Validation of Sterile Filtration

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This article discusses the basics of sterile filter qualification and validation, with emphasis on bacterial challenge protocol development and testing. Reference is made to Technical Report no. 26 of the Parenteral Drug Association.

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The Parenteral Drug Association (PDA) published the authoritative summary of best practices in sterile filtration and validation of sterile filtration in its 1998 technical report, "Sterilizing Filtration of Liquids" (1). It highlights the history of sterile filtration, explains how filters work, details selection criteria, and explains validation considerations and integrity testing methods. Much of this article is based on that monograph.

The purpose of sterile filtration validation is to prove that a particular filtration process generates a sterile filtrate. This is achieved by choosing a sterilizing grade filter that is compatible with the process, nontoxic, integrity testable, sterilizable, that does not adsorb formula components or add extractables to the process and can remove the bioburden associated with the product. The filter then is challenged with $>10^7$ colony forming units (cfu) of *Brevundimonas diminuta* (ATCC 19146) per cm^2 under process conditions and demonstrated by testing to produce a sterile filtrate.

Sterilizing grade filters

The removal rating of a given membrane filter type refers to the size, or narrower dimension, of microorganisms and particles removed by the filter, rather than the actual size or shape (*i.e.,* morphology) of the filter pore structure. The industry-accepted rating for a sterilizing grade filter is 0.2 or 0.22 μm, depending on the manufacturer, which is validated as capable of removing $>10^7$ cfu/cm^2 *B. diminuta* under certain extreme processing conditions. Tighter filters such as 0.1 μm filters that demonstrate the same bacterial retention also may be rated as sterilizing grade.

In validating and performing sterile filtration, it is essential to identify the bioburden or endemic microorganism(s) in a given process, to use the grade of filter that quantitatively removes the microorganism(s), and to demonstrate quantitative removal by test before using the filter in production. This is the essence of filter validation.

For filter manufacturers, a critical requirement is to provide users with a reasonably convenient, safe, easy-to-perform integrity test which confirms the integrity of the filter, the seals, and contiguous process equipment. Liquid-sterilizing filters can be integrity tested by the bubble point, forward flow or diffusion/diffusive flow test, or the pressure hold test. Hydrophobic filters can be tested with any of those methods or by the water intrusion test. Filter integrity tests are explained in another article in this issue.

Qualifying pharmaceutical filters

Safety and purity. Before they can be used in a sterilizing filtration process, filters must meet or exceed minimum safety standards.

The first step is to qualify the filter in several important areas of safety. To qualify as a pharmaceutical filter, the filter must be nontoxic, according to *USP*-specified tests (*e.g., USP* ⟨87⟩ "Biological Reactivity Tests," *in vitro*, *USP* ⟨88⟩ "Biological Reactivity Tests," *in vitro*, *USP* ⟨88⟩ "Biological Reactivity Tests," *in vivo,* including the Class VI Plastics Tests) and be tested free of pyrogen or endotoxin to acceptably low levels.

Pharmaceutical-grade filters also must have very low extractables levels, although neither USP nor the Food and Drug Administration have specified minimum or maximum levels. It is the responsibility of pharmaceutical manufacturers to set allowable specifications for extractables.

As a further indicator of safety and purity, filters also should rinse quickly when exposed to high-purity water, must be compatible with pharmaceutical process fluids and pharmaceutical products, and be both sterilizable and integrity testable.

All this pharmaceutical filter safety qualification information typically is provided in the filter manufacturers' validation guide and product literature for a specific filter.

Performance qualification. Filters must be qualified by the user to demonstrate that their performance in processing will meet or exceed minimum process requirements.

Performance qualification requirements

- flow rates
- throughput
- pressure and temperature resistance
- hydrophilic or hydrophobic
- membrane composition
- compatibility
  - membrane support layers, core, or cage
  - o-rings
  - housings

The filter must be tested to verify that it provides the flow rates required by the pharmaceutical process. The filter system must be sized to provide flow rates and volumes adequate to keep pace with filling machines or other production equipment requirements, with some reserve capacity for use in case of batch contaminant variability and premature plugging. The total liquid volume passed through the filters, its throughput, should be adequate to process a complete batch without interruption.

