no loss in viability.

If a process fluid is determined to be moderately bactericidal, then the process fluid is recirculated through the test and control filters under the worst-case test parameters (i.e., temperature, duration, volume, and flow rate or system / differential pressure) to pre-condition the filters. A short duration (preferably 120 minutes but may be reduced to 60 minutes dependent on the extent of loss in viability of *B. diminuta* in the process fluid) bacterial challenge is then performed on the test and control filters.

Wherever possible, Pall's preferred approach is to inoculate *B. diminuta* ATCC 19146 into the process fluid to perform the bacterial retention test, as recommended by regulatory and industrial guidance [4, 5, 6, 7]. However, for process fluids that are shown to be bactericidal, clearly, this approach is not possible. Under these circumstances, Pall recommends pre-conditioning the test filters with the process fluid under worst-case process conditions (as defined above), flushing the bactericidal fluid from the test filters, then performing the bacterial retention test in a surrogate fluid or a placebo (e.g., process fluid with the active product ingredient removed or the pH adjusted, if provided by the customer).

4 Technical Rationale

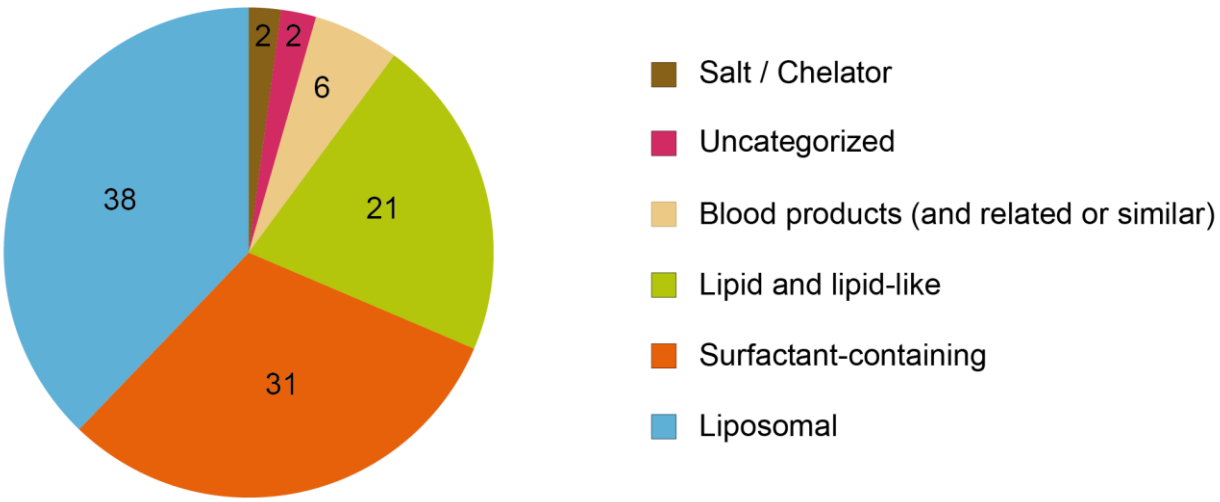
4.1 Selection of a Surrogate Solution

It is important to note that the purpose of performing a process-specific bacterial retention test is to demonstrate that the ability of the sterilizing grade filter to retain bacteria is not changed under worst-case process conditions. While Parenteral Drug Association (PDA) Technical Report 26 clearly states that the processing parameters should be simulated during the bacterial retention testing, it also indicates that the properties of the process fluid may impact the ability of the filter to retain bacteria ("...what effect does the product have on the filter and what effect does the product have on flora within the product?") [4].

Although bacterial penetration through 0.2 µm rated, sterilizing grade filters is not a common occurrence, a substantial body of evidence collected by Pall indicates that low surface tension fluids (< 68 mN/m), specifically those containing surfactant solutions, lipids and lipid-like solutions, and liposome solutions pose the greatest risk of allowing bacterial penetration through 0.2 µm rated filters [1, 2, 3]. This observation is summarized in Figure 1.

