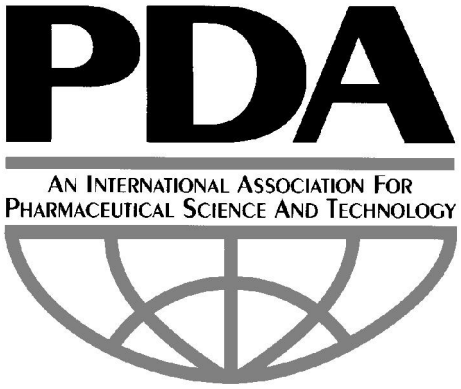

PDA Technical Report No. 26

Sterilizing Filtration of Liquids

Final Draft
August 28, 1997

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PDA TECHNICAL REPORT NO. 26
STERILIZING FILTRATION OF LIQUIDS

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1.0 INTRODUCTION

Sterilizing filtration is the process of removing all microorganisms, excluding viruses, from a fluid stream. A sterilizing grade filter must remove all microorganisms present in a fluid stream without adversely affecting product quality. This technical report is intended to provide a systematic approach to selecting and validating the most appropriate filter for a sterilizing filtration application.

Early, careful screening of potential filter types and configurations can result in fewer technical and regulatory problems, fewer delays, more efficient product processing and greater sterility assurance.

2.0 PHARMACEUTICAL FILTRATION/HISTORICAL HIGHLIGHTS

In the early 1900s, the first parenteral drugs were manufactured on an industrial scale. The need arose to find a suitable sterilization method for heat-sensitive products that could not be autoclaved in the final container, i.e., had to be aseptically processed. Later, filtration to remove subvisible particulates from parenteral preparations, particularly solutions introduced intravenously, was found to be important.

It is not surprising that pioneering work in the field of germ removal by filtration (sterilization by filtration) also was underway from about 1900 to 1930. In the industrial field, three paths were pursued: porcelain filter cartridges, asbestos-cellulose layers and membrane filters.

Initially, porcelain filters by Chamberlain were used in pharmaceutical production. However, problems with cleaning these permanent filters and the danger of cross-contamination led to their replacement. The first filter medium to be used on an industrial scale worldwide for almost 50 years was a cellulose-asbestos filter known as the Seitz EK Filter (EK - Entkeimung, "germ removal"). Since the mid-1970s this filter has been forced off the market because of the asbestos fiber issue. Collodion membranes, manufactured by users themselves, were employed in bacteriological laboratories as early as the first decade of the 20th century. The forerunner to the membrane filter was developed by Zsigmondy and Birchmann who patented a graded series of membranes in 1918. The Sartorius-Werke Aktiengesellschaft, Göttingen, Germany, refined the Zsigmondy process and in 1929 began the commercial production of membrane filters on a small scale. The first membrane disks led to the replacement of porcelain filter cartridges in pharmaceutical production. There still was a lack of efficient, large surface area, inexpensive membrane filters for use in large parenteral batch production. The membrane filter cartridges, especially the pleated cartridges that entered the market in the 1970s, were a step in the right direction.

There still were problems, as the retention rate for bacteria was unacceptable to the pharmaceutical industry. The problem was examined systematically, and a collaboration between filter manufacturers and pharmaceutical companies developed basic principles that would ensure safety in sterilization by filtration.

The homogeneity of the membranes in the sponge-like labyrinth system and the concern about the potential for interfering "large pores" initially led to the use of double-layer membranes. Additionally, defects in the cartridges arose from the folding and welding, or gluing of the membranes, as well as defective seals and damage during autoclaving. Newly developed integrity test equipment helped,

allowing accurate measurements to assess these defects. Improved filter manufacturing techniques eventually eliminated these defects.

Until the late 1960s, 0.45 μm-rated membranes were considered "sterilizing grade" filters, and were used successfully in the sterilizing filtration of parenterals. Such filters were qualified using 0.6x1 μm *Serratia marcescens*, a standard bacterium for qualifying analytical membranes used for water quality testing (ASTM, 1980). In the mid-1960s, however, Dr. Frances Bowman of the FDA observed a 0.45 μm "sterile-filtered" culture medium to be contaminated with an organism, subsequently shown to penetrate 0.45 μm-rated membranes repeatedly in small numbers at challenge levels above 10^4-10^6 per cm² (Bowman et al., 1967).

Bowman also observed that the next finer grade commercial membrane (nominally 0.22 μm-rated) effectively retained this organism at similar challenge levels. This 0.3x0.6-0.8 μm contaminant was identified as *Pseudomonas diminuta* (currently reclassified as *Brevundimonas diminuta*), and registered with the American Type Culture Collection (ATCC) as Culture No. 19146. This strain has been accepted widely by filter manufacturers and industry as the standard challenge organism for qualifying sterilizing grade membrane filters (ASTM, 1983; ISO, 1995).

Following the broad acceptance of *B. diminuta*, FDA incorporated demonstration of its retention in the definition of a sterilizing filter.

The use of this microorganism provides several advantages:
- Originally a process stream isolate, it is therefore a realistic potential problem organism.
- Generally regarded as nonpathogenic to humans, ordinary microbiology laboratories can use it without major biohazard concerns.
- It can be consistently cultured under controlled conditions to yield very small, monodispersed cells with a narrow size distribution. These can penetrate 0.45 μm filters reproducibly in small numbers at high challenge levels, thus representing a potential worst-case challenge.

The disadvantages of *B. diminuta* are:
- It is not viable in many pharmaceutical formulations.
- It may not be the smallest bacterium potentially encountered in all formulations, and thus may not represent bioburden organisms in terms of morphology and physiology.
- In spite of being stable, batch-to-batch variation in its morphology (size) should be examined.

3.0 HOW FILTERS WORK

It is widely believed that filters work by permitting fluid passage through their pores, retaining particles too large to fit through these apertures. This mechanism of particle arrest or capture is called variously sieve retention, physical capture, direct interception, size exclusion, etc. This view is based on the axiom of solid geometry that a particle too large to fit into a pore is incapable of passing through it.

Although size exclusion is an important, and perhaps for sterilizing filters the most reliable, mechanism of filtration action, it may not be the only one. Indeed, particles small enough to enter and pass through filter pores may be captured by becoming adsorptively attached to pore walls. The

effectiveness of adsorptive sequestration mechanisms depends upon filtration conditions. Many different operational conditions govern a filter's adsorptive removal of particles, including applied differential pressure, flow rate, number of particles present, and the liquid vehicle's makeup in terms of its surface tension, pH and ionic strength, among other factors. All must be considered and understood in filter validation.

The best way to assess whether an individual membrane filter can produce a sterile effluent is to challenge the filter with a large number of organisms and measure the retention. Unfortunately, this is a destructive procedure that leaves the tested filter contaminated by test organisms and subsequently unusable for product filtration. It is possible, however, to assess the suitability of a filter's sterilizing action by subjecting it to nondestructive integrity tests, which can be correlated to microbial retention.

3.1 Size Exclusion

As previously noted, a particle too large to pass through a filter pore becomes arrested by the spatial restraint. This serves to separate it from the conveying fluid in which it is suspended. This type of particle capture is called size exclusion. Size exclusion is a combination of surface screening and entrapment within the filter matrix.

If each particle challenging the filter is too large to pass through the pores, the number of particles does not matter; none will pass the filter. Filter efficiency, the completeness with which a filter retains particles confronting it, also is independent of the applied differential pressure, as long as that pressure does not deform the particle or the pore, causing a failure in sieve retention. Differential pressure is the difference in pressure on opposite sides of the membrane, and governs the flow of the liquid or gas through the filter.

3.2 Other Retention Mechanisms

Other mechanisms of particle removal include, but are not necessarily limited to adsorptive sequestration, inertial impaction and diffusional interception. Particles may be small enough to enter the filter pores but still may be captured by the filter, indicating that particle retention may depend upon other operating conditions governing the filtration. These effects are important if bacteria smaller than the pore size are present.

3.3 Bioburden Retention Probability

In situations where contaminants are not all retained by size exclusion, the outcome can be said to be probabilistic. Figure 1 shows a bacterium smaller than the pore size entering a pore. It may either traverse the pore to leave with the flow, or encounter the pore wall and become adsorptively arrested by Van der Waals forces, or residual or secondary valence forces. Which situation prevails is a result of probabilities. Regardless of the challenge level, for a filter that is not plugged, the probability that any particular particle will penetrate the filter is the same for any identical particle. The greater the number of particles, that is, the higher the challenge density, the more likely it becomes that some particles will escape capture. The lower the organism population confronting the filter, the better the filter performs.

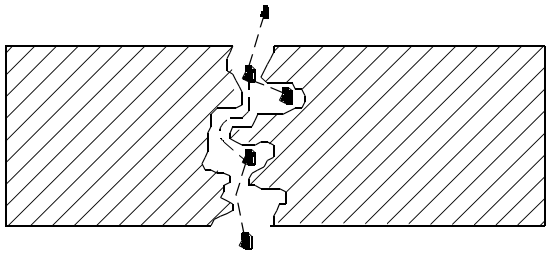

Figure 1 Alternate paths for particles entering pores.

The effects of differential pressure also must be considered. The lower the differential pressure, the greater the likelihood of organism retention. The longer a particle or organism remains within a pore passageway, the more likely it is to encounter the pore wall and be adsorbed. Conversely, at higher differential pressure, the velocity of the liquid through the pore increases and the residence time decreases, resulting in lower probability of particle capture. There is, therefore, a probabilistic nature to the adsorptive capture, expressed by the inverse relationship of adsorptive sequestration to the differential pressure level.

For particles not retained by size exclusion, various investigators have shown that such properties as pH, surface tension, ionic strength and viscosity may influence retention by microporous membranes. Such dependency may be indicative of adsorptive retention. The vehicle also may affect bacteria present, by changing their size or morphology. Such changes might result in a nonsterile filtrate.

It should be emphasized that the bioburden level can influence filtration process efficacy. For this reason, the bioburden should be kept as low as possible at each stage of the process. Filters exhibit maximum efficacy when the bioburden is minimized. Said another way, the probability of microbial passage is minimized when the bioburden is low. Bioburden should be routinely monitored.

3.4 Pore Size Rating

The rating of filters always has been controversial, primarily due to the lack of manufacturer uniformity in measuring pore sizes. Thus, pore size rating has limited value in predicting microbial retention or physical integrity test values, or providing a basis of comparison between different materials of construction and manufacturers.

Since the process of classifying a sterilizing grade filter by pore size has limited value, it has been replaced by defining the filter in terms of its bacterial retention (FDA, Aseptic Processing Guideline, 1987). Classically, a sterilizing grade filter has been defined as a filter which will retain 10^7 cfu of *B. diminuta ATCC 19146*/cm^2 of effective filter surface area, under specified conditions. A discussion of pore size ratings is found in Appendix A.

4.0 FILTER SELECTION AND CHARACTERIZATION

Selecting a sterilizing grade filter requires consideration of many important issues, such as materials of construction and their compatibility with the product. The selection also should consider the processing characteristics, including the volume of product filtered, flow rate, pressure differential, temperature and the chemical characteristics of the product.

The following sections consider important topics in determining the best filter for a given application. More detailed discussion on these topics is contained later in this report.