Small-scale sizing or *filterability* tests are used as the basis for extrapolating or scaling-up filtration systems. Sizing of final, sterilizing filters, and any upstream prefilters used to remove coarse contaminants and thereby extend the life of the final filters, is based upon anticipated flow rates and throughput in a given pharmaceutical liquid.

Sterilizing filters and filter housings, stainless steel, or disposable plastic, must be rugged enough to withstand the pressures and temperatures of normal process conditions, occasional runaway process conditions, and the temperature ranges of processing (minimum to maximum) and steam or autoclave sterilization, including both temperature ranges and duration of the sterilization cycle(s).

Sterilizing-grade filters for aqueous pharmaceutical liquids are normally hydrophilic, or water-wettable, membrane filters. In the case of solvent or chemical liquids to be filter-sterilized, hydrophobic, or nonwater-wetting, filters may be used. They can be wetted by a low-surface-tension liquid.

Compatibility. The filter system must be qualified to ensure that all product-contact surfaces of the filter and its constituent parts (membrane, support layers, core, cage, and end caps), o-rings, piping, hoses, seals, pumps, gaskets, and any other components of the sterilizing filtration system can withstand the hydraulic, thermal, and chemical challenges of the sterilization and production processes. None of these should extract into the filtered pharmaceutical product in any significant amount.

Chemical compatibility questions generally are resolved by reference to compatibility tables generated by manufacturers of elastomers or polymers used in o-rings, gaskets, and seals. Membrane compatibilities generally are well established for the commonly used membrane materials. Any specific questions can readily be resolved by testing during the qualification stage.

Sterilization of sterilizing filters. Sterilizing grade filters can be sterilized in a number of ways. Capsule filters can be gamma irradiated or autoclaved. Disk filter holders are autoclaved with the wetted filter in place. Cartridge filter installations frequently are sterilized by steam-in-place (SIP) operations.

Common steaming temperatures used in the United States are 121–135 °C, sustained for 30–60 min in the filter installation. Whatever time–temperature parameters are specified, it is critical that these parameters be validated by the pharmaceutical manufacturer under operating conditions.

Validation

There are four major elements of the filtration validation process:

- physical/chemical compatibility, usually established during the qualification phase before validation, is confirmed during the validation process
- binding and adsorption filter characteristics are measured in the qualification phase
- bacteria retention capability of the filter, which is established by challenging the filter with *B. diminuta*
- integrity of the process filtration installation, as verified by the filter integrity test.

Concerning integrity testing, the user must demonstrate that they know how to install, sterilize, and integrity test the filters. Filter integrity test values provided by the filter manufacturer are correlated to the bacterial challenge in the manufacturer's validation guide. Product integrity test values are correlated to the water or model solvent values.

Bacterial retention. The bacterial challenge test validates the ability of a filter to provide sterile effluent in a specific pharmaceutical liquid. It is also the ultimate compatibility test, because the bacterial challenge simultaneously tests the physical-chemical interaction of the liquid product and the filter, under process conditions. Any filter inadequacy caused by this interaction will be detected by the bacterial challenge.

Validation of bacterial retention normally is performed by the filter manufacturer or an independent laboratory, using 47-mm diameter disks to minimize the volume of pharmaceutical product required. Larger surface area filters also can be used.

Bacterial challenge tests are usually performed with an industry standard concentration of 10^7 cfu of *B. diminuta* per cm^2, using pharmaceutical product, whenever possible, for the most realistic validation. The high bacterial concentration used in the challenge test constitutes a worst-case scenario. The manufacturer qualifies the filter using a similar challenge.

B. diminuta is grown to produce monodispersed cells capable of penetrating a 0.45-μm filter, typically in accordance with ASTM Standard F838. Following that standard, the organism is cultured in saline lactose broth (SLB) and either used freshly cultured or concentrated into a frozen cell paste that is thawed immediately prior to use. A

0.4-µm rated membrane filter is used as a positive control; if the organisms pass through this control filter, it proves that they are alive, small, and nonaggregated, thus verifying that the challenge is a stringent test.

Validation parameters for bacterial challenge using pharmaceutical product. Key considerations for using pharmaceutical product liquid to validate sterile filters are listed in the following chart.