Figure 1

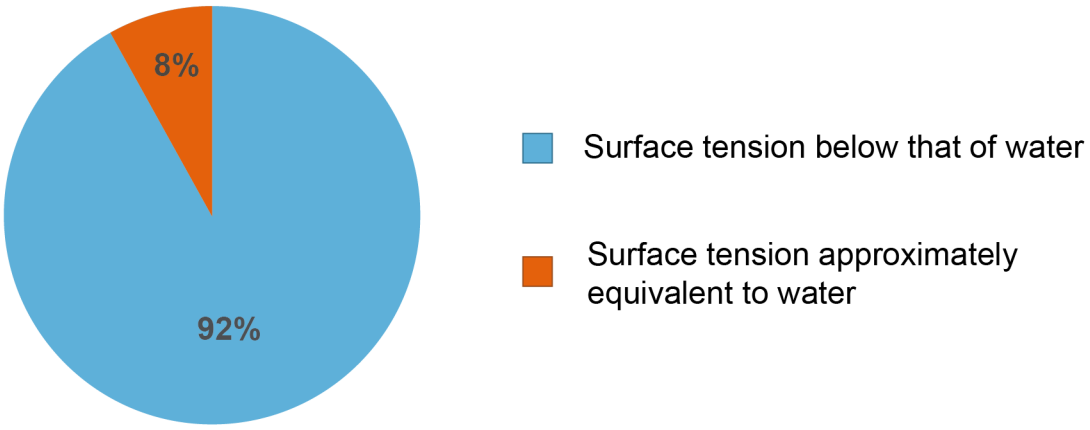
Categorization of process fluid types which result in increased risk of bacterial penetration (%) [3]



It is noteworthy that the majority of these 'high risk' fluid types have a surface tension value lower than that of water (Figure 2).

Figure 2

Comparison of surface tension values of fluids that show increased probability of bacterial penetration through a sterilizing grade filter [3]



While other fluid properties were evaluated against occurrences of bacterial penetration (e.g,. viscosity, pH), no correlation has been found except for surface tension value, if lowered due to the presence of surfactants, lipids, or lipid-like components etc. Indeed, Pall has previously demonstrated that sterile products can be obtained with very high viscosity fluids (160 mPa·s), even those which result in elevated differential pressures across the test filters [8, 9]. It can also be problematic to ensure a uniform bacterial cell suspension with very high viscosity fluids. The subsequent cell clumping may fail to penetrate the control 0.45 µm filter, resulting in an invalid bacterial retention test.

It is also worth noting that in Pall's experience, the probability of a bacterial penetration occurrence when filtering 'high risk' fluids is not correlated with the carrier fluid used for the bacterial retention testing (i.e. failures occur equally with a surrogate fluid compared to a liposomal fluid). This implies that the yet unknown mechanism causing the bacteria to penetrate can occur during the pre-conditioning phase of the testing.