4.1 Filter Types

Current available materials of construction include, but are not limited to polymers such as cellulose esters, nylon, polyesters, polytetrafluoroethylene, polyvinylidene flouride, polycarbonate, polypropylene, polyethersulfone and polysulfone. Minor components, such as surfactants may be added to the filter to enhance wettability, or render the filter membrane hydrophilic or hydrophobic. Hydrophobic means that the membrane pores are not easily penetrated by water or aqueous streams. Hydrophilic means that the pores of the membrane are readily penetrated by water or aqueous fluids.

Whether the filter is hydrophilic or hydrophobic also greatly affects its ability to properly serve in a given process. Generally, hydrophilic filters are used for aqueous based liquid processes, while hydrophobic filters are used for solvent, vent and gas applications.

4.2 Filter Configuration

During filter evaluation, it is best to consider all processing variables so the appropriate filter surface area and configuration can be selected. There are three basic types of filter configurations generally accepted for sterilizing products: flat stock membranes, preassembled capsules and membrane cartridge assemblies.

Flat stock membranes have been used for many years. Single membranes in reusable holders generally are considered suitable for small-scale sterilizing filtration. Multiple disc assemblies are suitable for larger filtration volumes. However, there are advantages to working with small cartridges or self-contained capsules, which are more practical and easier to handle than flat stock membranes. Mid-sized operations such as developmental pilot scale or clinical manufacturing are good candidates for self-contained, disposable capsule filters, while cartridge filters should be considered for larger scale operations or processes. Multiple cartridge configurations are suited to the large-scale production environment.

The appropriate filter surface area for a particular sterilizing filtration application can be estimated from laboratory experiments utilizing the identical filter membrane. Flow rate and particulate removal studies performed on 47 mm discs usually may be extrapolated to larger configurations, but the effects of filter configuration and the process stream must be considered. Considerations may include pleating, stacking and piping, among others. Since flow rates are very sensitive to system restrictions, undersized tubing and fittings should be avoided.

4.3 Particle Shedding

Particulate contamination from the filter and process must be evaluated and considered, since all filters may shed particles. Tests should be conducted at various flow rates in order to select the appropriate filter and establish process variables.

4.4 Extractables

The USP outlines a series of tests and guidelines for extractables from plastics. Typically, most filter candidates will pass these tests, but a relative measure between candidates can provide valuable information.

Potential extractable sources from sterilizing filters may include surfactant and wetting agents, additives used in the plastic component manufacture, manufacturing debris, and materials and oligomers of materials of construction.

Manufacturers can provide appropriate data on extractable levels and identities from their filters (Appendix B includes a detailed discussion). This may help users identify extractable components within their product filtrates. Extractables can be classified as toxic or nontoxic, and should be demonstrated to be nontoxic. It is the user's responsibility to demonstrate that the product does not contain objectionable levels of extractables from the filter. Processing conditions, including sterilization, must be considered when performing extraction studies.

Suitable analytical techniques for measuring extractable levels and types include "bulk" analytical methods, such as non-volatile residue and Fourier transform infra-red spectroscopy (FTIR), applied to an extract without fractionation, to avoid possible loss of unknown analytes in workup. Analytical techniques suitable for further measuring of extractable levels and types include gas chromatography (GC), high pressure liquid chromatography (HPLC), capillary electrophoresis (HPCE) and gas chromatography mass spectrometry (GC-MS). Where applicable, standardized and/or compendial methods should be followed, e.g., ASTM, USP.

Most filter manufacturers test for extractables using a standard solvent (typically water). The filter user is responsible for obtaining extractable data for the drug product formulation. When the product formulation precludes the use of standard analytical methodology, a suitable model may be used to measure the extractable levels. The model must, however, exhibit similar physical and chemical characteristics.

4.5 Chemical Compatibility

When considering chemical compatibility, it is important to include all of the filter system components under investigation. In addition to the membrane, these include support materials for the membrane, cartridge shell and housing material, and o-rings used to seal the cartridge and housing. A more subtle contributor to compatibility issues is the possible presence of filter component treatments.

Numerous chemical interaction possibilities exist in a filter system. The effects of these interactions must be adequately characterized prior to filter selection. A simple chemical compatibility chart will

often not provide enough information for predicting filter system compatibility, thereby requiring additional testing. Integrity testing is a "physical test" that relates to microbial retention and is a determinant of compatibility.

4.6 Thermal Stress Resistance

Steam is one of the major sterilization methods for filters. It is extremely important that the filter membrane and support structures be stable under the steam sterilization pressure, temperature and time process conditions. In addition, many processes may require the product filtration at elevated temperatures. Again, the filter must withstand the rigors of the process.

4.7 Hydraulic Stress Resistance

During product filtration, the filter will undergo varying pressure requirements. It may experience low to extremely high pressure differentials, due to the product processing characteristics. During validation, the process filtration pressures should be approximated. If the filter will be subjected to high hydraulic stress, this also should be simulated in the validation process.

4.8 Toxicity Testing

Filters should not be constructed of materials which are toxic or may affect product quality if released into the fluid stream. Filter suppliers typically provide toxicity testing data which may support customers' validation requirements. However, many filter users choose to submit typical stock filter samples for independent testing to supplement manufacturer information. Appendix B offers a detailed discussion of filter toxicity testing.

4.9 Bacterial Challenge Testing

The bacterial challenge test serves two major functions. The filter manufacturer uses it to classify filters as sterilizing grade if the filter provides a sterile effluent with a minimum of 10^7 cells of *B. diminuta ATCC 19146*/cm^2 of effective filter surface area.

Bacterial challenge tests also are required to validate the sterilizing filtration process of a specific product. The filter challenge test must be performed with actual product or, where justified, suitable surrogate fluid. This topic is dealt with in more detail later in this technical report.

4.10 Physical Integrity Testing

Integrity testing is required for all sterilizing filtration applications. Physical integrity tests are based upon the gas flow rate through a filter wetted with a suitable liquid, as a function of the applied test pressure. Hydrophobic filters used for liquid filtration also can be tested by measuring the membrane's resistance to water flow as a function of applied pressure. Manual and automated test methods are available. The chosen integrity test method and acceptance criteria must be validated and must correlate to bacterial retention.

5.0 PHYSICAL AND MECHANICAL CHARACTERISTICS

A number of characteristics should be considered in choosing a sterilizing grade filter. The filter's compatibility with the process is dependent primarily upon the materials of construction of the membrane, upstream and downstream supports, encapsulation materials and the cartridge sealing elastomers (o-rings and gaskets). While many of these materials meet a wide range of requirements, specific processes may favor one over another.

In addition to materials of construction, a number of other characteristics should be considered in a sterilizing filter. Some characteristics affect the filter's compatibility with a given process. The filter extractables (molecular and elemental contamination) should be considered seriously. Filter particle shedding also can be a factor. Filters have some extractables and may shed particles initially, but process compatibility helps to minimize this. Recognizing this, filter manufacturers generally recommend flushing the filter before use. Additionally, the filter should be sterilizable by the method of choice. In some cases, the filter must be able to withstand multiple sterilization cycles. The filter must be validated for its intended use, including sterilization and fluid stream processing. All filter components must meet applicable compendial requirements such as USP Biological Reactivity Tests, general chapters <87> and <88>.

Other physical characteristics which affect the filter's ability to perform properly include physical dimensions, configuration, end cap design (cartridges) and end connections (capsules). The physical characteristics are important from not only installation considerations, but because subtle differences can create bypass problems that could lead to process contamination.

5.1 Filtration Rate and Clogging (Throughput)

Sterilizing filter flow rates measure the amount of fluid flow through a given area, such as a square foot of media, cartridge or capsule, for a given period of time, usually one minute at a given pressure drop. Flow volumes typically are measured in terms of gallons, liters or milliliters. Alternately, the pressure drop for a given volume flow per unit of time may be expressed as pressure drop at a particular flow rate, e.g., psid/gpm. The sterilizing filter flow characteristic is determined by the amount of membrane surface area and the membrane's permeability. Membrane permeability is determined primarily by the total porosity (void volume), membrane thickness, and, to a lesser degree, effective pore size.

Several basic relationships exist between a filter’s flow rate and its porosity, thickness, effective pore size and surface area. The filter flow rate is proportional to the surface area of its membrane, but may not be linear. Generally, there is an inverse relationship between membrane thickness and flow. Flow rate also has a direct relationship with the membrane's effective pore size (retention) rating.

Construction of the filter device and housing design can affect its flow. For a fixed membrane area, permeability and construction, larger pore size rated filters typically provide higher flow.

The throughput or life of a sterilizing filter generally is determined by the membrane's capability to retain particles, and the amount of surface area exposed to the process. As the filter removes particles and bacteria, the flow begins to decline, unless differential pressure is increased. The

throughput life of a sterilizing filter is measured as the amount of time the filter can remain on-stream, before an unacceptably high differential pressure or loss of flow occurs. Pre-filters can appreciably affect filtration rate and filter life.

5.2 Fluid/Piping

The piping and cartridge housing used in the process must be compatible with process parameters and exhibit acceptable fluid dynamic principles.

5.3 Fluid/Filter

Many possible interactions exist between a process fluid and a sterilizing filter. The consequences of these interactions may manifest themselves in a variety of measurable changes. If the fluid and membrane are drastically incompatible, physical degradation of the membrane structure can lead to large membrane defects which will reduce filtration efficiency and cause contamination as the filter sheds particulates downstream.

Several membrane polymer types also can swell in the presence of certain chemicals, which may affect both flow and physical integrity test values. Measurable differences have been documented in both flow and physical test values as a result of this swelling. In addition to the deleterious effects of reduced flow through the membrane, physical integrity result changes can cause concerns about the membrane structure and its ability to retain particles.

Simple physical tests can detect these types of incompatibility. Consideration of any sterilizing filter exhibiting such characteristics after exposure to the process fluid should be re-evaluated.

5.4 Physical and Structural Limitations

Filters are limited by their physical strength and ability to retain integrity under handling and process stress conditions. While sterilizing membranes themselves may be relatively fragile, process filters typically incorporate resilient nonwoven polymeric layers and rugged plastic hardware which make the elements quite strong. These constructs still may be subject to damage under rough handling or severe process conditions.

There also are several pressure aspects that must be considered. The inlet pressure to the filter is important to ensure that there is no potential for structural deformation of the support structure. The differential pressure across the membrane must be accounted for, to comply with the filter manufacturer's recommended limits. Maximum differential pressure normally is stated as a function of temperature.

Another aspect that must be considered is the direction of the applied pressure. Filters may have different maximum pressure differentials in forward and reverse flow directions. These pressure differentials may also be temperature dependent. For example, a maximum limit of 75 psi (5 bar) differential at 25°C in the forward direction versus 50 psi (3.5 bar) differential in the reverse direction at 25°C and a maximum limit of 5 psi differential in either direction at 121°C.

When pressure limits are being evaluated, drug manufacturers should consider two areas of special concern. The first is in steam in place (SIP) sterilization pressure, where users should consider the effect of elevated temperature on the filter's differential pressure limitation. The second is hydraulic stress, which may increase dramatically if a valve is opened too quickly, or during a filling operation which does not incorporate a surge tank. In these cases, the filter may be exposed suddenly to pressures in excess of full line pressure. Differential pressure across the filter must not exceed the limit set by the filter manufacturer at the specified temperature.

6.0 STERILE FILTER VALIDATION/BACTERIAL RETENTION

Bacterial challenge testing, using ASTM method F838-83 or comparable methodology, is performed by the filter manufacturer to classify the retention capability of the filter membrane or device. The user, or designated test facility, then demonstrates complete microbial removal from each product or product family using a representative challenge microorganism. It is important to realize that these two filter testing concepts are not interchangeable and must be independently validated. The goal of these tests is to prove that the production process generates a sterile effluent.

