PDA Journal of Pharmaceutical Science and Technology

PDA

AN INTERNATIONAL ASSOCIATION FOR
PHARMACEUTICAL SCIENCE AND TECHNOLOGY

PDA TECHNICAL REPORT NO. 40

STERILIZING FILTRATION OF GASES

December 4, 2004

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Sterilizing Filtration of Gases

Technical Report No. 40
Supplement
Vol. 58 , No. S-1
January/February 2005

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1. INTRODUCTION/SCOPE STATEMENT

Sterilizing filtration of a process gas stream is defined as the complete removal of all microbiological contaminants, excluding viruses. Under certain circumstances, other contaminants such as viruses and plasmids can also be removed by filtration. Thus, in the pharmaceutical industry, particularly in the production of parenterals, there is a wide range of processes for which sterilizing filtration of air or other process gases is appropriate and applicable.

Early and careful screening of potential filter types and configurations can result in fewer technical and regulatory problems, fewer delays, more efficient processing and greater sterility assurance. Although other types of filters can be employed in the control of particulate matter and removal of liquid droplets by coalescence, the focus of this technical report is limited to hydrophobic membrane filter elements. The objective is to assist the reader in the selection, qualification, and validation of a filter that is appropriate for the application on hand. While most gas applications use hydrophobic filters, this does not preclude the use of hydrophilic filters in dry gas systems. For further information on the use of hydrophilic filters, refer to PDA Technical Report No. 26.

This report is intended to complement PDA Technical Report No. 26: *Sterilizing Filtration of Liquids***, and like other PDA Technical Reports it should be considered an educational guide rather than a mandatory or implied standard.**

2. HISTORICAL BACKGROUND

Filtration of air and other gases has always been used in a wide range of applications within the pharmaceutical and biotechnology industry. The first generation of gas filtration used depth filters, such as cotton wads or glass fiber, ranging in scale from small plugs within tubes or bottles to large packed towers. Typically, such depth filters were used in conjunction with elevated temperatures to prevent condensation of moisture within the filter medium.

The second generation of gas filters consists of depth cartridges of borosilicate or polypropylene composition. These cartridges are available in configurations that are easier to use, and they provide improved operator handling and safety. They also have more consistent retention characteristics as a consequence of improved nonwoven media (fleece) technologies and more appropriate methods of construction. The retention capability of such cartridges can typically be verified by oil droplet aerosol challenges such as the DOP (dioctyl phthalate) test (discussed later in Sections 8.3). Nevertheless, the depth media of these second-generation cartridges continues to present some limitations, particularly with regard to the potential for bacterial breakthrough. This led to the development of hydrophobic membrane filter elements.

Compared to the earlier depth media, hydrophobic membrane materials have the advantage that they are inherently more resistant to water blockage. While some of the early membranes had to be rendered hydrophobic by silicone coating or similar surface treatment, membranes made of inherently hydrophobic polymers have been available for over two decades. The most common polymers used for the production of hydrophobic membranes are polytetrafluoroethylene (PTFE), polyvinylidene fluoride (PVDF), polypropylene (PP), and polyethylene (PE). Hydrophobic membranes can be produced in more defined ranges of retention ratings than depth media, and their retention capability is considerably less dependent on operational parameters such as moisture content or differential pressure imposed during their use. Filter elements are available in various user-friendly configurations, including tubular, stacked disk, and pleated cartridges. One additional advantage over depth media is that the retention capability of hydrophobic membrane filters can be correlated to physical non-destructive integrity tests, as discussed later in this report.

3. HOW GAS FILTERS WORK

3.1 Size Exclusion

In gas filtration, as in liquid filtration, size exclusion (also referred to as sieve or mechanical retention) is one of the mechanisms of particle retention from a dry gas stream. As the name implies, this mechanism applies to particles that are too large to penetrate a given pore they encounter along the tortuous path through the filter material. Providing the particle is not deformed in the filtration process, this type of retention is independent of the velocity of the air stream through the filter. Retention by size exclusion is illustrated in Figure 1.

3.2 Retention of Smaller Particles

Retention of particles smaller than the actual pore size from a gas can occur by several additional mechanisms, such as diffusional interception, inertial impaction, and electrostatic attraction. These mechanisms capture particles that come in contact with—or close enough to—the filter material for relatively strong binding forces to come
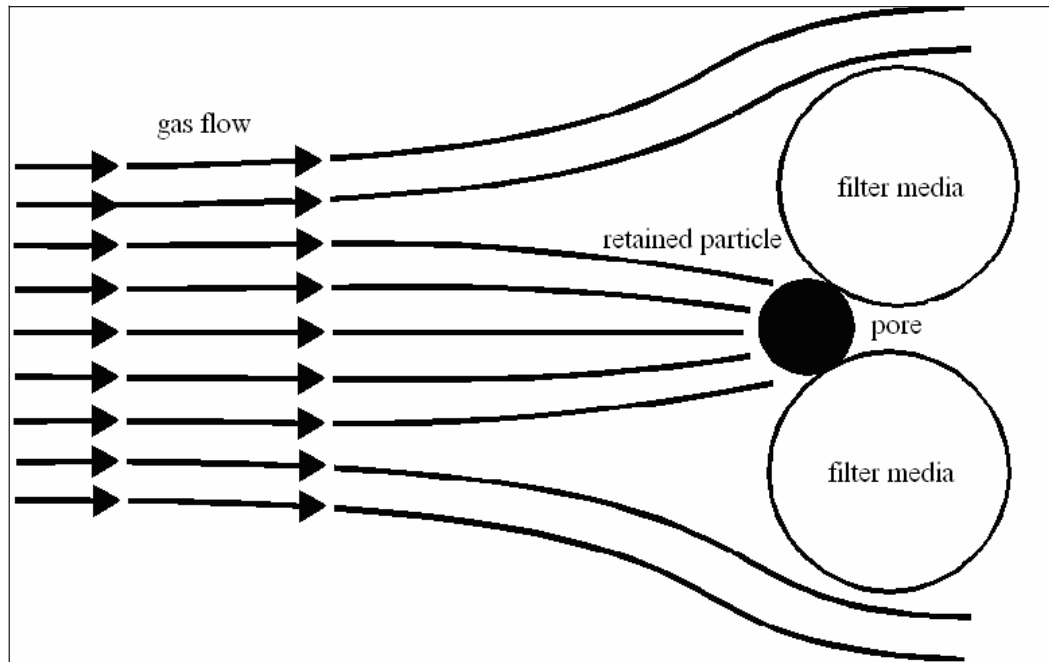
Figure 1: Particle Retention by Size Exclusion

into effect. These additional mechanisms are always present, but their contribution to the overall retention efficiency depends strongly on the size of the particle in question.

3.2.1 Diffusional Interception

In the diffusional interception mechanism, illustrated in Figure 2, particles stray out of the airflow streamlines due to Brownian motion caused by constant and random bombardment of the particles by air molecules. Brownian motion increases the probability of contact between the particle and the filter medium; hence, it increases the probability of particle retention. However, Brownian motion becomes more pronounced for smaller particle sizes. Thus, the retention efficiency by the diffusional interception mechanism increases as the particle size decreases.

3.2.2 Inertial Impaction

Figure 3 illustrates the mechanism of inertial impaction. The inertia of the particle keeps it from following the streamlines as the gas circumvents the filter material, causing it to collide with the filter medium. This effect becomes more pronounced at larger particle masses;
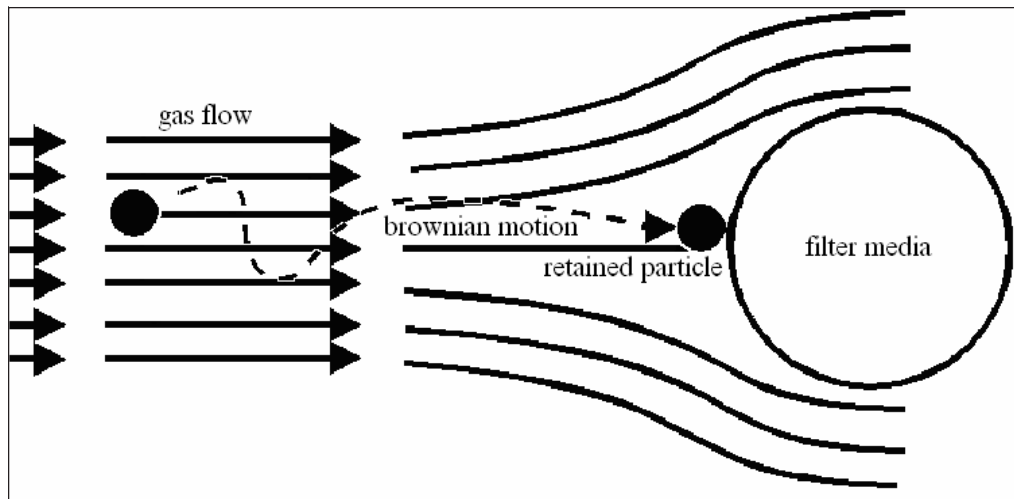
Figure 2: Diffusional Interception

hence, the retention efficiency by this mechanism increases with particle size. In the case of sub-micrometer hydrophobic membranes, the retention of large particles by this mechanism is less significant because these particles are typically retained by size exclusion. In coarse depth filters, however, inertial impaction plays a greater role by retaining particles that are less likely to be retained based solely on size exclusion.