Product contact time. The bacterial challenge using pharmaceutical product must be run for at least the same duration as a product batch will be run in processing. If the batch requires eight hours to filter, the challenge must be run for at least eight hours. It is good practice to run the challenge for a little longer to anticipate unusual processing circumstances.

Differential pressure and flow rates per unit area. Maximum process differential pressures and flow rates should be incorporated into the protocol. Often, it is virtually impossible to match both simultaneously. At the start of the challenge, when the filter is clean, extremely high flow rates per unit area are necessary to generate process-level differential pressures (pressure drop across the filter). But, as the test filter disks accumulate bacteria and pressure builds, the flow drops. One solution is to match flow rates at the start of the challenge and pressures near the end. Another solution is to decide whether pressure or flow is more relevant, and then develop a technically sound rationale to support the decision and use it in challenge testing.

Validation parameters

- product contact time
- differential pressure
- flow rates per unit area
- temperature
- bioburden
- integrity test correlation

Temperature. If the liquid temperature is outside the viable range of the challenge bacteria, it may be necessary to recirculate the product at process temperature, conditioning the filter first, and then challenge the filter at a temperature at which the bacteria survive.

Bioburden. Bioburden levels can influence process filtration efficacy. The probability of passage increases when the bioburden is high. The area-specific bioload (B_a) is the bioburden per unit area of filter (cfu/cm^2) or $B_a = BV/A$, where B is the bioburden in cfu/mL, V is the total volume to be filtered (mL) and A is the surface area of the filter in cm^2. It is therefore best to control the bioburden of the raw materials to avoid approaching or exceeding the validated limit.

Integrity test correlation. Filters used in the bacterial challenge must be integrity tested to form the correlation to retention. Because the user cannot use a destructive challenge test in processing, the filter manufacturer must supply a correlated, nondestructive integrity test that reliably assesses the integrity of a given filter installation. In performing the

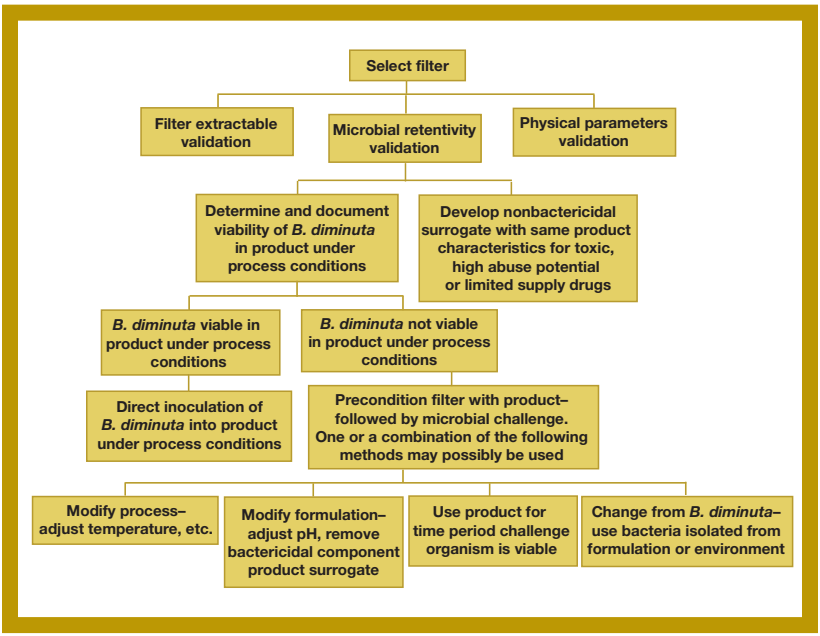

Figure 1: Establishing a microbial challenge protocol (1).

challenge test, three lots of filters typically are chosen, including a low-bubble-point lot, one with a bubble point specification that approximates the manufacturer's production limit. This is important because the end user is limited to using filters in their process that match or exceed the lowest bubble point validated in product.

Establishing the challenge protocol. The above chart highlights the steps needed to develop a suitable challenge protocol.