6.1 Factors Influencing Microbial Retention

Those factors potentially affecting microbial retention include filter type (structure, base polymer, surface modification chemistry, pore size distribution, thickness), fluid components (formulation, surfactants, additives), fluid properties (pH, viscosity, osmolarity, ionic strength), process conditions (temperature, pressure differential, flow rate, time) and the specific characteristics of the actual bioburden in the product.

One should also consider the potential of product formulations or process conditions to affect cell size or other physiological or morphological microorganism attributes which might allow their passage.

6.2 Considerations for Bacterial Retention Validation Studies

Sterilizing filtration process validation should include "worst-case" scenarios, using the filter membrane or device selected for the product.

The following should be considered when conducting product bacterial retention validation on membrane filters.

1. When possible, at least three filter membrane lots should be included in product bacterial retention validation studies. The exact number of filters and the test design will depend upon the process.

2. At least one of the filter membrane lots used for bacterial retention validation should have a pre-filtration, water wet, physical integrity test value at or near the filter manufacturer's specification limit. Other filter parameters, including thickness, should be representative of typical production membranes.

3. The membranes used for fabricating process filters must meet or exceed the integrity test value established during product-specific bacterial retention testing.

4. Membrane physical integrity test values from bacterial retention validation studies should be included in the test report. The physical integrity should be determined prior to challenge testing, using water, product or other wetting fluid for which specifications exist.

The appropriate physical integrity test value for a specific product/filter combination can be established independently of the microbial retentivity test by testing a number of different lots of product against a number of different lots of the same filter membrane. Using this data, a physical integrity test value for routine physical integrity testing that takes into account the manufactuer's lower physical integrity test limits can be calculated .

Although the physical test can be established independently of the microbial retentivity validation, it is only valid provided it agrees with the physical integrity values obtained for the filters used during microbial retentivity testing.

5. If the test organism is recovered downstream of any filter after the product bacterial challenge, an investigation must be performed. If such investigation confirms penetration of the filter by the test organism and the filter meets its integrity test specification, then the applicability of this filter under these process conditions must be reconsidered.

6.3 Challenge Organism Selection Criteria

The challenge bacteria should be small enough to challenge the retentivity of the sterilizing grade filter and simulate the smallest microorganism that may occur in production.

A sterilizing filter is defined as one that retains a minimum challenge of 10^7 cfu of *P. diminuta*/cm² of filter surface (FDA, 1987). FDA does not recommend any single protocol or challenge organism for validating the sterilizing filtration process for a given product. The appropriateness of any bacterial retention test protocol used to validate product-specific sterilization processes using filtration is considered on an individual basis.

Historically, *P. diminuta*, recently reclassified to *Brevundimonas diminuta ATCC 19146*, has been selected as the microorganism of choice.

B. diminuta to be used for challenge may be confirmed to be $\approx$0.3 μm-0.4 μm in diameter by $\approx$0.6 μm-1.0 μm in length, via an optical or scanning electron microscope equipped with an appropriate measuring device. The size of the challenge organism must be confirmed by demonstrating passage through a 0.45 μm-rated membrane as a positive control for each challenge performed.

B. diminuta penetrates 0.45 μm-rated membranes in small numbers at high challenge levels, typically showing a titer reduction of 10^4-10^6 (LRV = 4-6) and a corresponding probability of penetration of 10^{-4}-10^{-6}, i.e., only $1/10^4$-$1/10^6$ cells will not be retained by the membrane and subsequently cultured to a detectable colony. While not sufficiently retentive to serve as a sterilizing filter, this efficiency is entirely acceptable for an analytical recovery membrane.

6.4 Culture Maintenance

B. diminuta ATCC 19146 can be obtained in lyophilized form from the American Type Culture Collection (ATCC). After reconstituting per ATCC instructions, stocks can be maintained either refrigerated or frozen on appropriate media per standard microbiological practice. Maintenance of process isolates to be used for challenge should be determined for each isolate.

6.5 Culture Conditions and Standardization

Two standard methods have been recognized as suitable for preparation and maintenance of *B. diminuta* for challenge testing (ASTM, 1983). These are the Saline Lactose Broth (SLB) and the Frozen Cell Paste (FCP) methods. Both methods have been found to be effective in producing suitable suspensions of *B. diminuta* of approximately 0.3-0.4 μm in diameter by 0.6-1.0 μm in length (ASTM, 1983).

Alternate media and culture methods may be equally valid to prepare *B. diminuta*, provided they produce single monodispersed cells, suitably sized to reproducibly penetrate 0.45 μm-rated membrane filters. Alternate culture methods must be validated.

6.6 Effective Challenge Concentration

The bacterial challenge concentration should provide a uniform challenge over the intended process time to yield a final challenge level of at least 10^7 cfu/cm^2 of filter surface area. It is critical that all process parameters be accounted for in calculating the challenge concentration, including flow rate, time and pressure. Challenge concentration (cfu/ml) should not be confused with challenge level (cfu/cm^2).

6.7 Challenge Level

Membranes rated at 0.45 μm may provide sterile effluent with *B. diminuta* challenge levels $<10^7$/cm^2. A challenge level of $\geq 10^7$/cm^2 is, therefore, the minimum requirement for a sterilizing grade filter (historically a filter rated at 0.2 μm).

This level was derived from Bowman's observations that *P. diminuta* could penetrate a 0.45 μm-rated membrane at $>10^4$-10^6 cfu/cm^2 challenge level, and her suggestion for qualifying 0.2 μm-rated membranes as "sterilizing grade" with *P. diminuta* at 10^7/cm^2 to assure minimally sufficient sensitivity to detect oversized pores (Bowman et al., 1967).

6.8 Aggregation

The preferred bacterial challenge suspension is one of monodispersed cells. Retention testing will suffer sensitivity loss directly proportional to the degree of cellular aggregation. Aggregation should be avoided when performing a bacterial challenge, as it is not representative of a potential "worst-case" condition where single cells are incident on the filter.

Challenge stock cultures can be screened for aggregation by optical microscopy. If significant

aggregation is observed, one means of dispersing the challenge cells is to immerse the stock culture in an ultrasonic cleaning bath filled with cold water for 10 minutes. The cavitation action of the bath is effective in deaggregating bacterial cells without loss of colony-forming ability. This effect should be confirmed by optical microscopy and viable count.

Absence of significant aggregation is also confirmed by demonstrating penetration of 0.45 μm-rated membranes as a positive control for each challenge test performed.

6.9 Culture Viability

Viability of the *B. diminuta* suspension should be confirmed, using a suitable recovery medium, such as tryptic soy digest or Muller Hinton Agar. When performing filter challenges, viable titer should be determined immediately prior to and after the challenge. The upstream bacterial titer should be determined using an accepted microbiological testing method, e.g., standard plate count. The same culture medium also should be used to determine any recovery of *B. diminuta* downstream.

6.10 Challenge Test Methods

A standard method for qualifying microbially retentive membrane filters is described by the American Society for Testing and Materials (ASTM, 1983). Some filter manufacturers have described alternative bacterial challenge test methods, which are available as independently published validation guide documents. Demonstrating retention of *B. diminuta* in an aqueous vehicle other than a specific product may not be sufficient to validate the sterilizing filtration process for that product. In these cases, alternate testing methods may be required, which will be discussed in the viability section of this report. The following discussion and Figure 2 outline the steps to be considered when validating a specific filter for sterilizing filtration of a given product.

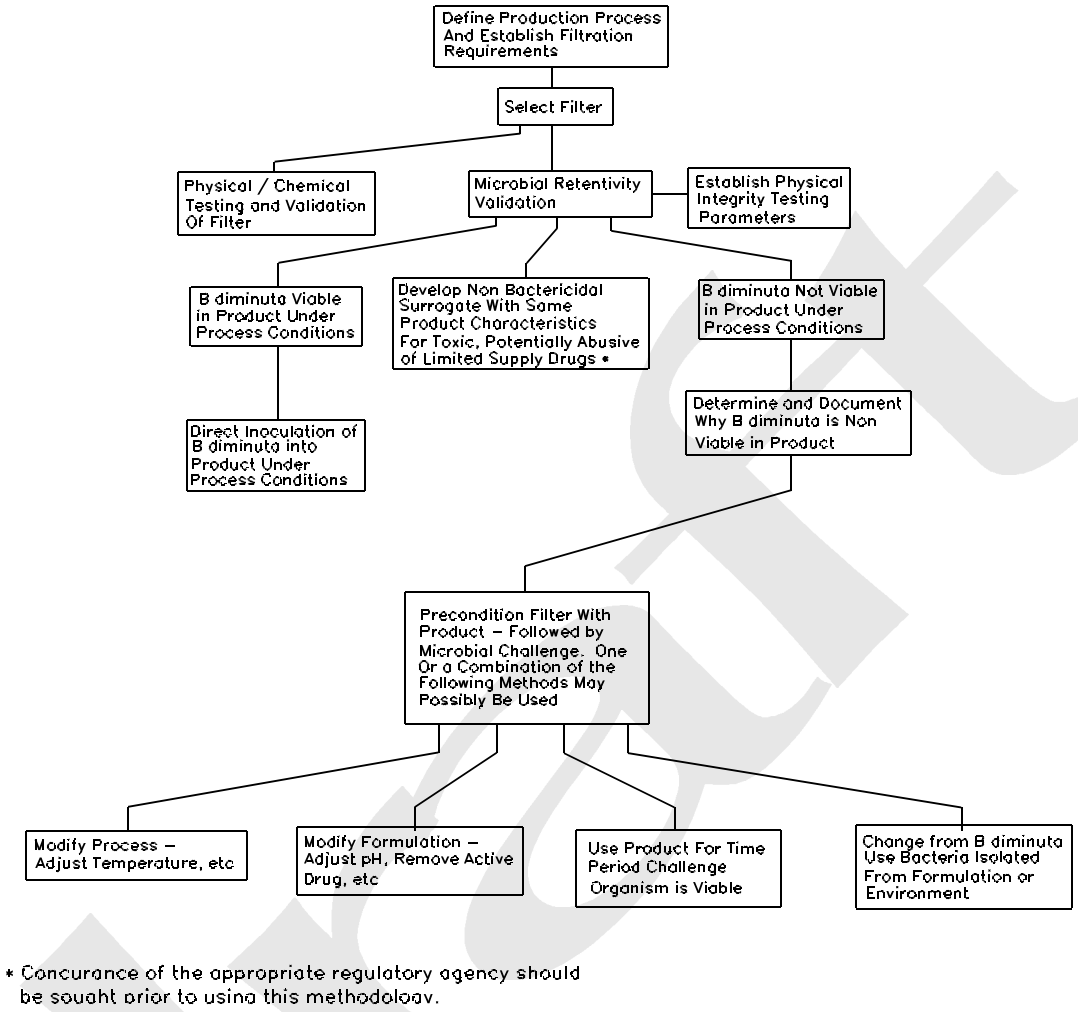

Figure 2 Key summarizing steps to be considered when selecting the appropriate validation strategy for a specific filter and product/process combination.