3.2.3 Gravitational Sedimentation

Particles can also deviate from the flow path and collide with the filter media by gravitational sedimentation. Much like inertial impaction, this mechanism is more pro-

Figure 3: Inertial Impaction

nounced for larger particles, particularly those of high density. In the case of sub-micrometer hydrophobic membranes, this mechanism is not very important, since particles of the size normally retained by gravitational sedimentation (as is the case in depth filters) are more likely retained by size exclusion.

3.2.4 Electrostatic Attraction

Depending on the nature of the particle and the filter material, intermolecular forces may retain particles that come in close proximity to or in contact with the filter material. They will be firmly retained, even during pressure pulsations, because the binding forces are many times stronger than the drag exerted by the gas stream flow upon the particle. Particularly, in the case of HEPA (High Efficiency Particulate Air) and ULPA (Ultra Low Penetration Air) filters, which are not within the scope of this document, electrostatic capture of particles of opposite charge is an effective mechanism. The effectiveness of such filters depends on two major parameters, the humidity of the air and the velocity. Humidity must be low because electrostatic charges are highest when air is dry.

Dry air also reduces the danger of condensate build-up within the filter matrix. Airflow velocity is highly important to creating a contact time that is sufficiently long to generate enough separation efficiency for incipient particles to be retained (Grant, 1988).

3.3 Net Retention Efficiency in Filtration of Dry Gases

The net effect on the retention of particles is the sum of the effects observed for the individual mechanisms. At a given gas velocity, very small particles are retained predominantly by diffusional interception. At the particle sizes where this mechanism becomes less effective, inertial impaction, gravitational sedimentation, and size exclusion become more pronounced. The size range in which the transition from diffusional interception to inertial impaction typically occurs includes particles of a size that are not captured by either of these retention mechanisms, as illustrated in Figure 4. The size of the particle least likely to be retained is termed the Most Penetrating Particle Size (MPPS).

The MPPS will not only depend on the type of filter material, but also on the gas velocity through the filter. Brownian motion becomes less pronounced at higher velocities and pressures; thus, at higher flow rates the probability of capturing a given sized particle by diffusional interception diminishes. The effect of air velocity on the inertial impaction mechanism is less pronounced, particularly in the case of tighter membrane filters, because inertial impaction is overshadowed by more efficient size exclusion (Liu et al., 1985; Grant, 1988). The net effect is a shift of the MPPS as a function of air velocity. Such information has to be taken into consideration in order to achieve the desired retention character-

Figure 4: Effect of Various Retention Mechanisms of Particles Retained from a Gas Stream as a Function of Particle Size

istics from a dry gas stream. Additional information on the theory of retention mechanisms is given in Appendix A.

3.4 Factors that Affect the Retention Efficiency

As discussed above, the most reliable mechanism of particle retention from a gas stream by membrane filters is size exclusion because the retention efficiency remains relativ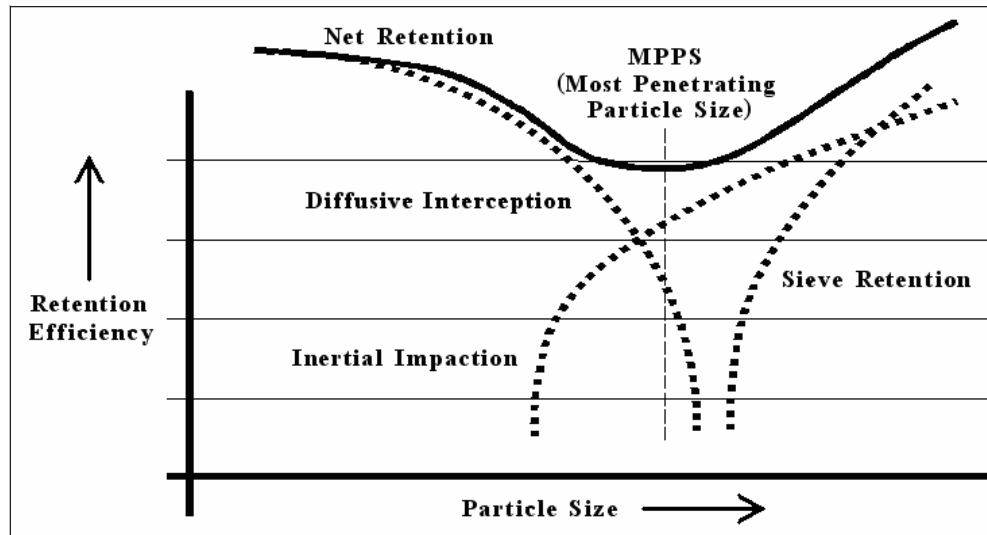ely unaffected by the use conditions imposed on the filtration process. This mechanism hinges mainly on the size of the contaminant to be retained in relation to the actual pore size of the membrane in question.

True pore size must not be confused with the nominal micron rating commonly assigned by filter manufacturers to the various types of filters offered. Such nomenclature is intended mainly for labeling purposes. However, due to the lack of uniformity in the rating from one manufacturer to another or due to different filter materials used, even from the same manufacturer, it is generally not advisable to select a filter for a given application based solely on the numerical micrometer rating. Since different retention mechanisms are involved in sterile gas filtration, a numerical pore size rating has even less meaning than it has in liquid filtration. Most manufacturers designate microbial retentive hydrophobic gas filters as 0.2 micron as a reference to "sterilizing" filtration of liquids. These membranes are, in fact, much more efficient in retention in dry gas streams (Liu et al., 1985).

Therefore, gas filters are best described by their performance in challenge tests, which are discussed in Section 7. For most applications, the presence of moisture in the form of condensate affects the filtration process adversely, but ways of keeping the filter element dry are discussed for various applications later in this report.

4. FILTER SELECTION AND SYSTEM DESIGN CRITERIA

Selection of a sterilizing gas filter requires consideration of many important issues, ranging from the retention efficiency through physical durability, compatibility with processing conditions, and ultimately the overall economics of the process. The relative importance of the selection criteria will depend strongly on the given application, but for many applications, the following characteristics need to be taken into consideration.

4.1 Retention Capability

In the pharmaceutical industry, hydrophobic membrane filters are useful in many applications. Very stringent retention expectations may be required for some of the more critical applications, while such requirements may not be necessary for others. In broad terms, the retention efficiency requirements can be classified in the following three categories:

The most stringent expectations are for sterile gas applications, where the filtered gas will be in direct contact with sterile final product or critical surfaces of the associated equipment. Examples include the filtration of compressed gases associated with aseptic filling equipment, blanket gas and venting of sterile bulk product holding tanks, and vacuum break applications in lyophilizers and critical autoclaves. Filters selected for such critical applications should be qualified with an appropriate liquid-based bacterial retention challenge test, and must have an appropriate physical integrity test that is correlated to the bacterial retention capability demonstrated in liquid filtration.

Moderately critical applications are those where the filtered gas will not be in direct contact with exposed sterile product or surfaces. Examples include many intermediate processing steps or the air supplied to a fermentation process. For such applications, filters qualified with aerosol-based bacterial challenge tests and with physical integrity tests that are correlated to aerosol retention capability are appropriate.

Applications that only require a reduction in bioburden have less stringent requirements. Because the retention expectation is similar to what is commonly expected from HEPA filters, dispersed oil aerosol challenges are often deemed acceptable to establish the retention capability of filters used in this type of application.

Classification of a given application into any of these groups and the suggested retention validation approach should be given careful consideration. Other applications may have additional or more specific requirements, such as the need for bacteriophage control in sensitive fermentations, or virus retention in critical applications. A number of articles that address the retention of different types of contaminants, including bacteria and phage, under various conditions and for various types of membrane have been published in the technical literature. However, the applicability of such data to a given situation must be carefully evaluated and justified by the filter user on a case-by-case basis.

4.2 Integrity Testing

As dictated by the application, it may be important to be able to verify the integrity of the filter element by means of a non-destructive physical test to assure the desired retention capability. More details and methods are discussed in Section 8 of this technical report.

4.3 Filtration Rate and Throughput

The flow rate of a filter at a given pressure differential depends on a number of factors, including the type of membrane and support materials, the thickness, porosity and pore-size distribution of the media, among others, as well as the retention characteristics.

For a given type of filter, the flow rate increases with the effective filtration area, but not necessarily in linear proportion. The appropriate filter area or number of individual filter elements for a particular sterilizing filtration application can be estimated from the clean gas flow versus differential pressure data, typically presented by the filter manufacturer in graphical or tabular format. An appropriate allowance for the extent of plugging to be tolerated has to be considered in the calculation of the

area necessary to meet the process objectives. It is necessary to consider the pressure drop of the entire system, including any pre-filters and any piping or ducts leading to and emerging from the sterilizing filter holder. Consideration should also be given to any special characteristics of an application, such as a high pump-out rate or steam-in-place requirements.

The throughput of the sterilizing filter can be increased considerably by the use of pre-filters. In addition, the useful life of the filter may not be limited by plugging, but rather by the number of sterilization cycles, the element can withstand before losing physical integrity.

4.4 Materials of Construction

Sterilizing membranes typically are manufactured from polymers such as PTFE, PVDF, polypropylene, or polyethylene. In addition to the membrane, assembled filter elements, particularly pleated filter cartridges, contain upstream and downstream support and drainage materials. Typically, these layers consist of a hydrophobic polypropylene non-woven medium (fleece), and the pleated packs are embedded in polypropylene by a thermal bonding process. Polypropylene is also a popular material for other cartridge hardware components, including the external cage, the inner support core, and the end caps. Some filter core elements and O-ring adaptors are reinforced with stainless steel or other materials to enhance the durability of the cartridge. Various O-ring materials are also available to suit the needs of a given application. The materials of construction have an impact on several key properties of the assembled filter element, such as thermal resistance, chemical compatibility and resistance to oxidation, and mechanical properties. The filter user should assess their suitability for a particular application.

