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Short Communication

An Insight Into Membrane Filter Validation

Sulagna Roy*

R and D Microbiologist, CBL, Ahmedabad, Gujarat, India

***Corresponding Author:** Sulagna Roy, R and D Microbiologist, CBL, Ahmedabad, Gujarat, India.

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Sterilisation by membrane filtration

Sterile filtration is an important building block of aseptic processing of drug products and is part of the aseptic manufacturing because aqueous product is sterile filtered during the filling procedure [1].

Objective of the sterile filtration process

By implication, the obvious objective of a sterile filtration step is the removal of any viable organisms that may be present in the bulk product solution as manufactured. This is typically accomplished by the use of "Sterilizing Grade" membrane filters, defined in the FDA "Aseptic Guideline" as those capable of totally retaining a challenge level of 10^7cfu / sq cm at a differential pressure of 30 psi. The FDA currently accepts *Brevundimonas diminuta* at this challenge level as a worst case model, but it is also acceptable to use natural flora.

A sterilizing grade filter is now defined as a filter that when appropriately validated, will remove all microorganisms from a fluid stream, producing a sterile effluent.

Validation

The used filter has to be validated for each filtered product. It is important to ensure as part of the process validation that the filtration step achieves the designed quality aspects in a reproducible and documented manner and that inadvertent side effects can be excluded. The most important quality target is of course sterility (bacterial retaining rate) whilst shedding of particulate matter or fibres from the filter into the solution, release of extractables or adsorption effects of the filter material regarding the active ingredients or excipients must also be avoided. The filter must be sterilised prior to use and filter integrity testing after sterilisation and after use are further requirements defined by EU GMP Guide [1].

Filtration validation typically includes Viability Testing, Bacterial Challenge Testing, Chemical Compatibility, Analysis of Extractables, Product Integrity Testing, Plant and Process Surveys, Systems and Integrity Tester Validation, Process Related Validation Studies.

Significance of validation

- Demonstrates the filter retains microorganisms to produce a sterile filtrate.
- Ensures the filter does not alter the product in an objectionable way.
- Ensures the product does not adversely affect the filter –compatibility.
- Ensures the physical parameters of the process do not adversely affect the filter or the product.

Microbiological challenge - bacterial retaining rate

The definition of a sterilizing grade filter is one that will produce a sterile effluent after being challenged by microorganisms at a challenge level of greater than or equal to $1x10^7/cm^2$ of effective filtration area [2].

Until the late 1960's, 0.45 µm-rated membranes were considered "sterilizing grade" filters, and were used successfully in the sterilizing filtration of parenterals. In the mid-1960's Dr. Frances Bowman observed a 0.45 µm "sterile-filtered" culture medium to be contaminated with a micro-organism, subsequently shown to penetrate 0.45 µm-rated membranes repeatedly in small numbers [2].

Brevundimonas diminuta (ATCC 19146) (equivalent to B diminuta MTCC 1287, previously Pseudomonas diminuta)

It is an aerobic gram-negative bacterium. Due to its small size, *B. diminuta* is a standard microbial organism for validation of

membrane filters for sterilization. *Brevundimonas diminuta* (ATCC 19146) has been proven to penetrate a 0.45 µm rated filter [3].

A standard procedure for the qualification characteristic for a 0.22 µm (or less) sterile filter is the adding of at least 10^7 *Brevundimonas diminuta* (ATCC 19146) per cm^2 of filter area. The supplier of the filter will perform a lot of microbiological challenges [1].

The *B. diminuta* cells ideal for performing filter validation should have:

- high cell concentration (preferably >1×10^8 CFU/mL),
- very small cell size (0.3 x 1.0um).
- a mono-dispersed population (preferably > 80%),
- high viability (preferably > 90%),
- high bacteriological purity.

B. diminuta cells are usually cultivated with deep fermentation techniques according to ASTM F838 - 83 procedure [4]. The final batch is grown aerobically to early stationary phase (approximately 2×10^{10} CFU/mL). However, this deep fermentation procedure often leads to aggregated and larger cells. Thus, there is a need for a better method for the production of *B. diminuta* cells suitable for the validation of sterilizing grade filter membranes.

Factors potentially affecting microbial retention

- Filter Construction (structure, membrane polymer, pore size distribution).
- Formulation components.
- Formulation properties (pH, viscosity).
- Process conditions (time, temperature, pressure differential, flow rate).

Microbial retention studies on filter devices

This involves spiking of the drug product with *Brevundimonas diminuta* according to ASTM 838 - 05. Challenge level is >10^7 CFUs / cm^2 filtration area. Validation Testing should simulate worst-case process conditions e.g., pressure differential, flow-rate, time, temperature [2].