6.11 Test Organism Viability

The test organism's viability should be verified by direct inoculation into the product. It is recommended that the microorganism for viability testing be grown in the same manner as that used for challenge testing, in order to preserve its morphological and physiological characteristics. The test exposure time should equal or exceed the actual process filtration time. If, after the exposure time, no more than a one log reduction in count is noted, the formulation can be considered nonbactericidal. If a reduction in microbiological concentration of more than one log is noted, the product should be considered bactericidal, and an alternate testing methodology may be considered (see following discussion).

6.12 Testing Procedure and Protocol Development

After the test organism's viability within the product has been established, the appropriate challenge methodology and protocol should be developed. For any testing procedure selected, test conditions should simulate the production process as closely as possible. Since challenge testing generally is performed in a laboratory, the methodology is scaled accordingly. The flow rate can be scaled to an equivalent flow rate per unit area, as expressed in ml/cm^2 of filter surface area. If filtration is regulated by pressure, the challenge pressure should be equal to the processing pressure. If questions arise during protocol development regarding acceptability of a testing methodology, it may be advisable to contact the appropriate regulatory agency for guidance.

6.13 Nonbactericidal Processes and Fluids

Direct inoculation of the product with the challenge bacterium is the preferred method of validating microbial retention. This is possible with products and process fluids that have demonstrated no bactericidal effects from the product or processing conditions. For these processes, the product should be directly inoculated with the challenge organism, at a concentration sufficient to deliver a minimum concentration of 10^7 cfu/cm^2 of filter surface area, under actual processing conditions, including time, pressure, flow rate and other critical variables (e.g., temperature). Minimize product adulteration by the inoculum by keeping the inoculum volume as low as possible, e.g., below 10 percent of the total volume.

6.14 Surrogate Fluids

It may not always be possible to work with the actual product, due to its toxicity, abuse potential or limited supply. The surrogate fluid should match the product as closely as possible in terms of its physical and chemical characteristics, without adversely affecting the challenge microorganism. Critical variables could include pH, ionic strength, osmolality, viscosity and surface tension. When using this testing method, the surrogate fluid is inoculated directly and challenged against the filter, following the guidelines for testing nonbactericidal products. If questions arise during protocol development regarding the acceptability of using this testing methodology, it may be advisable to contact the appropriate regulatory agency for guidance.

6.15 Bacteristatic/Bactericidal/Nondispersive Challenge Fluids

Performing bacterial retention testing on bactericidal products makes it more difficult to answer both questions relating to validation: what effect does the product have on the filter, and what effect does the product have on flora within the product. Bacterial retention testing performed on a bactericidal formulation or under challenge conditions adverse to microbial viability (e.g., elevated temperature) may not produce valid results.

To overcome these obstacles, an alternate testing methodology is required. This may involve modification of the challenge fluid or challenge conditions or a combination of the two. Following are some suggested methods for testing the bacterial retentivity of filters with bactericidal products. Other methods may be equally appropriate. As with any validation procedure, appropriate controls must be run to ensure the reliability of the data.

To evaluate the potential effect of the product/process on the filter, the filter may be preconditioned with the product under actual processing conditions, including flow rate, pressure, temperature and time. This preconditioning may be performed by recirculating the product through the test filter in a closed loop system, or by a single pass through the test filter. Preconditioning is followed by the challenge, which may require product or process modification. Following are some suggested methods which may be appropriate for the bacterial challenge test, following filter preconditioning. It may be advisable to contact the appropriate regulatory agency for guidance if there are questions regarding bacterial challenge testing strategies.

6.15.1 Product Use at Reduced Exposure Time

In many instances, the challenge organism may survive in the product under normal processing conditions, but not for the total processing time. The product should be inoculated directly with the challenge organism, sufficient to deliver a minimum challenge level of 10^7 cfu/cm^2 of filter surface area. This challenge should follow after preconditioning the filter with the product under process conditions.

The challenge bacteria can be inoculated into a sufficient volume of product to challenge the filter over the required exposure time, under the chosen filtration conditions.

An alternative is to expose the challenge microorganism in the challenge fluid under static conditions. After exposing the challenge bacteria in the product at process temperature, and preconditioning the filter by recirculation of the product under model process conditions, the filter could be challenged under worst case processing conditions (differential pressure and flow rate), minimizing the duration of the challenge, provided the challenge microorganisms remain viable during the actual challenge and recovery period.

6.15.2 Modify Process

This method for challenging bactericidal products is the easiest to implement, since it may involve changing only a processing variable, such as temperature. Using this approach maintains the product/challenge organism interaction. It does not account for all possible process/product interactions, but should allow the use of the standard challenge organism.

After preconditioning the filter with the product under actual process conditions, the bactericidal component of the process, e.g., temperature, is removed and the bacterial challenge performed.

The product should be inoculated directly with the challenge organism, at a concentration sufficient to deliver a minimum concentration of 10^7 cfu/cm^2 of filter surface area, under the modified product processing conditions.

6.15.3 Modify Formulation

Another option is removing the bactericidal component from the product for the bacterial challenge test, following preconditioning of the filter with the actual product. This may be

as simple as adjusting the pH to a nonbactericidal range, or removing or diluting the bactericidal component. When using this approach, the processing conditions of time, flow and pressure must be duplicated, ensuring that the challenge microorganism is in contact with the modified product and the test filter for the appropriate time.

The modified formulation should be inoculated directly with the challenge organism, at a concentration sufficient to deliver a minimum concentration of 10^7 cfu/cm^2 of filter surface area, under actual product processing conditions.

When using this approach, it is necessary to validate the interfering component dilution through experimentation to a level that eliminates its interference with challenge microorganism growth. The drawback to this method is that the challenge fluid is no longer identical with the original formulation, but it may be suitable.

6.15.4 Resistant Indigenous Bioburden Use

Although a product may be extremely bactericidal to *B. diminuta* under normal processing conditions, other microorganisms may survive under the same conditions. Another bacterial challenge method for bactericidal products is the use of "indigenous bioburden." Indigenous bioburden consists of bacterial isolates from the manufacturing environment or the product formulation, which have demonstrated the ability to survive within the product formulation, under actual production filtration conditions.

Acceptable challenge bacteria should be capable of surviving or being propagated within the product to a concentration sufficient to deliver a minimum concentration of 10^7 cfu/cm^2 of filter surface area, under actual processing conditions.

If these indigenous bacteria are not propagated in the actual product, their morphological and physiological characteristics may not be representative of the actual process isolates.

6.16 Filter Medium versus Device

The decision to test membrane discs or a full-size process filter is dependent upon the study goal. If the study is to validate the bacterial retention efficiency of a particular membrane material, the use of a small test membrane disc is sufficient.

The testing methodology used for determining physical integrity of process filters must yield results which are meaningful in terms of the bacterial retention testing. If a relationship between two different testing methods is demonstrated, different physical testing methods can be used.

6.17 Pressure Differential and Flow Rate

Maximum process pressure differentials should be incorporated in model challenge conditions. This must be the actual differential across the test filter, and not the total available system pressure. The pressure differential across the test filter during validation challenge should meet or exceed the maximum pressure differential observed during processing (within the filter manufacturer's design

specifications). This validates the filter's ability to retain bacteria in the product and provide a sterile effluent up to or beyond the maximum pressure differential specified in the user's documentation. The maximum pressure differential obtained during bacterial retention studies should not be exceeded during actual production. The actual process flow rates should be incorporated when designing the model challenge conditions. It may not be possible to mimic pressure differential and flow rate simultaneously during validation. The user should determine which is more relevant to the specific process and develop a rationale to support the decision.

6.18 Duration

Several factors related to process time may affect bacterial retention by membrane filters. These include filter compatibility, maintenance of integrity, changes in the bulk fluid during challenge and hold times, and occurrence of time-dependent penetration. While maintenance of integrity over time can be qualified independently of bacterial challenge, e.g., by a validated non-destructive integrity test, a bacterial challenge under model exposure conditions, including time, also must be considered.

6.19 Downstream Sampling

To ensure validation of complete bacterial challenge retention, analysis of the entire challenge effluent is necessary. This can be done either by direct passage through an appropriate grade analytical membrane (or membranes) installed downstream of the test filter, or by filtrate collection in a sterile vessel and subsequent filtration through analytical membrane(s). Installation of an analytical filter should not prevent achieving the desired differential pressure across the test filter. A true differential pressure across the test filter is a critical variable, and care should be taken not to affect this pressure.

Sampling a portion of the filtrate is insufficient to validate a sterilizing filtration challenge. A small number of cells may have penetrated the filter and remain undetected in the portion of the filtrate not sampled and analyzed.

6.20 Assay Membrane Selection

Typically, either a 0.45 μm or a 0.2 μm-rated analytical membrane is used to recover *B. diminuta* or other bioburden challenge bacteria.

6.21 Results Interpretation

Effective challenges of sterilizing membranes with *B. diminuta* or native bioburden should achieve influent total levels of at least 10^7 cfu/cm² effective filtration area, and demonstrate a sterile effluent. If the filter pressure rating is exceeded prior to achieving a challenge of 10^7 cfu/cm^2, testing should be terminated and the total challenge determined. Test filter samples representing three filter manufacturer's membrane lots can be considered sufficient replicates to demonstrate repeatability. To be acceptable as a sterilizing filter, no passage of the challenge organism is permissible in any of the three test filters, and positive and negative controls must be valid. If passage is found and no assignable cause can be determined, retesting may be required to confirm penetration.

If an assignable cause for the failure can be determined, it is permissible to retest the suspect lot of

filters. A minimum of three filters from the suspect lot should be rechallenged. To meet the test, no passage of the challenge microorganism is permissible.

6.22 Product Bioburden

The native bioburden always must be identified, characterized and quantified, since these organisms may have the potential to penetrate sterilizing grade filters. Bioburden quantification provides a basis for calculation to demonstrate the actual challenge in the pharmaceutical manufacturing process.

The calculation of bioload on an area basis can be performed. The area-specific bioload $B_a = BV/A$, where B is the microbial count (cfu/ml), V is the total volume of the process stream to be filtered (ml), and A is the total filter surface area in cm^2. If the value of B_a approaches or exceeds the validated challenge level, there is a risk that microbial passage may occur. Therefore B_a must be kept significantly below this validated limit to minimize the potential for nonsterile product. The morphology of the isolated organisms also must be considered in determining a safety factor. The best means of controlling this biological challenge level to the filter is by controlling the raw material bioburden and/or process.

6.23 Filter Configuration Change

Once a specific filter membrane and product/process combination is validated for bacterial retention, future changes in filter configuration may not require revalidation, provided the following requirements are met.

1. The filter membrane has not changed.
2. The flow rate per unit area is less than or equal to the validated parameters.
3. The filtration pressure does not exceed the validated parameters.
4. The exposure time does not exceed the validated time.
5. Appropriate extractable data are available for the filter configuration selected.

7.0 INTEGRITY TESTING

7.1 Integrity Testing Theory

The main objective of a nondestructive physical integrity test is to determine the presence of oversized pores or defects which compromise a given filter's retention capability without destroying the filter. Such test procedures must correlate to bacterial retention. The bacterial retention test is a destructive test and cannot be used to verify the integrity of a filter that will be used in production.

Typical microporous membranes used for sterilizing applications are nonfibrous, porous structures. Although the pores are generally irregular in shape, their formation is characterized by a given pore size distribution. These irregularly shaped pores have effective diameters. Effective pore size is a key variable in the retention process. For passage of a specific contaminant to take place, there must be an opening (pore or defect) that allows the contaminant to pass through the filter. The filter manufacturer should set physical integrity test limits for a given filter type, by bacterially challenging membranes over a range of test values until passage is observed.