4.4.1 Hydrophobicity

In order to reduce the potential for blockage by moisture accumulating within the filter element, particularly within the pore structure of the membrane, the materials of construction should be hydrophobic. Hydrophobicity is a reflection of the surface energy of the material compared to the cohesive energy of the liquid. Water, with a surface tension of 72 dynes/cm, is commonly the reference liquid. If the cohesive force exceeds the adhesive force, water will "bead up" rather than spontaneously wetting the surface, as shown in Figure 5.

The degree of hydrophobicity of a material can be expressed as the minimum surface tension of the liquid at which spontaneous wetting occurs, namely, the critical surface tension. Of the popular materials mentioned, PTFE is the most hydrophobic and polyethylene the least,
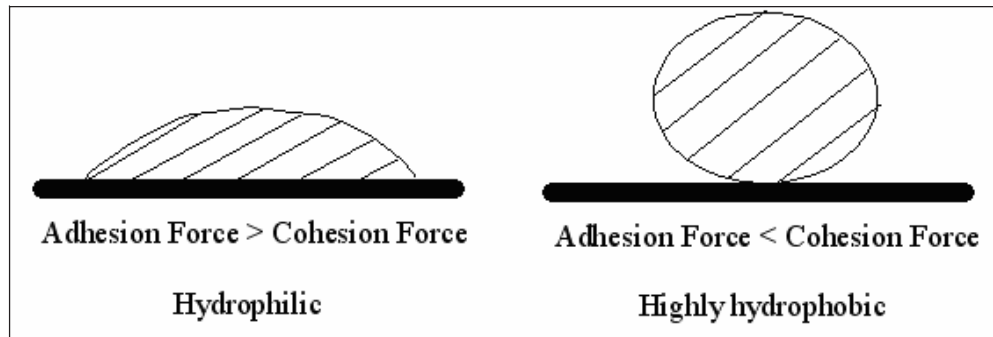
Figure 5: Interaction of Water with Hydrophilic and Hydrophobic Surfaces

as can be seen from the critical surface tension values listed below.

Polymer	Critical Surface Tension (dynes/cm)
PTFE	18
PVDF	25
Polypropylene	29.5
Polyethylene	32

4.4.2 Durability

The filter element has to be able to withstand the rigors of the intended application. Filter manufacturers typically provide information on the materials of construction and on the differential pressure limits that the filter element will withstand. Total inlet and differential pressure limits across the element are typically specified as a function of temperature and the direction of flow. Users must choose the filters in accordance with the needs of their particular processing requirements and must operate the filters within the specified limits to avoid loss of filter integrity and the associated reduction in retention capability. Loss of integrity can also be the consequence of degradation when filtering oxidative gases at elevated temperatures, particularly in long-term use. For such applications, the appropriate materials of construction must be selected or the filter change-out frequency should be adjusted to suit the materials.

Durability is particularly important in steam sterilization of the filter elements. In fact, the economics of some applications hinges on the number of steam sterilization cycles a given filter element can withstand without losing integrity. Sterilization of filter elements is discussed in more detail below.

4.4.3 Toxicity

It is important to assure that the filter system does not release toxic components into the process stream. Although extractables in liquids are of general concern, extractables cannot be generated in the absence of a solvent and hence are not a concern in dry gas applications. However, most elements should be and are generally constructed of materials that are not toxic based on standard toxicity test methods. These tests involve a static soak of the filter components in various solvents, followed by evaluation of the extracts and the plastic components themselves either in animal models and/or in mammalian cell culture tests. These tests are performed to ensure that filter membrane and support materials do not adversely affect the safety of the product. Filter suppliers typically provide toxicity data according to the standard United States Pharmacopeia (USP) Biological Reactivity Tests, *In Vivo,* for Class VI Plastics.

4.4.4 Particle Shedding

Particle shedding needs to be minimal for some applications, such as the filtration of compressed gases in conjunction with aseptic filling lines and breaking the vacuum at the end of freeze-drying cycles. On the other hand, it may be of little consequence in other processes, such as filtration of the air supply to a fermentor or filtration of the off-gas for such processes. Considering that all filters may shed particles, the possibility of media migration should be considered and assessed for those applications where it is deemed of importance (Decedue and Unruh, 1984; Hall, 1984; Meltzer, 1987a). Particle shedding can be evaluated using a liquid, where the higher viscosity of the liquid is considered to provide a more stringent test condition.

4.4.5 Gas/Filter Compatibility

Incompatibilities between the filter and the process are related to temperature, pressure, oxidation, or a combination of these factors. Excessive temperatures can result in deformation of components, while cryogenic temperatures can cause brittleness and stress fractures. Either extreme can potentially lead to loss of integrity. Oxidative gases, depending on temperature, concentration, and time in service (under flow conditions), can cause surface decomposition and particle generation from

susceptible support components long before there is any loss of integrity.

Due to flammability issues, polypropylene hardware filters should not be used in pure oxygen service. PTFE membrane filters with all-fluoropolymer materials of construction may be suitable for such severe conditions; however, if a sterile gas is required, users should verify that the filter is qualified as a sterilizing grade filter, as this may not always be the case.

4.5   Water Blockage

In many applications, the gas filter may come in direct contact with water or aqueous product solutions. Furthermore, condensation of water vapor can occur within the filtration system. Accumulation of water within the filter element will block the flow of gas, thus interfering with filter performance. The accumulation of water will also promote bacterial and fungal growth, and it should be avoided in all sterile gas applications (Meltzer, 1996; Rowe et al., 1996). Constructing gas filter elements from hydrophobic materials, as discussed above, reduces these adverse effects.

Even the most hydrophobic of filters can end up liquid-logged under some circumstances, particularly during steaming and integrity testing. This will invariably affect the flow characteristics in an adverse manner. The time that is required to dry the filter sufficiently to restore a useable or minimum required flow rate is referred to as the blow-down time. The more hydrophobic the filter media is, the shorter the blow-down time, provided that the filter assembly is properly oriented, with the outlet end pointed downward. For example, one vendor recom-

mends that flushing with a minimum of 75 cubic feet of air (with dew point below 40 ºC) per square feet of filter area be used as a rule of thumb for drying hydrophobic filters. Blow-down conditions and times required will vary for different conditions, filter materials, and air/gas dew points.

6.6 Design Considerations for Condensation Control

Accumulation of water within the filter element or the housing will also block the flow of gas, but this can be reduced by an appropriate system design that allows for drainage from the housing and the filter core. The amount of water droplets that reach the housing can be minimized by the use of coalescing pre-filters. The accumulation of moisture within the filter housing can be reduced by opening the lower housing vent. Accumulation of moisture within the filter membrane can be addressed by keeping the filter system at a higher temperature (typically 3–5 ºC above) relative to the process temperature.

Potential solutions to the problem include selecting a filter housing that is steam-jacketed or fitted with an electric heat tracer or insulating the housing and associated piping. Properly designed systems eliminate condensate from the filter assembly before it can accumulate (see Figure 6). Such systems must be thoroughly tested, as any blockage could be catastrophic to non-vacuum rated tanks and to the filter element itself.

When using steam-jacketed or heat-traced housings, it should be noted that many materials of construction (e.g., polypropylene cage, core, and membrane support layers) are susceptible to oxidation under extended periods of use at elevated temperatures (>80 °C). The high temper-
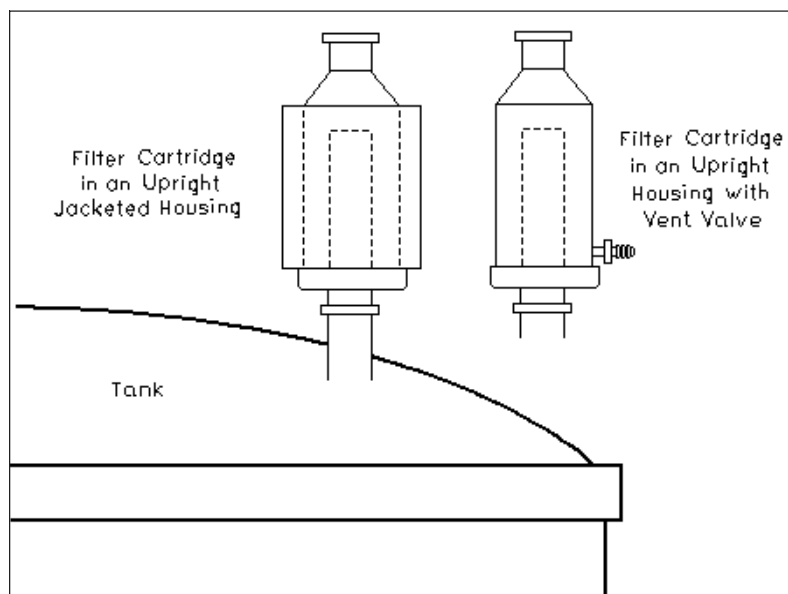

Figure 6: Examples with Filter Oriented for Drainage of Condensate with Steam Jacket or Open Lower Vent Valve

ature experienced in heated filter housings combined with high airflow can accelerate damage to oxidizable (polypropylene) filter components. It is best to avoid elevated temperatures above that recommended by the filter manufacturer, and it should be noted that specifications from many manufacturers will state a lower service life at elevated temperatures. For severe applications, oxidation-resistant materials (such as specially formulated polypropylene hardware and polyaramid support layers) are available.