After the filter is qualified and selected, filter extractables and microbial retentivity are validated. Physical parameters such as sterilization and integrity test methods also are validated. Viability of the test microorganism, *B. diminuta*, in the pharmaceutical product is compared to a control over time. If there is less than one log difference in the counts, the organism is considered viable under those conditions. Results are documented. If a pharmaceutical product is toxic, has high potential for abuse, or is in limited supply, a nonbactericidal surrogate fluid with very similar properties may be developed. It is recommended that the user consult with local FDA inspectors before developing this approach.

If the microbe is viable. If *B. diminuta* is found to be viable in the pharmaceutical product, it is directly inoculated into the product as the challenge microorganism.

If the microbe is nonviable. If *B. diminuta* is found to be nonviable under process conditions, the test filter is

Factors affecting microbial retention

- filter type: polymer, structure
- fluid properties: pH, viscosity, osmolarity, and ionic strength
- process conditions: temperature, differential pressure, and flow rate
- bioburden: quantity, composition

preconditioned with pharmaceutical product liquid, and one of several options can be followed, prior to conducting the bacterial challenge.

Options are to:

- modify the process by adjusting temperature or otherwise
- modify the formulation, by adjusting pH, removing bactericidal components, or using a product surrogate liquid
- use the bacteria for a brief enough time such that the challenge microorganism retains its viability during the test period
- switch the test organism from *B. diminuta* to a process isolate, or native bioburden endemic to the actual pharmaceutical process. It is recommended to consult first with FDA inspectors on this option.

If the product is bactericidal, the volume and time required to thoroughly rinse the filter free of the product must be established, after the product has been recirculated, prior to adding the bacteria and challenge solution. This is documented in an inhibition test.

Bacterial challenge. The challenge procedure typically consists of integrity testing the filters, autoclaving the apparatus, integrity testing post-sterilization and recirculating product through the filters at process flow rates or pressures. Bacteria are added according to the results of the viability study. In nonbactericidal products, the microorganisms are added at intervals throughout the challenge. Before introducing the bacteria, analysis membranes are installed downstream of all test and control filters. Analysis filters must have a pore size of at least 0.45 μm or tighter. Membranes that are 0.45 μm will retain 10^4–10^6 logs of *Brevundimonas*, which generally is accepted as adequate for a valid test.

Bracketing

- challenge extreme products
- carefully choose family groupings
  - API concentration levels
  - pH, other
- validates intermediate products

Factors affecting bacterial retention. The bacterial challenge procedure addresses the possible influences upon retention of the membrane polymer, liquid, process conditions, and bioburden level. Adverse effects from any of these parameters would result in bacterial passage.

The only factor not routinely addressed in the filter validation or microbial challenge process is composition of the bioburden. Composition of bioburden should be addressed prior to choosing the removal rating of the filter, during filter qualification.

Bracketing. It is not always necessary

to perform a microbial challenge on every pharmaceutical product. The practice of bracketing makes this acceptable. With a family of pharmaceutical products having high degrees of similarity but only slightly differing characteristics such as differing concentrations of active ingredient, we can perform the microbial challenge on the extreme products (*i.e.,* those having the highest and lowest concentrations) while products with intermediate-level concentrations need not be individually challenge-tested. However, we must generate data to show that these product extremes have been tested.

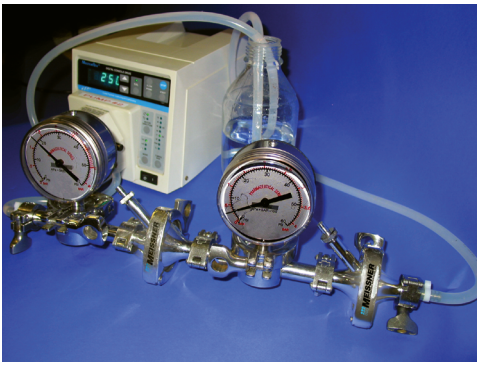

Figure 2: Bacterial challenge test apparatus showing differential pressure between two inline 47-mm diameter filter disk holders in series, with the challenge filter upstream and the analysis filter downstream, connected by sanitary piping to a peristaltic pump which recirculates the challenge microorganism.