Gas flow properties of wetted filter membranes can be evaluated over a range of pressures. After completely wetting the entire filter membrane, gas is introduced onto the upstream side of the membrane at a low pressure. Capillary forces keep the liquid from being expelled from the pores. Most traditional integrity tests are based upon the fact that wetting the filter membrane with a suitable liquid reduces the flow of a test gas through it, particularly at low test pressures. As pressure is increased on the upstream side of the filter (with the downstream side open to atmospheric pressure), the upstream gas can dissolve into the wetting liquid. Since only atmospheric pressure is on the downstream side of the filter, gas can come out of solution because the pressure of the gas is lower downstream. This gas concentration gradient, due to the pressure of the gas on the upstream side, allows diffusion through the wetted membrane. Diffusion will increase as the pressure on the upstream side is increased. If the amount of gas that diffuses to the downstream is measured, the following characteristic graph can be obtained for the given membrane filter.

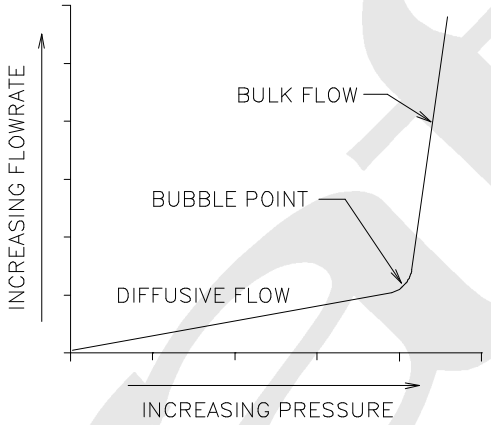

Figure 3 describes the relationship between measured air flow downstream of wetted filter membranes. The wetting liquid is held in the pores of the filter membranes by capillary forces. As gas pressure is increased on the upstream side, gas flow through the membrane can be measured on the downstream side of the filters.

Two characteristic portions of the curve act as the basis for membrane filter integrity testing. The linear portion on the low end of the pressure axis shows diffusive gas flow through the liquid held in the pores of the membrane. As the pressure is increased, there is a characteristic bend in the curve followed by another linear portion. This bend indicates the transition between diffusive gas flow and bulk or viscous flow. Bulk gas flow occurs after the bubble point of the largest pores has been exceeded. Above this point, the majority of gas flow is due to free flowing gas through open pores, with a minor portion of the flow due to diffusion through the pores of the membrane that are still wetted.

Looking specifically at quantifying the diffusive flow experienced during integrity testing of a thoroughly wetted membrane, test gas movement (at sufficiently low pressures) follows well established laws of diffusion. In its simplest form, the diffusive flux of test gas to atmospheric pressure, as a function of the test pressure applied, is described by

$$N = \frac{DHP}{L}\varphi$$

(Equation 1)

N = the diffusive flux of the test gas
D = the diffusivity of the test gas through the wetting liquid
H = the solubility coefficient of the test gas in the wetting liquid
P = the gauge pressure applied to the upstream side of filter system
ϕ = the overall porosity of the structure
L = the thickness of the wet layer (thickness of the membrane corrected by a "tortuosity" factor)

The molar flux should be expressed in moles per unit area and unit time, but since these are measured at a fixed set of atmospheric pressure and temperature conditions, moles of gas can be converted to volumetric (ml/min or cc/min) units. Because the wetting fluid, the test gas, the filter thickness, porosity and area are fixed, the expression for a volumetric diffusive flow further reduces to

$$F = HP$$

(Equation 2)

Note that the molar flux of gas is independent of the actual filter pore size, providing the pores are filled with the wetting liquid. Further, Equation 2 predicts a linear relationship between the diffusive flow and the applied test pressure. This relationship ceases to exist if the applied test pressure exceeds that required to displace the wetting liquid with gas. Once the bubble point pressure is reached, bulk or viscous flow of air will occur, in addition to the diffusive flow. This viscous flow of test gas through the pores from which the liquid has been displaced will obey Newton's laws of viscous transport, often modeled by the Hagen-Poiseuille equation for flow through cylindrical tubes.

$$Q = \frac{\pi \Delta P d^4}{128 \mu L}$$

(Equation 3)

Q = the volumetric flow rate of the test gas
ΔP = the applied differential pressure (or gauge pressure if collected at atmospheric conditions)
d = the capillary diameter of the pore
μ = the viscosity of the test gas
L = the length test gas must travel to the downstream side, or the length of wetted pores through the membrane

The pressure at which a given pore will open to viscous flow can be estimated from the cylindrical capillary relationship attributed to Laplace, often referred to as the "bubble point equation."

$$P = \frac{4k\gamma \cos\Theta}{d}$$

(Equation 4)

k = correction factor for the shape of the pores
γ = the surface tension of the wetting liquid
cos Θ = the contact ("wetting") angle between the liquid and the membrane
d = the diameter of the pores

To demonstrate the dependence on the liquid used to wet the pores and its interaction with the filter material, Equation 4 shows an inverse relationship between the pore diameter and the test pressure required to free it from the wetting liquid. If the wetting liquid and membrane surface chemistry are held constant, the expression can be simplified to read

$$d = \frac{K}{P} \qquad \text{(Equation 5)}$$

where K is a correction factor accounting for shape as well as wetting properties for a given membrane/liquid combination. The value of the constant, and therefore the bubble point, in relationship to its retentive capabilities for a given contaminant is established empirically.

The theory behind integrity testing can best be summarized by the extended integrity test profile in Figure 3, depicting the gas flow properties of a wetted filter as a function of the applied test pressure. The linear portion at the lower test pressures corresponds to the diffusive flow regime described by Equation 1 or 2, while viscous flow becomes the main transport mechanism for the steeper portion at higher pressures. The transition from diffusive to bulk flow (diffusive plus viscous flow) represents the maximum end of the pore size distribution, as the larger pores are being voided of their wetting liquid. The relative size of the membrane's largest pores can be estimated from the test pressure using Equation 4.

7.2 Relationship Between Integrity Test Results and Bacterial Retention

A physical integrity test is meaningful only when it can be related to specific filter retention characteristics. For sterilizing grade, 0.2 μm-rated membrane filters, the industry standard test is a microorganism challenge using *B. diminuta (ATCC 19146)*. The organism and minimum challenge level (10^7 cfu/cm^2 filter area) are specified in ASTM F838-83. This test method serves as the basis for testing done by filter manufacturers for membrane classification and filter lot release.

Validation of filter retention capability requires challenge testing to detect the passage of the challenge bacteria. Since such tests cannot be performed on a filter intended to be used in production, a correlation is made to a nondestructive physical integrity test in the laboratory.

Retentive capability can be assessed by challenging successively tighter samples of the specific membrane under standardized test conditions and analyzing the bacterial passage results. Typically, the retention pattern observed will allow identification of physical integrity test values above which the probability of bacterial passage is nil. The integrity test value established by this exercise is linked to the sterility of the filtrate. This scenario is depicted in Figure 4.

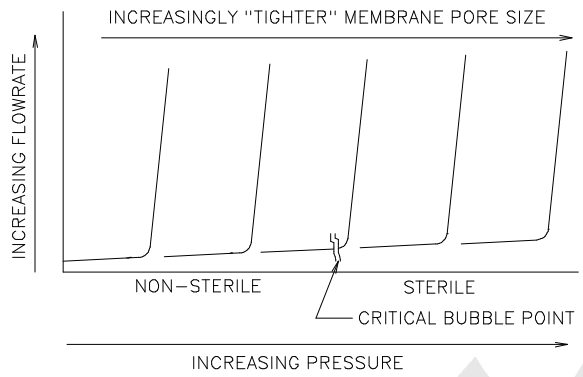

Figure 4 shows a typical relationship between the gas flow profile values and filtrate sterility from a series of five filters differing only in pore size. This demonstrates the transition from failure (filters allowing passage of the contaminant) to complete retention (sterile filtrate).

The traditional, nondestructive tests used to verify sterilizing grade filter integrity include bubble point, diffusive flow/forward flow, and pressure hold/decay (a variation of diffusive flow/forward flow). These test methods can be used for both hydrophilic and hydrophobic membrane filters and can be performed manually or with automated integrity test instruments. Each integrity test method has its advantages and limitations.

Bubble Point

The bubble point relates to the effective diameter of the largest pores present in the membrane and these pores, along with membrane thickness and pore tortuosity, directly influence the retention properties of the membrane. The usefulness of the bubble point diminishes as a function of increasing filter area, since diffusive gas flow below the bubble point tends to obscure it.

Diffusive Flow

While diffusive flow does not bear a direct relationship to pore size, reflecting the total porosity and thickness of the filter, bacterial challenge studies and the long history of satisfactory use show that an empirical and reliable correlation has been established between organism retention levels and diffusive flow values. Multipoint testing enables plotting of the diffusive flow curve from low pressures through the bubble point, combining the advantages of the bubble point and single point diffusive flow integrity tests. Small area membrane filters exhibit low diffusive flow, minimizing the usefulness of the test.

Validation Testing

Validation studies should establish the relationship between the chosen integrity test method and bacterial retention and serve as a basis for establishing appropriate parameters for the pre and post use integrity testing of production filters. Multipoint diffusive flow may prove useful in this regard since it reveals the slope of the diffusive flow curve, the bubble point and any shifts which may be due to the effects of different wetting fluids (e.g., water, product or surrogate). Once the relationships have been established for the filter type, reliable single point integrity testing of individual production filters is made possible.

Integrity Testing of Production Filters
The method for integrity testing of production filters should be chosen to provide reliable results based on the nature of the filter, product, and processing conditions. Bubble point, multipoint and single point diffusive flow tests can be used, recognizing that each has strengths and limitations that must be evaluated in terms of the particular circumstances of the test.

Filtrate Sterility Assurance
Integrity testing of sterilizing grade production filters pre and post use is a fundamental element of sterility assurance. However, integrity testing alone is insufficient to assure the sterility of the filtrate. At least two other elements must be in place: the production controls and quality assurance systems used by the filter manufacturer to ensure the quality and uniformity of the filter membranes and fabricated filters, and the validation studies used to show that a particular combination of product, processing conditions and sterilizing grade filter will meet the requirements of the bacterial challenge test.

Once the three elements are in place, integrity test limitations are minimized and any of the described integrity tests may be used, as appropriate. It is the responsibility of the filter user to assure all three elements are and continue to remain in place.

Appendix D contains a further discussion of filter integrity test methods.

7.3 Product-Wetted versus Water-Wetted Integrity Testing

Use of an appropriate wetting fluid, typically the filter manufacturer's recommended reference wetting fluid, is critical in obtaining a correct pass/fail integrity test value. In some cases, however, it may not be practical to follow the manufacturer's recommendation. Use of a drug product as the wetting fluid may change the integrity test values relative to those that would be obtained with the filter manufacturer's specified wetting fluid (e.g., water).

Testing is required to directly establish specific product physical integrity testing values. Integrity test results generated using product as the wetting fluid are compared to those using the filter manufacturer's recommended reference wetting fluid on the same membranes to determine a product-wetted specification. This product-based specification can be related to the bacterial retention capabilities of the membrane, assuming chemical compatibility between the product and the filter membrane has been demonstrated. Additionally, the reproducibility of these values should be established.