5. EXAMPLES OF SPECIFIC APPLICATIONS

Most applications for hydrophobic membrane filters can be satisfied with a filter that meets as many of the following ideal characteristics as possible:

- The filter must retain microorganisms, even under adverse conditions such as high humidity.

- The filter should have high thermal and mechanical resistance, sufficient to endure long-term applications under demanding use conditions.

- The filter should withstand multiple steam sterilization cycles.

- The filter should allow high gas flow rates at low differential pressures.

- The membrane should be hydrophobic to resist blockage by condensate.

- The filter construction should be optimized for long, dependable service life.

- The filter must not release fibers.

- The filter must be integrity testable with a test correlated to removal efficiency.

- The filter should be easy to install and maintain.

- The filter's materials of construction should be compatible with the proposed application (e.g., oxygen service)

The relative importance or need for such properties can best be illustrated by a few sample applications.

5.1 Product Contact Gases

The broadest, most critical use of sterilizing grade hydrophobic membrane filters is for gases that are in direct contact with pharmaceutical products. For example, nitrogen gas is widely used to blanket oxygen-sensitive

solutions to reduce degradation. Any gas that comes in contact with solutions should be sterile to maintain low bioburden in terminally sterilized products or to maintain sterility in aseptically filled products. This includes process gases used in tanks or headspace gases used to flush product vials and ampoules.

Due to the critical nature of these applications, hydrophobic membrane filters that are validated to a rigorous liquid-based microbial retention challenge are recommended. In many critical applications, redundant filters in series are frequently employed, but not required. Filters must be routinely integrity-tested in use to assure their efficacy. Membrane materials should be chosen to reflect the conditions of use, especially if filter units are steamed- or sterilized-in-place.

5.2 Fermentor Inlet Air

The volume of air required to maintain the fermentation process depends on the process and the volume of the culture, and filtration systems should be sized accordingly. In large fermentor applications, the air supply may be millions of cubic meters per year and require large filter assemblies. The air supply needs to be reliable to provide proper oxygenation of the culture and sterile in order to avoid costly contamination problems in the process. Filters used in fermentation processes should meet high microbial retention standards and provide high flow rates at a relatively low pressure drop (1–5 psig). Membrane materials for such applications should be hydrophobic, of high void volume, yet show reliable microbial retention capability. Construction of the filter cartridges is optimized to avoid water blockage. The elements also require a high thermal and mechanical stability, because for the process to be economical, they have to withstand many sterilization cycles at elevated temperatures.

5.3 Fermentor Off-Gas

Membrane filters are employed increasingly for fermentor off-gas applications. The challenges in this application are the high moisture content and the high level of microbial contamination of the fermentor exhaust gases. As the gas stream cools, condensation occurs. This, in turn, can result in an undesirable increase of the head pressure within the fermentor. As indicated above in Section 4.6, water blockage can be avoided by choosing the proper design, protecting the final filter with coalescing pre-filters, and heat tracing the filter housings to avoid condensation (Keay, 1991; Orchard, 1991). Off-gas systems should be designed to prevent condensate and coalesced aerosols from reaching the filter. This is often accomplished by having the off-gas condensate drain back into the fermentor.

Also, there is a potential for foam to be carried over into the off-gas, which can lead to blockage of the filter. Therefore, systems should be designed and operated to avoid foaming. Foaming is typically reduced with addition of anti-foam agents or modification of the fermentation media. In difficult processes, it may be necessary to install a mechanical separator to eliminate foam and the potential for filter blockage.

5.4 Vent Filters on Compendial Water and Product Holding Tanks

When liquid is added to or drawn from a tank, an equivalent volume of air is displaced from or into the tank. To avoid bacterial contamination of the contents in critical applications, the air has to be filtered through a sterilizing grade vent filter. The same is true when a holding or transport tank is steam-sterilized because the air that enters the tank at the end of the sterilization cycle has to be sterile. In addition to the rigor of the steam cycle, another challenge presented by this application is blockage of flow due to entrapment of moisture within the membrane.

The need to avoid blockage of flow through the vent filter is particularly important at the end of a steaming cycle. As the tank cools, condensation of steam creates a vacuum that can be estimated from the ideal gas law or steam tables (Cole, 1977; Meltzer, 1987b). At 100 ºC, for instance, each liter of steam that condenses will occupy only about 0.6 mL, an almost 1700-fold decrease in volume. Because the bulk of the condensation will take place rapidly, the vent filter should be properly sized to deliver the equivalent of the tank volume of air in a small period of time. If no appropriate measures are taken to prevent the disruption of airflow through the vent filter, the resulting vacuum in the tank may damage the tank. The issue is less problematic in tanks that are vacuum-rated, a feature that makes them considerably more expensive.

Other design features can also prevent tank implosion. For instance, the vent filter can be connected to a source of compressed air, at a pressure high enough to displace the moisture lodged within the pore structure. The preventive measures cited above (in Section 4.6), such as heat-traced housings, should be seriously considered. Special care needs to be exercised in the sizing of the filter to avoid the problems associated with blockage in this application. It is also prudent to fit sealed tanks with a suitable rupture/implosion disc. However, reliance on this feature risks product loss and implies a significant amount of downtime to replace the disc, as well as repeating the cleaning and steaming process.

5.5 Lyophilizer and Autoclave Vacuum Break

The air (gas) that enters the chamber of a lyophilizer will come into direct contact with the sterile product. Likewise, the air that enters an autoclave will come in contact with sterile commodities or equipment. Hence, the gas supplied to reduce/break the vacuum at the end of the lyophilization/autoclave cycle must be sterile in these cases. Disruption of airflow due to condensation can adversely affect the operation, and appropriate measures to prevent this should be taken. The filter element in such applications needs to be sterilized, most often by steaming in place. The filter manufacturer's recommendations for steaming or sterilization should be adhered to, particularly if steaming in the reverse direction is required. Because the filter may be subjected to repeated steaming or sterilization cycles in such applications, it should be durable, and should be integrity-tested on a regular basis to assure the expected microbial retention level (see Section 8.5 for considerations for integrity test practices). Ease of integrity testing, placement of the filter, and easy access to the filter are critical in this application.

5.6 Gas Used for Drying and Transfer/Fill Line

Some components (such as rubber stoppers) and large equipment (such as holding tanks) are typically rinsed in water for injection and dried after steaming. Drying is especially critical if they are to be used in oil-based sterile product formulations. Often, compressed air is used to accelerate the drying process.

In addition, in many processes, the sterile bulk product must be transferred from the sterile holding tank to the filling line. This is often accomplished by pressurizing the head space in the holding tank with a suitable gas.

The gas in such critical applications must be sterile and free of particles, and a suitable filter must be chosen. Filters in such applications should be routinely sterilized and integrity-tested to assure the expected microbial retention capability.

5.7 Blow-Fill-Seal Equipment

Large amounts of sterile compressed air are needed to run blow-fill-seal operations. Often, the equipment is fitted with several different air filtration systems in order to provide sterile air to individual process steps, such as in molding the primary container or shielding critical portions of the machine to prevent the ingress of environmental air containing bacteria and particulate matter. The filtered air contacts critical surfaces as well as the product during the filling step; thus, a high level of bacteria retention must be assured through proper filter se-

lection and validation (Wilson, 1994). Because form-fill-seal operations are typically run for extended times, the filters used must be durable and reliable. The filters must be routinely sterilized and integrity-tested to assure the expected retention performance.

5.8   Environmental Air in Isolators

Isolator technology has been gaining popularity over the past few decades for critical applications, such as sterility testing, aseptic filling, and weighing and handling of sterile and even non-sterile potent compounds. Depending on the application, isolators can be run at positive or negative pressure relative to the surrounding environment. Whichever the mode of operation, filtration of make-up and exhaust air plays an important role. Hydrophobic membrane filters can be used as an alternative to conventional depth filters, such as HEPA filters to accomplish the air exchange between isolators and the surrounding environment. The more demanding the operation, whether it be retention of toxic powders from the exhaust or the admission of sterile air, the more demanding the retention validation and integrity test program that should be implemented.

6.   STERILIZATION OF HYDROPHOBIC MEMBRANE FILTERS

The filter element to be used in a sterilizing filtration process must be sterile for the operation to be successful. The most common sterilization method for hydrophobic membrane filter cartridges is steaming under pressure. Steam sterilization may be hindered by the poor heat transfer characteristics of the plastic components, the large void volume within the filter pores that traps air, tortuous paths for steam penetration, and the limited stability of some of the materials of construction at elevated temperatures. Therefore, filter users should validate the sterilization of the filter to document that an appropriate level of sterility assurance has been achieved (Kovary et al., 1983).

The filter element must withstand the stresses associated with the sterilization process by which it is rendered sterile, without loss of physical integrity (Myers and Chrai, 1982; Steere and Meltzer, 1993). In many applications, "sterilizing grade" hydrophobic membrane filters are re-used or left in service for long periods. Hence, they should withstand multiple sterilization cycles. Generally, users should follow the filter manufacturers' sterilization guidelines to avoid excessive exposure because the filter membrane or other components may be susceptible to damage if not sterilized properly. Users should contact the filter manufacturer to determine sterilization limits.

6.1   Sterilization in Steam Autoclave

Most sterilizing membrane filters are qualified by their manufacturers for autoclave sterilization to temperatures of at least 121 ºC and up to 140 ºC. Temperatures higher than 140 ºC may make many of the plastics used in the construction of filter devices unstable and may adversely affect the physical integrity of the filter. However, higher temperatures may be used if it can be validated that the filter is not adversely affected.