Extractables. Another important aspect of sterile filter validation is extractables testing. It is important that the filters are not themselves a source of physical (*e.g.,* particles) or chemical contaminants.

Adsorption analysis

- binding of formulation components to filter
- potential for OOS ingredients
- identify any problem
- address problem if necessary

The nonvolatile residue (NVR) test from *USP* ⟨661⟩ Containers is used to quantify the amount of extractables released by a filter in a particular process stream. NVR typically is tested by using a model solvent, rather than pharmaceutical product. It involves soaking the filter, boiling off the solvent, drying the residue, and weighing it. The weight of residue per filter is calculated. Pharmaceutical products seldomly are used for NVR assay, because their constituents mask filter extractables. Various analytical techniques may be applied to identify filter extractables.

Adsorption. The flip side of the phenomenon of filter extractables is the phenomenon of filter adsorption. The filter should not remove active ingredients, excipients, carriers, diluents, proteins, preservatives, or any other formulation component. It is important to test the product to ensure that the filter does not cause any ingredient to fall below specification.

With most sterilizing grade filters, adsorptive levels are so low as to be insignificant and well within limits

Table I: Tests commonly performed by filter users and the filter manufactures—general industry practices.

	Filter user	Filter manufacturer	
Criteria	**Filter device**	**Membrane disk**	**Device**
Bacteria retention/integrity test relationship data	—	Q	Q
Integrity test			
water/solvent	V	Q, R, L	Q, R, L
product	V	—	—
Integrity test methodology and selection	V	R	—
Bacterial retention			
water, SLB, etc.	—	Q, L	Q, L
product	V, membrane disk	—	—
Bacterial retention/integrity test methodology	V	Q	Q
Effects of chemical compatibility on filter integrity	V	Q	Q
Toxicity testing	—	Q	Q
Extractables	V	Q, R, L	Q, R, L
Effects of sterilization methods on filter integrity	V	Q	Q

Q denotes qualification testing process specific; V denotes validation testing; R denotes recommendation for validation; and L denotes filter lot specific release criteria.

set by pharmaceutical manufacturers. However, if an adsorption problem is experienced with a certain formulation, it should be addressed.

Redundant filtration. One final circumstance to be addressed in validation is the use of redundant filters, two final filters of identical rating deployed in series as "insurance" against nonsterile filtration. This practice is common in Europe and with some American manufacturers of serum, tissue culture media, and biologicals.

If both filters are needed to achieve sterility in a given process, both must pass the post-use integrity test in process operations. If only one filter in the series is necessary to validate a sterile filtrate, then only one is required to pass the integrity test.

Where redundant filters are installed, the validation process also must address the impact of the second filter on extractables levels and adsorption (*i.e.*, twice as much).

PDA Technical Report No. 26

Filter validation recommendations. PDA's Technical Report No. 26 summarizes the principles and best

practices of sterile filtration and its validation. In this article, we have described details of some of the more significant aspects of filter validation that follow the recommendations of "TR26."

TR26 recommends that the pharmaceutical manufacturer perform a process-specific validation which includes:

- establishing an integrity test methodology and demonstrating integrity of the sterilizing filter
- performing bacterial retention studies
- having a correlation between bacterial retention and the integrity test method
- verifying chemical compatibility
- performing extractables testing
- evaluating the effects of sterilization on filter integrity.

Summary and conclusion

The validation report summarizes the protocols, results, and interpretations of all testing performed. It also provides parameters and protocols for performing steam or autoclave sterilization and integrity testing. The report is an important documentation of the validation process. It should be read and understood by all end users involved in validating the specific sterile filtration process. The report also serves as an important reference document in an FDA inspection.

While filter manufacturers perform much of the qualification and validation work in sterile filtration validation, it is crucial for the pharmaceutical processor to remember that the pharmaceutical or biopharmaceutical company bears complete responsibility for:

- filter validation
- use of the validated sterile filtration system in compliance with current good manufacturing processes and the recommendations of the filter manufacturer as found in the validation report and supporting documentation.

Pharmaceutical users of sterile filtration are urged to read PDA's Technical Report No. 26, to follow its guidelines, and to work closely with their filtration manufacturer in resolving any questions or issues relating to sterile filtration and its validation.

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