The procedure used to determine product-specific integrity test values should take into account the drug product and such factors as surface tension and viscosity. A representative lot of the drug product should be used to determine product attributes and for any additional testing that may be required. Integrity tests are conducted on three filter membranes with known water-wetted bubble point values (for bubble point testing), using the test product as the wetting medium. Using multiple filter membranes is important because the bubble point test depends upon the interaction of the fluid with the membrane. The ratio of the results in the two fluids allows determination of the physical integrity using integral filters with a range of integrity test values.

Determination of the ratio of the diffusive flow/forward flow test values in any two fluids (e.g., product and water) is not affected by the filter. Instead, the ratio depends on the diffusion constant and the solubility coefficient of the test gas in these fluids, not the fluid's interaction with the filter. Therefore, for the diffusive flow/forward flow test, one filter (or more) can be used and potential variability in test results can be addressed by averaging several replicate determinations in the reference fluid (e.g., water) followed by replicate determinations on the product-wetted filter.

Product-based integrity test specifications should generally be developed based upon experimental results rather than mathematical calculations (e.g., simply comparing surface tensions). Bubble point values are affected by both surface tension and contact angle, and diffusion values are affected by the solubility of the test gas in the wetting fluid and its diffusion constant through the wetting fluid.

It may not be possible to determine product-specific integrity test specifications using surface tension or bubble point measurements alone. Examples include for diffusive/forward flow testing, non-aqueous solutions and high viscosity fluids and, for bubble point testing, fluids containing surface active agents. The solubility and the diffusion constant of the test gas in product, and interactions between product components and the filter can affect product-specific integrity test specifications. In such cases appropriate tests, as described below, should be conducted on filter membrane discs having known water-wetted bubble point values (for bubble point testing) or representative filter assemblies (for diffusive flow/forward flow tests) using the test product as the wetting medium. The scaled-down study is only the first part of the validation. The second part is obtaining additional on-going product attribute data. This may include measuring the product surface tension periodically and comparing it to an established standard, or periodically measuring the bubble point ratio.

For determining product-specific bubble point specifications, testing is conducted as follows:

- Multiple samples of filter membranes are selected for testing. Since the bubble point refers to specific values relating to the membranes' pore size and the wetting liquid surface tension, filter discs can be used.
- Install the membrane discs in the appropriate holder and rinse them with water or specified solvent (solution).
- Perform the bubble point test on each disc.
- Either completely dry the filter discs or rinse the membranes with an appropriate amount of the product in question, to ensure complete removal of the wetting fluid used in the first integrity test.
- Perform the bubble point test with the product. Generate rationale and use to establish bubble point specifications.

The bubble point limit for product can be calculated using the following formula, which correlates the product-wetted filter membrane data to the filter manufacturer's minimum physical integrity testing limit.

$$PBP_{\min} = PBP_{avg}\left(\frac{MWBP_{\min}}{WBP_{avg}}\right)$$

(Equation 6)

Where:
PBP_{min} = Minimum Product-wetted Bubble Point
PBP_{avg} = Average Product-wetted Bubble Point
(Values determined on the membranes used for bacterial retention testing.)
$MWBP_{min}$ = Manufacturer's Minimum Water-wetted Bubble Point
(Filter manufacturer's published minimum validated water-wetted bubble point limit.)
WBP_{avg} = Average Water-wetted Bubble Point
(Values determined on membranes used for bacterial retention testing.)

Similarly, testing can be conducted to determine product-specific diffusive flow/forward flow test specifications for a high area pleated filter cartridge.

First, the test pressure for the product-wetted diffusion test can be calculated using the following formula:

$$TP_{PW} = PBP_{avg}\left(\frac{MTP_{WW}}{WBP_{avg}}\right)$$

(Equation 7)

Where:
TP_{PW} = Product-wetted Test Pressure
PBP_{avg} = Average Product-wetted Bubble Point (See Equation 6)
MTP_{WW} = Test Pressure specified by the filter Manufacturer for water
(or other test solvent)
WBP_{avg} = Average Water-wetted Bubble Point (See Equation 6)

Then, the product-wetted diffusional/forward flow limit can be determined as follows:

- Conduct several replicate water-wetted diffusion tests on the filter assembly (small scale devices may be used as long as an accurate determination of flow is possible) at the test pressure specified by the filter manufacturer.
- Dry the filter assembly and conduct several replicates of the product-wetted diffusion test at the appropriate test pressure determined above.
- Calculate the diffusion limit using the following formula:

$$DFL_{PW} = DFL_{WW}\left(\frac{DF_{PW}}{DF_{WW}}\right)$$

(Equation 8)

Where:
DFL_{PW} = Product-wetted Diffusional Flow Limit
DFL_{WW} = Water-wetted Diffusional Flow Limit
DF_{PW} = Product-wetted Diffusional Flow
DF_{WW} = Water-wetted Diffusional Flow

7.4 Upstream Testing without Downstream Manipulation Utilizing Automated Integrity Test Instruments

Some manual integrity test methods require downstream manipulations that could compromise the sterility of the system. Automated integrity test units perform the integrity test from the upstream (nonsterile) side of the filters. Use of automated integrity testers assures that sterility is not compromised during filter integrity testing and offers several advantages over manual tests. These include:

- increased sensitivity through the pressure transducers or mass flow meters
- minimized operator variability
- better reproducibility of results
- documented hard-copy printout of test results
- software security
- assurance of system sterility (upstream connections only)

Automatic integrity test equipment, both hardware and software, must be validated. Users should contact the instrument manufacturer for validation documentation and information concerning the validation of the particular instrument.

Validation requirements would be similar to those for other process test equipment, with similar Installation Qualification/Operational Qualification (IQ/OQ) testing. This will include:

- programming evaluation - test parameters, test methods, programming the unit and the tests
- unit sensitivity evaluation
- unit startup
- unit calibration
- performing the tests
- integrity test performance evaluation - bubble point, diffusive flow/forward flow, pressure hold
- testing other functions - volume determination, failure modes, rejecting invalid entries
- test printout evaluation
- computer software evaluation
- password protection qualification
- peripheral function evaluation - date/time clock, memory, cleaning

7.5 When a Sterilizing Grade Filter Should Be Integrity Tested

Whether to test filters in-place or externally will depend upon actual process requirements. The advantages of in-place testing were discussed previously. It generally is regarded as a CGMP requirement that filters or filter systems routinely be integrity tested both prior to and after use.

If one filter has been validated to achieve sterilization with a specific product, then the single sterilizing filter must satisfactorily pass integrity testing before and after use. However, to ensure against the loss of product due to potential failure of the sterilizing filter, many pharmaceutical manufacturers opt to use an additional sterilizing grade filter in the filter train. This additional filter must be satisfactorily tested before use, but does not require post-use integrity testing unless the primary sterilizing filter fails. In that case, the secondary filter must satisfactorily pass integrity testing

before and after use, if it is to serve as the sterilizing filter.

In those processes where redundant (or serial) filtration is a regulatory requirement or has been validated to achieve sterilization of a specific product, the filter train is considered to be a sterilizing unit and all sterilizing grade filters within it must satisfactorily pass integrity testing before and after use. It may not be possible to establish the pre-filtration integrity of either filter in a series after sterilization, because sterility downstream of the first filter may be compromised. In this case, the filters should be tested both prior to sterilization and after use.

The two filters can be integrity tested individually and, when required, can be aseptically connected. This ensures that both filters are integral. For processes requiring in-series testing (e.g., where both filters are sterilized in series), they still must be integrity tested individually. The first filter can be tested by connection to its inlet. The downstream side of this filter is the upstream side of the second, so all gas moving from upstream of the first filter, through either diffusion or bulk gas from a bubble point test, is now between the two filters. This is because the second filter also is wet and will not allow gas flow until either the pressure has increased to the point where diffusion can occur or the bubble point is exceeded. To assure that the downstream side of the first filter is at atmospheric pressure, the vent valve or integrity test port on the second housing must be opened to allow the gas to escape. The integrity test on the first filter then can be performed as usual, however the sterility of the downstream side of the first filter, the upstream side of the second filter and the connecting tubing may be jeopardized.

To test the second filter, there must be a valve between it and the first filter. Closing this valve isolates the first filter from the second. Attach the integrity test hose to the integrity test port on the second housing (with the vent valve that was opened for the test of the first housing closed) and integrity test the second filter as usual. If this testing approach is followed, all steps must be performed aseptically and the gas used for the test must be filter sterilized, to prevent contaminating the connection between the two filters. All valves must be completely open during sterilization to permit steam penetration.

Integrity tests can be performed prior to sterilization and, preferably, after sterilization. A presterilization test will confirm that an integral filter of the proper pore size has been installed. An integrity test after sterilization will provide the same information as a presterilization test, and check whether the filter was damaged as a result of improper sterilization procedures. If an integrity test is performed post-sterilization, steps must be taken to ensure that the downstream side of the system remains sterile. An upstream integrity test can be used when downstream aseptic conditions must be maintained. Assurance of microbial retention throughout critical fluid filtration must be confirmed by a post-filtration physical test.

7.6 Failure Analysis/Troubleshooting

If a filter fails an integrity test, the following steps are recommended, at minimum:

- Assure that the appropriate integrity test has been selected.
- Confirm that the correct test parameters are being used.
- Confirm that the correct wetting fluid is being used.

- Ensure that there are no leaks in the test system.
- Ensure that the process temperatures have remained within specification during testing.
- Confirm that the equipment has been properly calibrated.
- Assure that the test setup is properly assembled and functions properly.
- Confirm that the correct filter has been installed.
- Re-wet the filter according to filter specifications and repeat the test.
- If the filter integrity test fails again, after fulfilling the above criteria, the filter fails the test.

8.0 FILTER STERILIZATION

One of the major elements of a successful sterilizing filtration process is the sterility of the filtration assembly. Filter membranes and components may be susceptible to thermal, mechanical, chemical or physical damage, if not sterilized properly. The failure may be due to excess temperature, high differential pressure or degradation of the materials of construction. It therefore is recommended that filters be sterilized according to the manufacturer's recommendations, and that each process be validated for its intended use.

8.1 Steam Sterilization

The most common sterilization method is steam under pressure. This usually is done in an autoclave or in situ (Sterilization in Place, SIP). The sterilization process is complex due to the poor heat transfer characteristics of the plastic components, the large void volume within the filter pores which trap air, torturous paths for steam penetration and the stability of the materials of construction at elevated temperatures. Steam sterilization validation studies must demonstrate that the sterilization cycle results in a probability of nonsterility of 10^{-6}.

Steam sterilization parameters must be considered carefully when designing a sterilization process for any filter. Refer to the filter manufacturer's literature for additional information.

8.1.1 Autoclave Sterilization

Most sterilizing filters are qualified for steam sterilization by their manufacturers to at least 125°C. Temperatures higher than this may make many of the plastics used for filter construction unstable, and may affect the physical integrity of the filter or increase the level of extractables. However, higher temperatures can be used if it can be demonstrated that the filter is not adversely affected.

Filter assembly preparation for autoclaving is very critical. It is important to protect the sterile filter components with a suitable microbial barrier for sterilization. The barrier must allow the steam to enter the filter for sterilization. It is critical that the filter inlet and outlet are free to breath, so that differential pressures are not created during the various sterilization stages which may damage the filter membrane. If the filter is sterilized outside its housing, it should be properly supported during sterilization to prevent deformation. The user should refer to the manufacturer's specifications when developing a sterilization cycle for the filter assembly.