Use of other natural flora

ASTM 838 - 05 (2005) uses *B. diminuta* as the standard challenge organism. 2004 Aseptic Processing Guidance suggests the use of native bioburden isolate when appropriate [2]. The cell count and nature of the bioburden will likely be different in actual filtration settings, particularly if a model such as *B. diminuta* was used in the validation runs. In light of publications that document the passage of presumably smaller organisms through *B. diminuta* retentive membranes, this could adversely affect the expected retention in an actual filtration setting. For this reason, validations using naturally occurring flora will likely eventually be preferred over any model organism testing, even if natural flora may also vary from time to time.

To reach concentrations of 10^7 / sq cm using natural flora it may also be necessary to increase the inherent bioburden of the actual product by additional steps, such as allowing incubation beyond the regular processing time or the concentration of cells by cross-flow filtration.

Source of variability: size and shape

B. diminuta varies in size and shape, depending on how it is cultivated (Leahy and Sullivan, 1978). As such the size of *B. diminuta* is described as 0.3 x 1.0um. When the organism is grown at 30°C and incubated for 24 hours in saline lactose broth, a minimally nutritional medium for this microbe, cocci-like cells of 0.3 x 1.0um size are obtained. This microbe is typically cultivated to develop as spherical a form as possible, since spheres are least amenable to retention.

The smaller the test organism, the more likely that its removal by a filter would assure the sieve retention of larger organisms. Experimental studies show that penetrating organisms can shrink if they are cultivated in conditions that are nutritionally inadequate. In such cases filtration in 0.22um –rated membranes would fail to yield a sterile effluent.

Method

(i) Selection of 0.22um filter discs.

(ii) Preparation of culture media solution of *Brevundimonas diminuta* (*Pseudomonas diminuta*) with concentration of > 10^7 CFUs:

CFUs

Medium – minimum essential medium with a high osmolarity like:

(a) Saline lactose broth (1.3 g of Lactose Broth dry powder in 100 mL of hot distilled water with 970 mL of sodium chloride solution) to control size and dispersion characteristics or in (b) Growth

medium A (7.5 g of Trypticase Peptone, 2.5 g of Yeast Extract, 0.5 g of Sodium Chloride and 0.35 g of magnesium sulfate added to 1.0 L of hot distilled water) that gives high titers but a larger size, because medium A is more nutrient rich. So saline lactose broth is a more optimal medium. The headspace is about one half of the volume of the chamber. The chamber is preferably a disposable pre-sterilized bag. The chamber is preferably rocked at a rate of 15 rocking/minute. Aeration rate is maintained at 0.8 L/min for 28+/- 2 hours. Temperature is maintained at 30+/-2 ◦ C The size of *B. diminuta* cells is determined using ocular micrometre. Monodispersion is determined by optical microscopy [3-5].

(iii) Passing the culture through the sterile 0.22um filter discs at a challenge level > 10^7 CFUs/cm^2 filtration area.

(vi) Passing of the culture through a second assay filter disc of 0.45um.

(v) Both the discs (0.22um and 0.45um) are placed aseptically onto agar plates and incubated.

Integrity tests - bubble point value

Currently practiced membrane filter integrity tests are based on the observation that membrane samples wetted out with a suitable liquid are essentially impermeable to viscous flow of gases at low pressures. As the applied pressure is increased, the liquid within the largest pores present in the sample will eventually start to dislodge, allowing the test gas to flow through them forming visually observable streams of bubbles.

The pressure at which bubbling commences is referred to as the bubble point of the membrane, a value which has been used to characterize the integrity of small area membrane samples At higher bubble-point values, the retention becomes more "reliable," and a pressure, or a narrow range, above which "total" (better than the challenge level or 10^7 cfu / sq cm) retention is observed, can readily be identified. This pressure is often referred to as the "critical" bubble-point value.

Bibliography

1. Gisela Greger, "Basic Requirements For Aseptic Manufacturing Of Sterile Medicinal Products -A Comparison Between Europe And USA". Master of Drug Regulatory Affairs (2004): 27-28.

2. Validation of Sterilizing Grade Filters, Sartorius Corporation.

3. Vargas D. Process to cultivate Brevundimonas diminuta for filtration validation. US 20070092961 A1 (2007).

4. ASTM. Standard Test Method for Determining Bacterial Retention of Membrane Filters Utilized for Liquid Filtration 938-944.

5. ASTM F838-05 (2005).

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