Preparation of the filter assembly for autoclaving is very critical. It is important to protect the open ports on the filter assembly using a suitable microbial barrier that prevents contamination of the sterile filter components post-sterilization. The barrier must allow the steam to enter the filter assembly for adequate sterilization. It is critical that the filter inlet, outlet, vents, and drain ports are open to allow for steam penetration. The filter cartridge should be properly supported during sterilization to prevent deformation. For proper steam penetration, the air must be removed from the filter assembly, including the void spaces within the filter element. This can be achieved effectively at the cycle initiation by a series of vacuum cycles or flushing the chamber with pulses of steam pressure. In a gravity displacement autoclave, the filter should be oriented horizontally, or with the outlet pointed downward, to facilitate removal of cold air and condensate from the core. Typically, a slow exhaust or liquid cycle is employed. The user should refer to the filter manufacturer's specifications when developing a sterilization cycle for the filter assembly. Larger filters or filters attached to tubing or ancillary equipment may require adjustments to the sterilization parameters. For all applications, the autoclave cycle should be validated to yield the desired level of sterility assurance.

Drying of the sterilized filter is generally accomplished by applying a post-cycle vacuum and air purge. Sterilization of the filter in a steam autoclave requires that the filter be installed aseptically at the site of the application. In such applications, an integrity test may also be performed off-line prior to installation of the filter. If these cases, the integrity test should be performed in a suitable environment, such as a HEPA filtered work station.

6.2   Steaming-in-Place (SIP)

Sterilizing grade hydrophobic membrane filters should be sterilized and integrity-tested in place whenever feasible. Sterilization in place eliminates the need for aseptic installation, thus avoiding associated contamination risks (Agalloco, 1990). If the filter cannot be steamed-in-place together with the remaining process equipment, valve arrangements may be used to isolate the filter from

the process equipment during steaming of the filter. Note: Valves must be selected to ensure that they can be steam-sterilized adequately. Consideration must be given to the valving to allow venting of trapped air from the system. Also, by using two filters and appropriate valves, one filter can be online while the other is being steamed. With such an arrangement, it is feasible to steam filters periodically without interrupting the process.

Although the SIP approach offers distinct advantages, it also presents some limitations and some challenges. For one, the process is limited to filter housings that can resist steam pressures of 15–30 psig. Therefore, fully disposable capsules or filter assemblies with polypropylene or polycarbonate shells are not suitable for SIP. Capsules specifically designed for SIP will require more temperature-resistant materials. The steam pressure should be increased gradually in order to reduce thermal shock to the filter, and the steam supply should be modulated so as not to exceed the specified maximum differential pressure. The filter assembly must be oriented with the opening of the core pointed downward to minimize accumulation of condensate. The bleed points of the system should be designed to allow condensate removal either via a small continuous steam bleed or the use of steam traps. Users should ensure that the pressure differential across the filter element stays within the allowable range in order to avoid damaging the filter.

At the completion of the SIP cycle, it is imperative that a positive gas flow be maintained during the cool-down period to minimize the potential for condensate formation in the filter. The gas should be allowed to bleed freely from all condensate points until the system is dry and has cooled to the operating temperature. As mentioned in the specific example of vent filters for holding tanks, it is extremely important that free flow of gas be established through the hydrophobic filter to prevent collapsing of any non-vacuum-rated tank.

6.3 Other Sterilization Methods

It is feasible to sterilize hydrophobic membrane filter cartridges by other means, including gas sterilization, and in some cases by ionizing radiation. Filter users conduct these alternate sterilization methods themselves much less frequently when compared to steam sterilization, and these will not be discussed further in this report. Filter users interested in the application of these alternate sterilization methods can consult applicable guidelines issued by the Association for the Advancement of Medical Instrumentation (AAMI).

In some cases, the filter units are subjected to a defined exposure to specific irradiation conditions by the vendor, and are supplied as "gamma-irradiated" units. The vendor may not make claims regarding sterility of the supplied units or the sterility assurance of that process. When using units provided by the vendor with a sterility claim, filter users should evaluate the sterilization process validation performed by the vendor to ensure that an appropriate level of sterility assurance has been achieved.

7. VALIDATION OF FILTER RETENTION CAPABILITIES

There is no specific standard that defines the retention requirements for a membrane filter used to sterilize gases. There are several approaches to qualifying the retention capability of gas filters, ranging from liquid bacterial retention tests to aerosol challenge tests with bacteria or spores, as well as with phages/viruses. In the aerosol test setting, a range of different organisms has been used to demonstrate the retention performance of such filters, which makes a direct comparison between filters very difficult.

Liquid bacterial challenge testing represents a worst-case condition for sterilizing gas filters because the retention efficiency in liquids is much lower than in gases. Because of the enhanced retention efficiency in gases, bacterial (spore) aerosol challenges are always less rigorous than a liquid challenge, even though it does represent the way the filter is challenged in a dry gas process. Finally, phage/viral aerosol challenge tests may be the least rigorous because in gas filtration the smallest particle sizes are not the most difficult to retain, as evidenced by the concepts presented in Section 3 on gas retention mechanisms and MPPS.

7.1 Liquid Bacterial Retention Test

Liquid bacterial challenge testing represents a worst-case condition for sterilizing gas filters because the retention efficiency in liquids is lower than in gases. This is demonstrated by the fact that filters shown to be retentive for phages/viruses in aerosol challenges will not retain them in liquids. In addition, a liquid challenge test provides retention information for high humidity process conditions, such as extreme moisture after filter sterilization or water droplets entrained in the gas being filtered. Commonly, the liquid bacteria challenge test is performed to American Society for Testing and Materials ASTM F838-83 standards or comparable methodology. The test can be performed on membrane filters in disc, capsule, or high-area cartridge configurations.

7.1.1 Challenge Organism

A "sterilizing grade" filter, when appropriately validated, will remove all microorganisms from a fluid stream, producing a sterile effluent. The microorganism *Brevundimonas diminuta* (ATCC 19146), when properly grown, harvested and used, is a common challenge microorganism for 0.2-µm rated filters because of its small size (0.3-µm mean diameter). (FDA Sterile Drug Products Produced by Aseptic Processing, 2004)

Historically, a sterilizing grade filter for liquids has been defined as one that retains a minimum challenge of 10^7 cfu of *Pseudomonas diminuta* (reclassified to *Brevundimonas diminuta*) per cm² of filter surface (FDA Aseptic Processing Guidance Document, 1987; PDA Technical Report No. 26, 1998), and this criterion has been carried over to the liquid challenge of gas sterilizing filters.

Details on maintenance, preparation, and characterization of *B. diminuta* challenge suspensions are given in Appendix B.

7.1.2 Challenge Concentration and Effective Challenge Level

The bacterial concentration in the challenge suspension should deliver a uniform challenge over the intended test time to yield a final challenge level of 10^7 cfu per square centimeter of effective filter area. If necessary, the challenge suspension can be diluted by adding the necessary volume of vehicle, typically water (a model for condensate) or saline lactose broth. The actual challenge suspension used (not just the stock suspension) should be subjected to cell count, cell size, viability, and aggregation as discussed in Appendix B. It is critical that all parameters be accounted for in calculating the challenge level, including the flow rate and time, or volumetric throughput, and the concentration of the bacterial suspension. The challenge concentration (cfu/mL) should not be confused with challenge level (cfu/cm²).

7.1.3 Pre-Challenge Integrity Test

The filter element to be challenged must be integrity-tested prior to and after the actual challenge test in order to relate the observed retention to a non-destructive physical test result. The pre-challenge test can be performed by any of the integrity tests (Bubble Point, Diffusive/Forward Flow or Pressure Hold/Decay, or Water Intrusion) discussed in more detail in Section 8, while the post-challenge integrity test can be readily performed only by a Bubble Point Test or diffusion-based tests.

If the water intrusion integrity test approach is chosen, water is added to the upstream portion of the housing that holds the dry filter element to perform the test. For a Bubble Point or the Diffusive/Forward Flow test approach, it is necessary to wet the filter using a low-surface-tension liquid, such as 25% (v/v) t-butyl alcohol in water, 60% isopropyl alcohol in water, or other suitable liquid recommended by the manufacturer.

7.1.4 Challenge Test Method

Standardized methods for qualifying microbial retentive membrane filters are described by the American Society for Testing and Materials. Some filter manufacturers have used alternative bacterial challenge test methods, which are typically presented in their validation guide documents.

Because gas filters are hydrophobic, it is necessary to initially wet the filter using a low-surface-tension liquid (such as 25% (v/v) t-butyl alcohol in water or 60% isopropyl alcohol in water), prior to an aqueous bacterial challenge test.

In the case of a Bubble Point Test, and of the Water Intrusion Test, a wetting step is needed prior to the liquid challenge. In the case of a Bubble Point Test, the wetting fluid has been expelled from at least parts of the filter membrane, and in the Water Intrusion Test approach, the hydrophobic membrane remains essentially dry. In the former case, the wetting step needs to be repeated with the low-surface-tension liquid, while in the latter, it is done after the intrusion test by replacing the water added to the housing with the low-surface-tension liquid. In the case of the Diffusive/Forward Flow Integrity Test, the filter is already alcohol-wet at the end of the integrity test, and a separate wetting step is unnecessary.

In all cases, once the filter is adequately wetted, the alcohol solution has to be displaced so as not to interfere with the challenge test. Typically, a 4–5-minute water flush at 1 liter per minute per 10-inch cartridge will suffice. The adequacy of the flush should be established prior to and/or confirmed during the test. Once the filter has been pre-wetted and flushed, the challenge suspension can be filtered.