One of the major difficulties encountered in autoclave sterilization is air removal. Air can be removed effectively at the cycle initiation by pulling a series of vacuums or flushing the chamber with steam. Filter assemblies should be sterilized at a minimum of 121 °C. Larger filters or filters attached to tubing or ancillary equipment may require adjustments to the sterilization parameters. For all applications, the required sterilization cycle of each loading pattern must be validated.

8.1.2 Sterilize-in-Place

Another method of sterilization using steam is Sterilize-in-Place or Steam-in-Place (SIP). This sterilization method is limited to filters in housings that can withstand steam pressures of 15-30 psig. However, the differential pressure limit established by the filter manufacturer for each type of filter should not be exceeded.

Condensate removal from the filter and equipment is a major concern with SIP. Design of the system to insure free drainage and condensate removal is extremely critical. Although some filters will allow reverse steam flow, it generally is recommended that steam flow during sterilization be forward, to reduce the probability of damage. Steam pressure should be increased gradually, to reduce thermal shock to the filter and enhance condensate removal. To regulate the sterilization temperature within the filter and system, the condensate bleeds and supply pressure regulator should be modulated until the desired sterilization temperature is achieved, while minimizing the differential pressure across the filter. It is important that a small continuous steam bleed be maintained from all bleed points throughout the sterilization period.

At the completion of sterilization, it is extremely critical that a positive pressure be maintained by air or other suitable gas during the cool down period. If a dry filter and system are required, the gas should be allowed to bleed freely from all condensate points until the system is dry and cooled to operating temperature. It is extremely important that a free flow of gas be established through hydrophobic filters used to vent tanks during the cooling process. Failure to establish a free flow of gas may result in subsequent damage to tanks and equipment.

8.2 Irradiation Sterilization

This method of sterilization has several advantages: high sterility assurance level; no residual gas components; dry filters; and minimal interference by packaging components with the sterilization process. However, it also has some negatives: some filter components are incompatible with irradiation; and this process is not as readily available as others.

Some polymers used in filter manufacture have limited resistance to irradiation sterilization. Irradiation sterilization, as all sterilization methods, requires validation to establish sterility and stability. The spores of *Bacillus pumilis* have been established as the indicator microorganism of choice. The methodology for establishing the dose for components sterilized in this manner is outlined in the 1994 AAMI document *Sterilization of health care products-Requirements for validation and routine control-Radiation sterilization*.

8.3 Gas Sterilization

The most common sterilization gas is ethylene oxide, which is used at 100 percent or diluted in a carrier gas. In addition to environmental and safety concerns, other factors are formation of ethylene chlorohydrin and ethylene glycol reaction products, and ethylene oxide that may remain in the filter matrix.

Parameters influencing gas sterilization are preconditioning factors, gas concentration, relative humidity, temperature and time. Since there is no established correlation between lethality and the interrelationship between the sterilization parameters, destruction of a suitable biological indicator is the preferred method for validation and process monitoring. Spores of *Bacillus subtilis* have been established as the indicator of choice.

For successful filter sterilization by ethylene oxide, the filters must be dry and wrapped in a manner which allows for the free entry of humidity and gas into the filter matrix. Following sterilization, the package also must permit the removal of residual gas and other undesirable byproducts. The gas concentration used, as well as the other parameters, must be established during the development and validation processes.

Appendix A

Pore Size Estimation

Pore size is most commonly regarded as the diameter of a pore. Not all membrane pores are exactly the same size. The range of pore sizes and the relative population of each size often is described as the pore size distribution.

A common method for estimating a membrane's largest pore size is the bubble point. In this test, a membrane is wetted with an appropriate test fluid and gas pressure is applied to one side. The pressure at which the first stream of bubbles (or first measurable bulk gas flow) appears is the bubble point. The inverse pressure/pore size relationship is given by the following equation:

$$D = \frac{CS}{P}$$

(Equation 9)

Where:
D = Pore diameter
C = Constant
S = Surface tension of wetting fluid
P = Test pressure

For any pore structure other than a cylinder, modifying factors must be added to the equation. Since membranes do not have a uniformly cylindrical pore structure, the bubble point is a measurement of the complex interaction of the wetting fluid, membrane and test gas. Therefore, while the general inverse relationship holds that higher bubble point pressures indicate tighter membrane structures (smaller pore sizes), an actual pore diameter cannot be determined definitively from a bubble point measurement.

Single point diffusional flow tests commonly are used to verify a cartridge filter's integrity in a process stream. In this test, a wetted membrane is subjected to a specified pressure below the bubble point, and gas flow through the membrane is measured. While the results of these tests have been shown to relate to bacterial retention, they do not measure pore size. A diffusional flow test is a reflection of the test pressure, membrane thickness and porosity, and test gas diffusivity across the wetting liquid.

Appendix B

Toxicity and Filter Extractable Testing

Assessment of extractables which may be introduced during filtration is an important consideration in evaluating the suitability of a filter for a particular application. In addition to the potential adverse effect of extractables on the filtered product, the presence of extractables may be related to degradation of the filter, ultimately affecting its ability to perform as intended.

It is important to assure that the filter system does not release toxic components into the process stream. Since most microporous membranes and filter devices are made from plastics and other polymers, plastics toxicity testing is indicated. An appropriate test battery is described in the USP 23, <87> Biological Reactivity Tests, In Vitro and <88> Biological Reactivity Tests, In Vivo. These tests involve a static soak in various solvents. The extracts and the plastic components themselves are evaluated in two animal models and in cell culture. This testing is done to ensure filter membrane and support materials do not adversely affect the safety or stability of the filtered product.

Analytical testing of polymeric compounds which make up the filter ensures that no extraneous contaminants are present, other than those consistent with filter component materials. A basis for this testing is USP <661> Containers. Although this chapter refers to containers, the testing may be applied to any plastic component used in the manufacture of sterile injectables. Additional Testing is recommended as the amount of filter material used in the USP test does not allow a true perspective of what may potentially be transferred to the processing stream. Usually, another extraction procedure involving an exhaustive, low-volume soak (either dynamic or static) in an appropriate model solvent is performed. It is important to include the pretreatment of the filter, usually autoclaving or steam-in-place, as well as the processing conditions to which the filter will be exposed, e.g., elevated temperatures. Therefore, the user should be actively involved in designing the appropriate studies, based on the specifics of the process.

Components extracted during the above testing should be identified and quantitated insofar as possible using appropriate analytical techniques (1-3). The filter extracts can be analyzed for materials such as high and low molecular weight oligomers, additives and low volatility compounds. Standard analytical techniques such as total organic carbon (TOC), Fourier transform infrared spectroscopy (FTIR), gas chromatography-mass spectrometry (GC-MS) and reverse-phase high performance liquid chromatography (RP-HPLC) may be used.

The amount and type of extractables should be considered when determining the suitability of the filter for the intended application. Flushing the filter prior to use often reduces the extractables potentially entering the process stream.

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Appendix C

Filter Validation Recommendations
Responsibilities of the Filter User and the Filter Manufacturer -
General Industry Practices

Criteria		Filter User	Filter Manufacturer	
		Filter Device	Membrane	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)		
Bacterial Retention/ Integrity Test Methodology		(V)	(Q)	(Q)
Effects of Chemical Compatibility on Filter Integrity		(V)	(Q)	(Q)
Toxicity Testing			(Q)	(Q)
Extractable		(V)	(Q/R/L)	(Q/R/L)
Effects of Sterilization Methods on Filter Integrity		(V)	(Q)	(Q)

Q = Qualification Testing
V = Validation Testing - Process Specific
R = Recommendation for Validation
L = Filter Lot Specific Release Criteria

Establishment of Process Requirements
- Methods
- Scaleability
- Establishment of test limits

* SLB = Saline Lactose Broth

Appendix D

Nondestructive Physical Integrity Test Methods

Bubble Point Test

The bubble point test is based upon the premise that membrane pores can be represented as a multiplicity of capillaries, and the knowledge that liquid is held in a capillary tube due to surface tension and other factors. The minimum gas pressure required to force liquid out of the pores is a direct function of pore diameter. The bubble point test is used to determine the pressure at which there is bulk flow of the test gas through the largest pores in the membrane, which are the first from which liquid is displaced. Since the observed bubble point is a function of the wetting liquid contact angle and the pore size, results are specific to each membrane polymer/surface chemistry and structure. While bubble point is an indication of the largest pores, it cannot be used directly to calculate their diameter. The bubble point test also does not indicate the number of largest pores, which can affect the probability of bacterial retention.

The test can be performed manually by subjecting a wetted filter membrane to gas pressure on the upstream side. Tubing on the membrane's downstream side is immersed in a liquid. The differential pressure at which a steady stream of bubbles, or in some cases the displacement of liquid, is subjectively observed is the bubble point. The typical test equipment for performing a manual bubble point test is described in Figure 5.

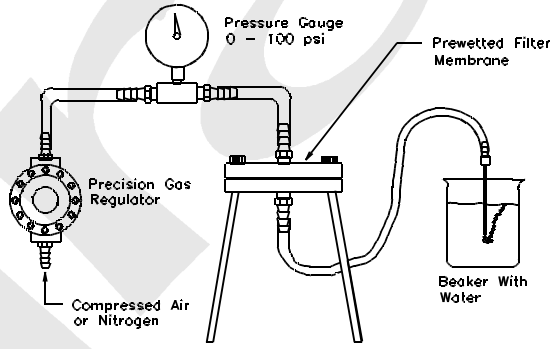

Figure 5. Typical manual bubble point test setup.

The manual bubble point test is a sensitive visual technique for disc filters and other small surface area membrane filters, and is performed routinely as a quality assurance test for cast membranes. The bubble point test detects minor defects and out-of-size pores, and can be correlated with the bacteria retention test.

Points to Consider when Performing the Manual Bubble Point Test:

- Pressure should be increased slowly in a step-wise manner.
- Allow pressure to stabilize at each step in the pressure increase. The true bubble point value may be overshot if pressure is increased too rapidly. The test cannot be repeated without rewetting the membrane.
- Minimize downstream connections and avoid kinking the downstream tubing.
- Check for leaks in the system.
- Clearly define the bubble point as bulk air flow. The most common error is misidentifying bubbles that are not the result of bulk flow. Some bubbles will result as a result of diffusion across the wetted membrane. Free flowing air is the indication, not the first bubble.
- Manually performed bubble point test determination can be subjectively different from one operator to another, therefore, operators should be properly trained to conduct the test and to interpret test results.
- Keep upstream volume to a minimum. With extremely high upstream volumes, longer stabilization between pressure increases may be required.
- Maintain the temperature within the required range.

Advantages

- quick (short stabilization time)
- directly related to the membrane pore size
- easy to perform manually, for small area filters by a trained operator
- effective for filters with low membrane surface areas

Limitations

- Sensitivity is limited for large membrane area systems when test is conducted manually.
- Manual methods may confuse diffusive flow below the bubble point with the actual bubble point.
- Manual testing requires downstream, sterile side manipulations.

Automated integrity test units determine the bubble point using incremental pressure hold tests or direct mass flow measurements. The pressure hold tests are performed at increasing pressures, with the bubble point identified as some point where pressure decays become non-linear, based on an algorithm specific to each instrument manufacturer.

Note: Filters may plug during processing. As filter membranes begin to plug, larger pores may be blocked, which can increase the bubble point. This increase should not be evaluated as a change in the filter cartridge's integrity. Should the integrity of the filter be lost during processing, a decrease in bubble point would result. (Theoretically, if a filter is completely blocked with particles, there would be no achievable bubble point value.) The same phenomenon similarly would decrease diffusive flow (forward flow). This emphasizes the importance of the pre-use integrity test.