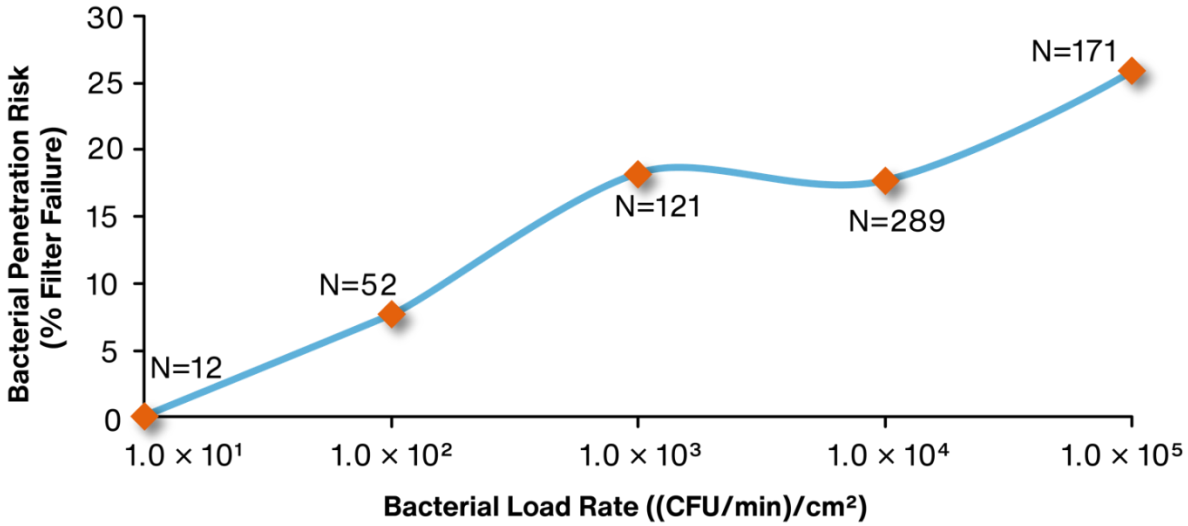
Consequently, to ensure a 'worst-case' bacterial retention test, when a surrogate fluid is used as the carrier for the bacterial retention test, Pall's policy is to primarily select a fluid that has a similar surface tension to the process fluid but has been shown not to impact viability of *B. diminuta*. However, for circumstances where the surface tension of the process fluid is like water, but the viscosity is much higher than water, then it is justified to select the surrogate fluid based on viscosity to simulate increased differential pressures across the filter.

4.2 Challenge Duration

Further evaluation of tests with 'high risk' fluids that resulted in confirmed bacterial penetration showed that the rate of delivery of the bacterial challenge may play an important role. Based on a sample size of 645 data points, Figure 3 shows the trends observed when considering the rate of delivery of the bacterial challenge to the test filters.

Figure 3

Impact of rate of delivery of bacteria to the test filters vs. probability of bacterial penetration [3]



This data suggests that for 'high risk' fluids, there is a greater probability of bacterial penetration when higher levels of bacteria are delivered to the filter over a shorter duration.

Furthermore, it is important to consider the principles of the bacterial retention test design. For a valid bacterial retention test, the minimum industry challenge level of ≥ 1.0 x 10^7 colony forming units (CFU) per cm^2 of effective filtration area (EFA) must be met. If a test filter was damaged during the filter pre-conditioning phase to the extent that bacteria could not be retained, *B. diminuta* penetration through the compromised area(s) of the filter would be observed upon delivery of the bacterial load. Therefore, the critical factor is that the filter is challenged with ≥ 1.0 x 10^7 CFU/cm^2 to determine the retentivity of the test filter, and as already discussed, for 'high risk' fluids, delivery of the bacterial challenge over a shorter duration but at a higher concentration may represent a worst-case test.

Finally, it is important to acknowledge that the process fluid (or surrogate fluid) is only one factor that can impact a bacterial retention test. Other critical factors that must be considered during the design of the bacterial retention test includes flow rates, volume, differential or system pressures across the filter, temperature and duration. By incorporating all these factors into the bacterial retention test design, along with a low surface tension surrogate fluid, it is Pall's position that this can be considered a worst-case test design, which meets all regulatory requirements for filter validation.

In conclusion, the technical rationale outlined in this paper represents Pall's approach for conducting standard, process-specific bacterial retention studies. Other approaches may be applicable for non-standard fluids and/or processes. However, it is recommended that alternate approaches be reviewed with Pall and the appropriate regulatory authorities, prior to testing using modified technical rationale.

5 References

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PALL CORPORATION

Corporate Headquarters
Port Washington, NY, USA
+1-800-717-7255 toll free (USA)
+1-516-484-5400 phone

European Headquarters
Fribourg, Switzerland
+41 (0)26 350 53 00 phone

Asia-Pacific Headquarters
Singapore
+65 6389 6500 phone

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