Challenges of liquid sterilizing filters are typically conducted at a set differential pressure or controlled flow rate. (Note: The differential pressure across the test filter should not be confused with the filter inlet pressure.) When the differential pressure is a critical variable, it should be measured appropriately, particularly if assay filters are installed in-line. If reasonably non-restrictive tubing is used downstream of the test filter, the differential pressure of interest is the inlet pressure minus the

pressure observed upstream of the assay filter. To reduce the influence of downstream restrictions, instead of using an in-line assay filter, the effluent of the test filter can be accumulated in a vented sterile receiving vessel for subsequent analysis. Tests conducted at a controlled flow rate can also be influenced by downstream restrictions, and may be similarly handled.

7.1.5 Post-Challenge Integrity Test

A post-challenge integrity test must be conducted to verify that the filter did not suffer damage during the challenge procedure. The result is important if passage is observed, because it can provide an explanation for the observed retention failure.

The Water Intrusion Test should not be performed after the challenge because viable and non-viable contaminants will affect the hydrophobicity of the filter. In addition, any filter media still wetted after the challenge test will alter the results of a Water Intrusion Test.

Furthermore, the post-challenge test cannot be conducted in water, as the hydrophobic filter was now artificially wetted by the intrinsically non-wetting aqueous challenge solution. Because the aqueous solution is easily expelled from the hydrophobic pore structure, applying gas at any pressure will not produce a meaningful test result.

However, the post-challenge integrity tests can be readily carried out after flushing the filter in the same low-surface-tension liquid used in the pre-challenge test, and then using a Bubble Point Test or the Diffusive/Forward Flow Test.

7.1.6 Effluent Analysis

To ensure demonstration 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). Sampling only a portion of the filtrate is insufficient to validate a sterilizing filtration challenge because a small number of cells may have penetrated the filter and remain undetected in the portion of the filtrate not analyzed.

Either a 0.45-µm or a 0.2-µm-rated analytical membrane is used to recover *B. diminuta* (Bowman et al., 1967; Leahy and Sullivan, 1978; Carter, 1996). *B. diminuta* grown in and used for microbial challenges under the standard conditions (see

Appendix B) penetrates 0.45-µm-rated membranes in small numbers at high challenge levels, typically showing a titer reduction of 10^4–10^6 (Log Reduction Value, 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 analytical membrane (Trotter et al., 2002). While not sufficiently retentive to serve as a sterilizing filter for liquid filtration, this efficiency is acceptable for an analytical recovery membrane under ASTM challenge conditions.

7.1.7 Interpretation of Results

There is no standard for liquid challenges of sterilizing grade gas filters, but most of the commercially available filter types claim to meet the criteria for sterilizing grade liquid (hydrophilic) membrane filters. Effective challenges of sterilizing membranes with *B. diminuta* or other test organism should achieve influent total levels of at least 10^7 cfu/cm² effective filtration area and should demonstrate a sterile effluent.

7.2 Aerosol Bacterial Retention Test

A wide range of bacteria, especially spore-forming types, can be used in aerosol challenge tests, but there is no regulatory- or industry-accepted standard that has to be met. Because of enhanced retention efficiencies, an aerosol challenge is less rigorous than a liquid challenge; however, it does represent the way the filter is challenged in a dry gas process.

7.2.1 Aerosol Bacterial Challenge Organism

Bacterial aerosol challenge tests can be performed with the same microorganism used in liquid challenge tests, *Brevundimonas diminuta* (Duberstein and Howard, 1978). However, utilizing this organism in aerosol challenge tests requires careful selection of challenge conditions. For example, low humidity or high velocity during the challenge procedure could result in reduced viability of the challenge organism.

Bacterial spores are of particular concern for sterilizing gas applications due to their resistance to desiccation and prolonged viability in gases. Thus, spores such as those of *Bacillus subtilis* have also been used in aerosol challenge procedures. Spores are more resistant to desiccation, but may not be as likely to pass through filters as vegetative cells, which tend to be smaller in size.

7.2.2 Preparation of the Challenge Suspension

The bacterial/spore suspension to be used in the aerosol bacterial challenge should be prepared by accepted mi-

crobial procedures. Spore preparations should be heat-shocked to kill any vegetative cells and, in some cases, to activate spores to germinate. To avoid interference of other culture media ingredients, the final step in the preparation of the suspension to be aerosolized should be the washing and resuspending of the cells in an appropriate sterile liquid, such as sterile deionized water or buffer.

Characterization of the resulting suspension (viability, titer, cell size, and aggregation) should be performed as described for *Brevundimonas diminuta* in Appendix B.

7.2.3   Aerosol Bacterial Challenge Conditions

7.2.3.1 Challenge Size

The aerosol system must demonstrate the ability to generate particles of a suitable size for the challenge. An Andersen Sampler can be used to demonstrate that the system is capable of generating aerosolized droplets as small as 0.65 μm.

The Andersen Sampler is a cylindrical, bio-aerosol sizing device consisting of an inlet cone and typically six (and up to 7–8) classification stages. The sampling stages have 300–800 precision-machined orifices. Petri dishes containing an agar-based bacteriological medium appropriate for the microorganism(s) in question are placed in the sampler below each stage. Air is pulled into the top stage, at a specified flow rate (typically 28.3 LPM or 1 SCFM), onto and around the petri dish, and then through the perforations onto the next dish below it. The perforations of each stage are smaller than the previous one. Air impinging onto the top agar plate is traveling at relatively low speed, and only the larger airborne particles impact the top agar surface. Progressively smaller droplets impact the progressively lower dishes as their momentum increases due to increasing air velocity passage while passing through the smaller holes. Aerosol size can be assessed after incubation of the media and counting where growth occurs.

7.2.3.2 Challenge Conditions

Test parameters include the challenge duration, gas velocity through the filter, relative humidity, and aerosol size distribution. As higher flow rates (generally from higher pressure differentials) generally serve to enhance inertial impaction of aerosolized bacteria, lower flows may represent worst-case conditions (Jornitz, 1999).

Critical challenge parameters, such as average relative humidity, temperature, differential pressure across the test filter, flow rate of the carrier gas, and flow rate of the challenge suspension in the nebulizer, should be maintained at acceptable levels for bacterial viability and the desired test conditions.

7.2.3.3 Challenge Concentration and Level

The concentration of the bacterial suspension fed to the nebulizer (challenge concentration) should be adjusted to yield no more than one cell per droplet. Evaporation of the droplets in a dry air stream further reduces aerosol particle size. Theoretically, this can yield a dry aerosol suspension. If using a vegetative challenge organism, precautions should be taken not to desiccate the challenge organism, resulting in unacceptable viability. Viability of the cells under the resulting conditions must be verified. Viable cell count in the aerosol must be determined by an acceptable test method.

The challenge concentration and volume/flow rate selected should deliver a uniform challenge over the intended processing time to yield an appropriate final challenge level per unit of filter surface area. Challenge concentration (cfu/mL) should not be confused with challenge level (cfu/cm^2).

The specific challenge level will depend on the organism used and the challenge conditions (such as gas velocity and relative humidity). The most relevant organism, challenge level, test conditions, and the retention efficiency required will vary depending on the specific application.

7.2.4   Challenge Test Methods

The bacterial aerosol is generated from the suspension prepared as indicated above by means of a nebulizer. A pre-challenge integrity test must be performed, which will necessitate drying of the filter prior to the challenge test. The organism suspension is delivered to the nebulizer at a constant flow rate by means of a peristaltic pump or from a bowl at constant pressure. Ideally, the nebulizer produces droplets that contain no more than one cell each. The carrier gas, sterilized by filtration, is delivered to the system upstream of the nebulizer at the desired airflow rate. The gas stream with aerosolized bacteria then passes through the mixing chamber. Oversize droplets will impinge on the walls of the mixing chamber, while the main aerosol stream will travel along the piping and through the test filter. Any test organisms that pass through the test filter are collected by impingement on a suitable medium, typically a sterile buffer solution or growth medium, and the effluent gas is filtered through a sterilizing grade filter before being exhausted to the environment.

The challenge can be performed with a split (dual) stream procedure that permits simultaneous monitoring of both the input and the filtered streams (see Figure 7). In this procedure, a two-channel timer is used to direct gas flow,

on an alternating basis, between the effluent impingers downstream of the test filter and the control impingers on the upstream side. This enables simultaneous and direct monitoring of the input and effluent, and it eliminates the need to perform separate control and challenge tests.

Alternatively, the aerosol challenge can be performed with a single-stream system. However, a control test to determine aerosolization efficiency must then be performed before or after the challenge test to establish the challenge level. This control test is performed in the same manner as the filter test experiment, except that no filter is installed in the system. The control test measures bacterial losses in the test system due to impingement of larger droplets on the piping or any viability losses when determining the challenge level.

In either approach, impingers must be at their flow rate specification for optimum recovery. For higher gas flow rates, it may be necessary to use multiple parallel impingers. After the challenge is completed, the collection liquid in the impinger can be subjected to a cell count by an acceptable microbiological method, typically by incubation of an analytical membrane used to filter the collection liquid, and a titer reduction is calculated.

For some high flow rate challenges, it may not be possible to analyze the entire effluent due to practical limitations regarding the number of impingers. In such cases, the inability to sample the entire effluent does not affect the calculation of titer reduction when the challenge organisms are recovered from the collection fluid in the impinger. However, when the impinger collection fluid is negative for the challenge organism, appropriate statistical methods should be used to account for the incomplete sampling of the effluent. Typically, the amount of challenge organism that would have to be present in the portion of the effluent sampled in order to achieve a positive result with a high confidence limit should be calculated, and this value taken into account when calculating the titer reduction. Confidence limits at 95% are desirable. For guidance on calculating titer reductions, refer to Appendix 3 of ICH Guidance for Industry Q5A on Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin (International Conference on Harmonization, 1997).