Diffusive Flow/Forward Flow Test

This test method is generally used for membrane filter systems with large surface areas, such as pleated filter systems with multiple filter elements. In a wetted membrane filter under pressure, gas molecules migrate through the liquid-filled pores at differential pressures below the bubble point via a diffusion process following Fick's Law of Diffusion. The overall rate of diffusion for a filter is proportional to its membrane surface area and the solubility and diffusivity of the test gas in the wetting liquid. In small surface area filters, such as flat disc filters, this gas flow is very low and may not be measurable. In large surface area filter systems, it is significant and can be measured to perform a sensitive integrity test.

The diffusive flow/forward flow test quantitatively measures the sum of diffusive flow through the membrane and flow through any open pores. The user should use the manufacturer's recommended testing method for each filter. Diffusive flow measurements may be performed downstream of the wetted membrane under constant test pressure, or on the upstream side by measuring the air flow required to maintain constant test pressure. These tests are performed on membrane filters by first wetting out the filter with an appropriate liquid, and draining excess fluid. The upstream side of the filter is pressurized and the diffusive flow is measured. Test equipment for a diffusive flow/forward flow integrity test with flow measurement on the downstream side is illustrated in Figure 6. The inverted burette filled with liquid for the collection of diffused gas can be replaced with an appropriate gas flow meter.

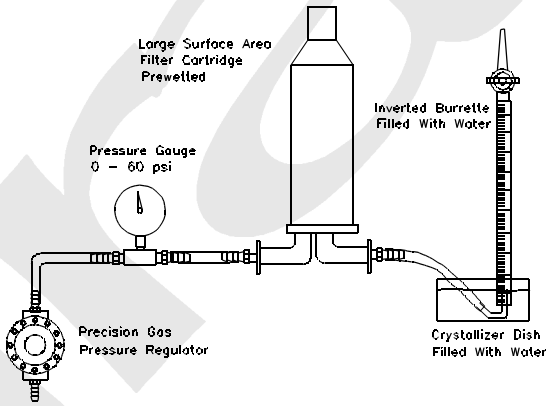

Figure 6: Typical test setup for manually determined diffusive flow rates.

Points to Consider when Performing the Diffusive Flow/Forward Flow Test Manually:

- Minimize downstream connections and downstream volume. Avoid kinking downstream tubing.
- Check for leaks in the system.
- Use a volume collection vessel with the proper resolution.
- Keep upstream volume to a minimum. With extremely high upstream volumes, proper stabilization time must be allowed.

- Maintain a stable testing temperature throughout the testing period.
- Allow sufficient equilibration time after pressurizing the system.

Advantages

- Increased sensitivity for large membrane surface area systems when test is conducted manually.
- Objective, quantitative measure of gas flow.
- Does not require downstream manipulation when performed in upstream test mode.
- Does not require upstream volume determination.
- Correlatable to bacterial retention.

Limitations

- More sensitive to temperature fluctuations than bubble point test.
- Requires downstream manipulations when performed in downstream mode.

Pressure Hold/Pressure Decay Test

The pressure hold (or pressure decay) test is an indirect method of upstream diffusive flow/forward flow testing. In this method, the filter housing is pressurized to a predetermined setting, then isolated from the pressure source. Gas diffusion across the membrane is quantitatively measured as a decay in pressure over a specific period of time. The allowable pressure drop is unique to a given filter and system and is calculated by taking into account the diffusive flow specification at a given pressure and constant temperature, the upstream system volume and the duration of the test. This test has been indirectly correlated to bacterial retention.

The pressure hold/decay test measures the diffusive gas (typically air or nitrogen) flow through all the wetted pores, and the bulk gas flow through larger nonwetted pores, when applying a predetermined gas pressure to a wetted filter. Pressure decay on the upstream side is then measured, after isolating the incoming gas supply. The pressure decay is a function of the gas flow through the filter. If the pressure decay is below the maximum allowable value, the physical integrity test passes. If the decay and concomitant pressure drop exceed the correlated maximum allowable value, the test fails.

Upstream integrity tests are particularly useful in critical fluid applications since they can be performed without compromising the sterility of the downstream system. The pressure hold/decay test will confirm the integrity of the entire assembly, including housing seals. Therefore, failure analysis for lack of seal integrity is required prior to reaching the conclusion that the filter element itself is not integral.

In performing the pressure hold/decay test, the physical conditions must be held constant, as the test is affected by interactions of temperature, system volume and filter area. The pressure hold/decay test equipment is illustrated in Figure 7. This test commonly is performed preprocess, post-sterilization of the filter assembly, and again after sterilizing filtration.

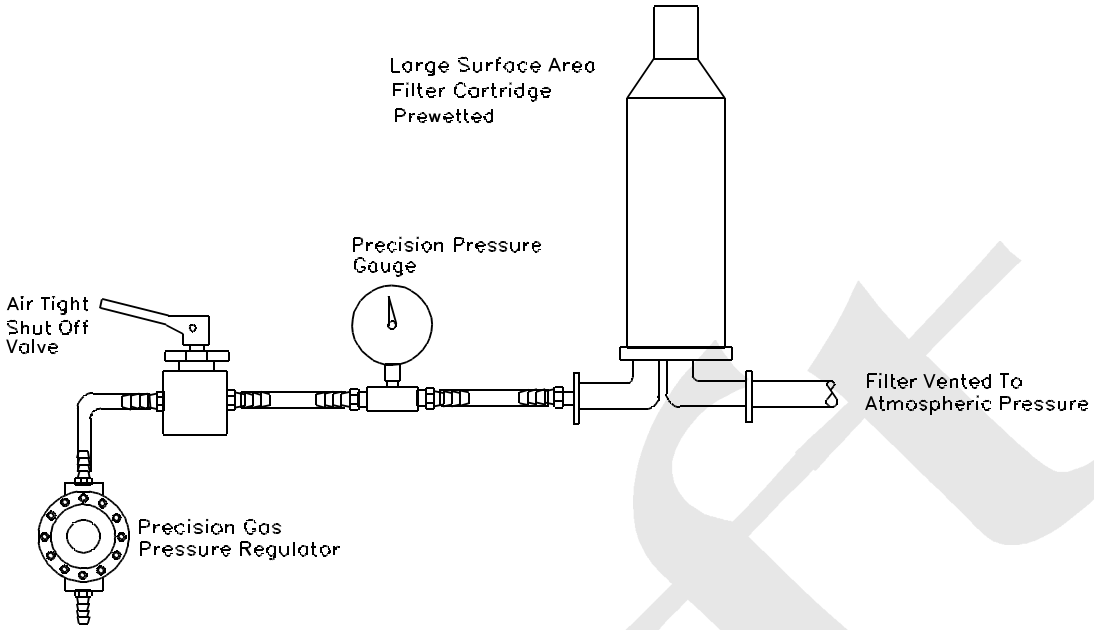

Figure 7: Typical test setup for the pressure hold/decay test.

Recommendations when Performing the Pressure Hold/Decay Test:

- There should be no leaks in the system.
- Upstream volume must be determined or accounted for to assure a proper maximum allowable pressure drop calculation. It is recommended that upstream volume be kept to a minimum.
- The downstream side of the filter is vented to the atmosphere.
- Allow sufficient stabilization time before beginning the test; stabilization time increases as upstream volume increases.
- Use a pressure gauge with the proper level of accuracy for determining the pressure drop during testing.
- Test time may need to be extended if the pressure drop rate is extremely small relative to the sensitivity of the gauge.
- Maintain the specified temperature and assure that the temperature does not change during the integrity test. Note that lower upstream volumes will result in increased sensitivity to temperature changes.

Advantages

- For large volume systems, with large allowable diffusion rates, pressure drop measurement for the fixed upstream volume is easy to perform.
- No downstream manipulations are required.

Limitations

- For a given filter area, the sensitivity of the test decreases as the upstream volume of the

system is increased.
- The resolution of the test is limited by the resolution of the pressure sensor used.
- Process conditions for the test, including temperature and gas used for pressurization, must be defined for the issuance of integrity test values. The upstream system also must be defined. This would include the housing used and the number of cartridges in it, as well as the volume.

Correlation Between Pressure Hold/Decay and Diffusive Flow/Forward Flow

Diffusive flow prior to the initiation of any bulk flow through the first opened pore can be expressed by the following equation, derived from Fick's Law and Henry's Law.

$$V_s = (M/\rho_S) A_F \frac{D(p_2 - p_1)K}{H\,L}$$

(Equation 10)

The measured diffusive flow, resulting from a specified differential pressure, can be related to the microbial retentive capability of a filter membrane.

The relationship between this diffusive flow, measured in the nondestructive Pressure Hold/Decay Test, and the destructive microbial challenge test is the objective of the validation for each filter medium and each filter configuration.

Equation 10 shows that diffusive flow is proportional to the difference between the test pressure and atmospheric pressure. Since the pressure in the upstream volume decreases, due to the effluent diffusive flow, the flow also decreases.

From the ideal gas equation

$$p_2 V_{up} = NRT$$

(Equation 11a)

the change of state per time dt can be derived:

$$\frac{dp_2}{dt} V_{up} = \frac{dN}{dt} RT$$

(Equation 11b)

With

$$-V_s \rho_s = \frac{dN}{dt}$$

(Equation 12)

and equations 10 and 11b can be written as:

$$\frac{-dp_2}{dt}\frac{V_{up}}{RT} = \frac{MA_F D(p_2 - p_1)k}{LH_C}$$

(Equation 13a)

Hence,

$$\frac{1}{p_2 - p_1}(-dp_2) = \frac{RT}{V_{up}} MA_F \frac{D}{LH_c} k dt$$

(Equation 13b)

and carrying out the integration between limits and simplifying yields the result:

$$p_{2I} - p_{2II} = (p_{2I} - p_I)(1 - \exp[\frac{-RT}{V_{up}} MA_F \frac{D}{LH_c} k(t_{II} - t_I)])$$

(Equation 13c)

To obtain an equation for the pressure decay (as a function of volume flow in the standard state) , introducing equation 10 in 13c and using $p_S = \rho_S$ RT/M yields:

$$p_{2I} - p_{2II} = (p_{2I} - p_I)(1 - \exp[\frac{-(t_{II} - t_I)}{V_{up}} \frac{V_s p_s}{p_{2I} - p_I}])$$

(Equation 14)

Measurements of pressure and time are necessary for the determination of Vs because the retentive capability of the filter membrane is related to the flow Vs, as shown in Equation 14. Additionally, the upstream volume must be known and the temperature in the upstream volume must be constant.

Nomenclature for Symbols Used in Equations 10-14

A_F = area of the filter
D = diffusion constant for gas/liquid system
Hc = Henry's Law coefficient

k	=	membrane specific correction factor
L	=	length of diffusion path
N	=	moles
M	=	molar mass
p_1	=	atmospheric pressure
p_2	=	upstream pressure
$p_2 - p_1$	=	differential pressure
P_{2I}	=	pressure at t_I
P_{2II}	=	pressure at t_{II}
p_{2I}-p_{2II}	=	pressure decay
$p_{2I} - p_I$	=	entered test pressure
R	=	ideal gas constant
T	=	temperature
t_{II}-t_I	=	entered test time
V_{up}	=	upstream volume
V_s	=	standard volume flow
ρ_s	=	density of gas in the standard state

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