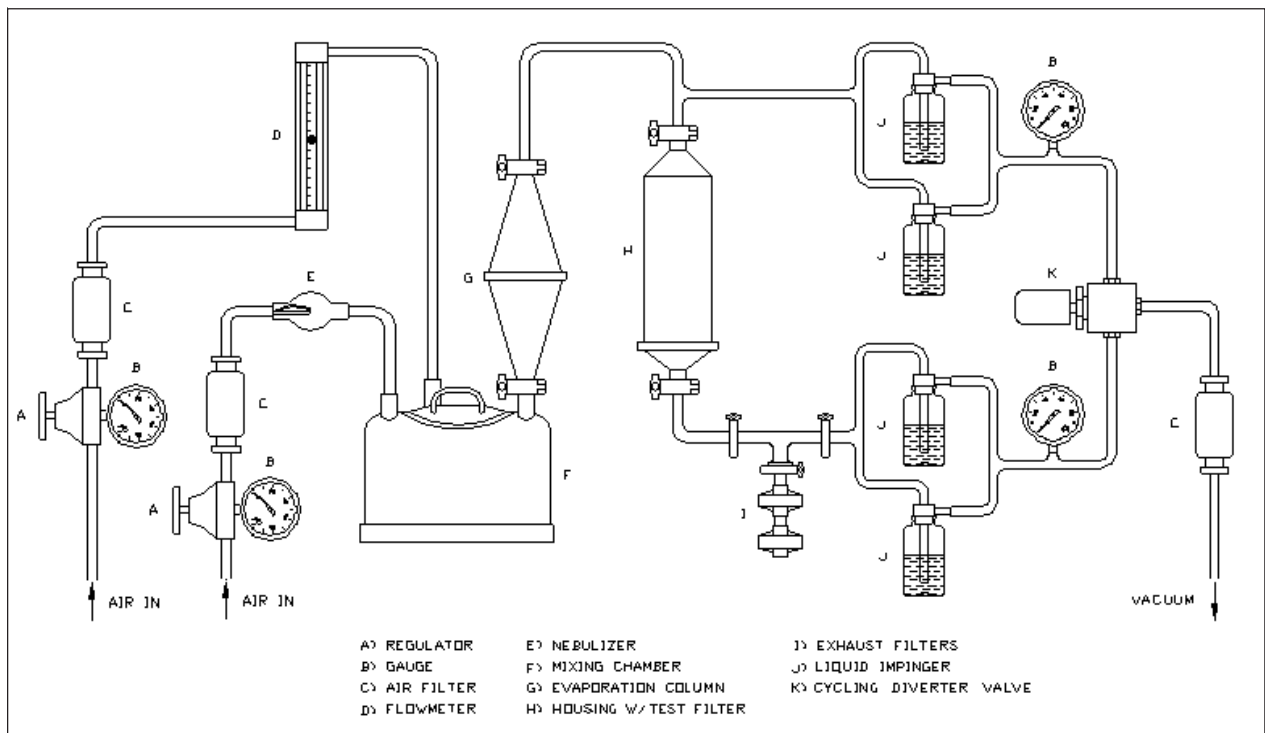|		
A) REGULATOR	E) NEBULIZER	I) EXHAUST FILTERS
B) GAUGE	F) MIXING CHAMBER	J) LIQUID IMPINGER
C) AIR FILTER	G) EVAPORATION COLUMN	K) CYCLING DIVERTER VALVE
D) FLOWMETER	H) HOUSING W/ TEST FILTER	

Figure 7: Schematic of Aerosol Challenge Test (Split Stream Approach)

7.2.5 Interpretation of Results

There are no industry standards for aerosol challenge tests; thus, filter users have to select filters with the desired retention efficiencies to match their specific requirements. For an aerosol challenge of a sterilizing gas filter to be deemed acceptable, a pre-established challenge level must have been delivered to the filter surface and a pre-established titer reduction must be demonstrated under the selected challenge conditions.

7.3 Viral (Bacteriophage) Aerosol Challenge Tests

As discussed earlier, the retention of particles smaller than the actual pore size from a gas stream depends on mechanisms (i.e., diffusional interception and inertial impaction) that are not operative in a liquid challenge. Thus, most filters used for the sterilization of gases will not retain viruses in a liquid challenge test. However, some filter types may be qualified for virus retention using aerosol viral (bacteriophage) challenge tests. Aerosol tests have the benefit of more closely resembling gas filtration and can be used to determine removal efficiency of these filters in gas service for viral species that are not normally removed under liquid conditions.

However, the utility of viral aerosol challenges is debatable due to the inability to precisely measure aerosol particle size. Viruses typically used in aerosol challenges range in size from 25 nm to 180 nm. Cascade samplers used for assessing aerosol particle size can demonstrate that the aerosolized viral particles are smaller than 650 nm, but cannot precisely establish the actual size. In addition, based on the concepts presented in Section 3 on gas retention mechanisms and MPPS, the smallest particle sizes are not the most difficult to retain. Although the MPPS will depend on the filter type and gas velocity, it has been shown to be generally in the 200–300 nm range. For these reasons, small phage/viral aerosol challenge tests (<200 nm) may be the least rigorous challenge compared to bacterial challenges, either liquid or aerosol.

There are no specific standards for viral aerosol challenge tests, and a variety of microbiological contaminants are used, including fX-174, PP7, MS2, and T1 bacteriophages.

7.3.1 Viral Challenge Organism

Viral aerosol challenge tests are often performed with a model bacteriophage. Bacteriophages are typically chosen over mammalian viruses for health and safety reasons, and for their relative ease of use, which requires no sophisticated equipment. In addition, bacteriophages can be of particular concern for bacterially based fermentations because they infect and cause lysis of bacterial cells.

Aerosol challenge methods have been developed for several representative bacteriophages, such as T1, fX-174, MS2, and PP7, ranging in size from 25 to 180 nm. Challenges can be performed with individual or mixture of bacteriophages (Bradburne et al., 1992).

Bacteriophages used in challenge testing are generally obtained from the American Type Culture Collection (ATCC), and should be maintained in accordance with ATCC recommendations, or using appropriate microbiological practices. Challenge suspensions can be prepared by adding the phage at an appropriate multiplicity of infection (MOI) to a culture of the host bacteria in logarithmic growth phase. The MOI reflects the number of phage particles needed per host cell to achieve infectivity, and can vary depending on the phage. After a suitable infection period, the culture is lysed, typically by the addition of chemical lysis agents such as chloroform or lysozyme and EDTA, to release the phage from the host cells. Cell debris is removed from the lysed cultures by low-speed centrifugation. The resulting supernatant can be purified by passing the suspension through hydrophilic 0.2- or 0.1-μm-rated filters. Alternatively, the supernatant can be concentrated (if needed) and purified further by cesium chloride (CsCl) gradient centrifugation or other appropriate purification techniques.

7.3.2 Viral Aerosol Challenge Conditions

7.3.2.1 Challenge Size

Viruses typically used in aerosol challenges range in size from 25 nm to 180 nm. Anderson samplers used for assessing aerosol particle size can demonstrate that the aerosolized viral particles are smaller than 650 nm, but cannot precisely establish the actual size. Depending on the extent of drying, the aerosol particles may be larger than reported size of the phage. Additional drying is recommended to further reduce the droplet size; however, if the organism is susceptible to loss of infectivity upon desiccation, caution should be taken not to over-dry.

7.3.2.2 Aggregation

The preferred viral challenge suspension should be one of monodispersed bacteriophage. Retention testing will suffer sensitivity loss directly proportional to the degree of phage aggregation. Aggregation should be avoided when performing a bacteriophage challenge, as it is artificially enhances physical removal. This is typically done by pre-filtering the viral spike suspension through 0.2- or 0.1-μm-rated filters.

7.3.2.3 Challenge Conditions

Test parameters include the challenge duration, gas velocity through the filter, relative humidity, and aerosol

size distribution. As higher flow rates (generally from higher pressure differentials) generally serve to diminish diffusive interception of aerosolized virus, higher flows may represent worst-case conditions

Critical challenge parameters, such as average relative humidity, temperature, differential pressure across the test filter, flow rate of the carrier gas, and flow rate of the challenge suspension in the nebulizer, should be maintained at acceptable levels for viral infectivity and the desired test conditions.

7.3.2.4 Challenge Level/Infectivity

The viral titer in the nebulizer should be diluted sufficiently to yield no more than one virion per droplet. Evaporation of the droplets in a dry air stream further reduces aerosol particle size. Theoretically, this can yield a dry aerosol suspension. Infectivity of the viral challenge should be confirmed, using an appropriate test method for that organism.

The viral challenge concentration and volume/flow rate selected should deliver a uniform challenge over the intended processing time, to yield an appropriate final challenge level per unit of filter surface area. Viral challenge concentration (plaque forming units, or pfu/mL) should not be confused with challenge level (pfu/cm^2).

The specific challenge level will depend on the bacteriophage used and the challenge conditions (e.g., dry or humid). The most suitable bacteriophage, challenge level, test conditions, and retention efficiency will depend on the application and filter type.

7.3.3 Challenge Test Methods

The test conditions for viral aerosol challenge tests in general follow the procedures outlined for bacterial aerosol challenge tests described in Section 7.2.3.

After the viral (bacteriophage) aerosol challenge is completed, the buffer in the effluent impingers downstream of the test filter can be analyzed for the test particle. Analysis is done by an infectivity (plaque) assay. The titer reduction is calculated after a statistical correction for the effect of sampling only a portion of the impinger fluid volume is made. Please see Section 7.2.4 for more information.

When sampling only a portion of the impinger fluid, a presence/absence test can be performed on the remaining impinger fluid. This remaining effluent is mixed with growth media and host culture and is incubated for a length of time. During the incubation time, any virus present will be amplified, due to its ability to replicate, thus increasing the sensitivity of detecting possible virus passage.

7.3.4 Interpretation of Results

For a viral aerosol challenge of a sterilizing gas filter to be deemed acceptable, a pre-established challenge level must have been delivered to the filter surface and an acceptable titer reduction must be demonstrated under the selected challenge conditions.

8. PHYSICAL INTEGRITY TESTING

The main objective of a nondestructive physical integrity test is to verify that the filter is of the correct retention rating and capable of performing its stated function. Such tests can reveal the presence of oversized pores or defects that compromise a given filter's specified retention capability, without damaging the filter. The physical integrity test can also be used to confirm the retention capability of a given filter element, providing a correlation has been established between the results of physical integrity tests and the retention capability of that particular type of filter using appropriate challenge tests as described in Sections 7.1 and 7.2

As discussed extensively in PDA Technical Report No. 26, integrity testing of hydrophilic membrane filters used in the sterilization of liquids relies on the measurement of the flow of a test gas through filter elements, wetted with a suitable liquid, as a function of the test pressure applied. Variations on how the measurement is taken and interpreted result in the Bubble Point, Diffusive/Forward Flow, and Pressure Hold/Decay Tests. These tests are also applicable to hydrophobic filters, providing that a liquid of sufficiently low surface tension as to completely wet the hydrophobic pore structure is chosen as the integrity test fluid. Various types of aqueous alcohol solutions are typically employed in Bubble Point, Diffusive/Forward Flow, and Pressure Hold/Decay Tests of hydrophobic membrane filters.

The integrity of hydrophobic filters also can be tested by measuring the dry membrane's resistance to wetting with water as a function of the applied pressure. This test approach is referred to as Water Intrusion or Water Breakthrough/Penetration testing. The advantage of this approach is that the membrane remains dry, thus requiring shorter blow-down time after the integrity test is performed. In addition, solvents such as alcohol are not required.

Practical aspects of both test approaches are covered below, and additional information on the

theory behind these tests is offered in Appendix D. Pressure sensors and flow meters involved in any of the test procedures should be included in an appropriate calibration program as suggested in GAMP 4, Good Automated Manufacturing Practice Guide to Validation of Automated Systems in Pharmaceutical Manufacture (International Society for Pharmaceutical Engineering, 2001)

In certain applications, an aerosol integrity test can be used to verify the performance of hydrophobic membrane filters. The main advantages of this method are that the filter is tested in the gas phase and the test times may be considerably shortened.

8.1 Traditional Tests Using Wet Filters

As indicated above, the traditional approaches used to test the integrity of hydrophilic filter elements can also be used for hydrophobic membrane filters. Because water is not a suitable wetting liquid, lower surface-tension liquids, such as aqueous solutions of isopropyl or tertiary butyl alcohol, are typically employed. The use of pure alcohols is problematic due to higher gas diffusion and evaporation rates. For the test results to be meaningful, the wetting liquid and the test gas, typically compressed air or nitrogen, should be specified. Furthermore, because the physical properties of the wetting liquid and the test gas (e.g., surface tension, diffusivity, and solubility) are affected by temperature, these tests must be conducted at the temperature recommended by the filter manufacturer, or a temperature for which test limits have been established by the filter user.

8.1.1 Manual Bubble Point Tests

Manual Bubble Point Tests are based on the observation that at a low applied pressure the bulk flow of a test gas through a membrane filter is essentially blocked if a liquid fills the pore structure. As the applied pressure is increased, eventually a pressure high enough to dislodge the liquid from the largest pores is reached. As the pressure is increased further, it causes liquid to be expelled from the larger pores, resulting in increasing levels of bulk gas flow. When sufficient amount of bulk flow takes place through a set of the largest pores, passage of the test gas through the filter can be observed as a "steady stream of bubbles" emanating from the downstream tubing when placed in a test beaker filled with water, as indicated in Figure 8. The pressure at which this occurs is the bubble point pressure.

As discussed in more detail in Appendix D: Theoretical Aspects of Integrity Testing, the numerical value of the

bubble point pressure depends on the filter pore size distribution and morphology, any surface modification chemistry associated with it, and the surface tension and wetting characteristics of the liquid used to fill the pore structure for the test. In practical terms, the result of a bubble point observation can also be affected by procedural details such as filter area, rate of pressure increase, upstream volume, downstream volume and tubing size, and, more importantly, operator skill and interpretation (Hoffman, 1984; Sundaram et al., 2000a, 2000b). If all other parameters, including filter area and test method, are kept constant, the higher the bubble point pressure, the smaller the effective pore size.

If the bubble point pressure observed in a given test is as high as or higher than the minimum value specified, the filter is deemed integral and can be placed in service. An apparent bubble point below the specified minimum value may be indicative of a defective filter, and should be investigated (see Section 8.6). Even though the manual bubble point test is somewhat subjective, it is recognized as a sensitive, visual technique for judging the integrity of disc samples and other small-surface-area membrane filters. The minimum bubble point specification of a given type of membrane should be supported by the bacteria retention data.

Points to consider when performing a Manual Bubble Point Test:

- Increase the pressure slowly and in small increments in order to avoid overshooting the true bubble point value. It is possible to use larger increments initially and begin the bubble point test at approximately 75–80% of the expected value.

- Allow the pressure to stabilize after each pressure increase.

- Minimize downstream connections and avoid kinks in the downstream tubing.

- Check for and repair leaks in the system.

- Bulk flow of the test gas through the test membrane indicates that the bubble point pressure has been reached. A steady stream of freely flowing gas bubbles is the proper endpoint of the test, not the first isolated bubble. Alternatively, displacement of fluid downstream is also indicative of bulk gas flow.

- Keep upstream volume to a minimum to shorten stabilization time.

- Maintain the temperature steady and within the required range.

- If the test is to be repeated, the membrane must be re-wetted with the same integrity test fluid.

- If desired, an alternate integrity test fluid may be used for which limits are available.

Attributes and shortcomings:

- The test is relatively easy to perform for small- to medium-area filters, but manually performed Bubble Point Tests are subject to interpretation. Therefore, operators must be properly trained.

- A relatively short stabilization time is required.

- The test is not recommended for large-area membrane systems.

- The test result can be correlated to the bacterial retention capability of the type of membrane tested.

- The test is invasive, i.e., it requires manipulations on the sterile downstream side of the filter element.

8.1.2 Manual Diffusive/Forward Flow Test

This test is based on the fact that at pressures below the bubble point of the filter membrane, gas molecules migrate through the liquid within the pore structure of a wetted filter in accordance with Fick's law of diffusion (Hoffman, 1984; Waibel et al., 1996). The rate of diffusion through the membrane depends on the solubility and the diffusivity of the test gas in the liquid used for wetting the filter. It is directly proportional to the applied test pressure, the effective surface area, and the total porosity of the membrane. It is inversely proportional to the thickness of the membrane and the viscosity of the liquid. While low for membrane disc samples or small-area filter elements (less than approximately 0.2 ft^2), the diffusive flow of gas through larger membrane filter systems, such as single- or multiple-pleated filter cartridges, can readily be measured quantitatively by means of test setups such as the ones depicted in Figures 9 and 10.

8.1.2.1 Downstream Measurement Method

As illustrated in Figure 9, the Diffusive/Forward Flow Test at the specified test pressure can be conducted by collecting the downstream test gas in a graduated device over a water trough for a suitable length of time. Lower flow rates can also be established by timing the advance of a soap film within a graduated pipette or any other suitable flow measurement approach.

If the flow measured in a given test is less than or equal to the maximum allowable value at the specified test pressure, the filter is integral and can be placed in service. A passing result indicates that the retention capability of the filter tested has not been compromised. A flow rate higher than the maximum allowable limit may be indicative of a defective filter, and should be investigated further (see Section 8.6).

Points to consider when performing Diffusive/Forward Flow Tests manually:

- Perform the test at a validated test pressure.

- Allow sufficient equilibration time after pressurizing the system.

- Maintain a stable test pressure and temperature throughout the test.

- Use a suitably sized graduated cylinder/burette. Avoid kinking any downstream tubing.

- Check for and repair leaks in the system.

Attributes and shortcomings:

- Developed to provide increased sensitivity for large-surface-area systems.

- Provides an objective, quantitative measure of the flow of the test gas, but the gas volume observed will depend on prevailing atmospheric conditions.

- The test results are indicative of the filter's bacterial retention capability.

- The test is sensitive to temperature fluctuations.

- The test is invasive, as it requires manipulations on the sterile downstream side of the filter element. It can be performed off-line after the filtration process.

8.1.2.2 Upstream Measurement Method

As illustrated in Figure 10, the Diffusive/Forward Flow Test can also be conducted on the upstream (non-sterile) side of the housing with the use of sensitive gas flow meters, particularly mass flow transducers (Schroeder, 1995). The typical test is carried out as follows. With a wetted filter in the housing, the test pressure is adjusted against the closed isolation valve. Once the test pressure is set, the valve is opened to allow pressurization of the system. The regulator keeps the system at a constant pres-
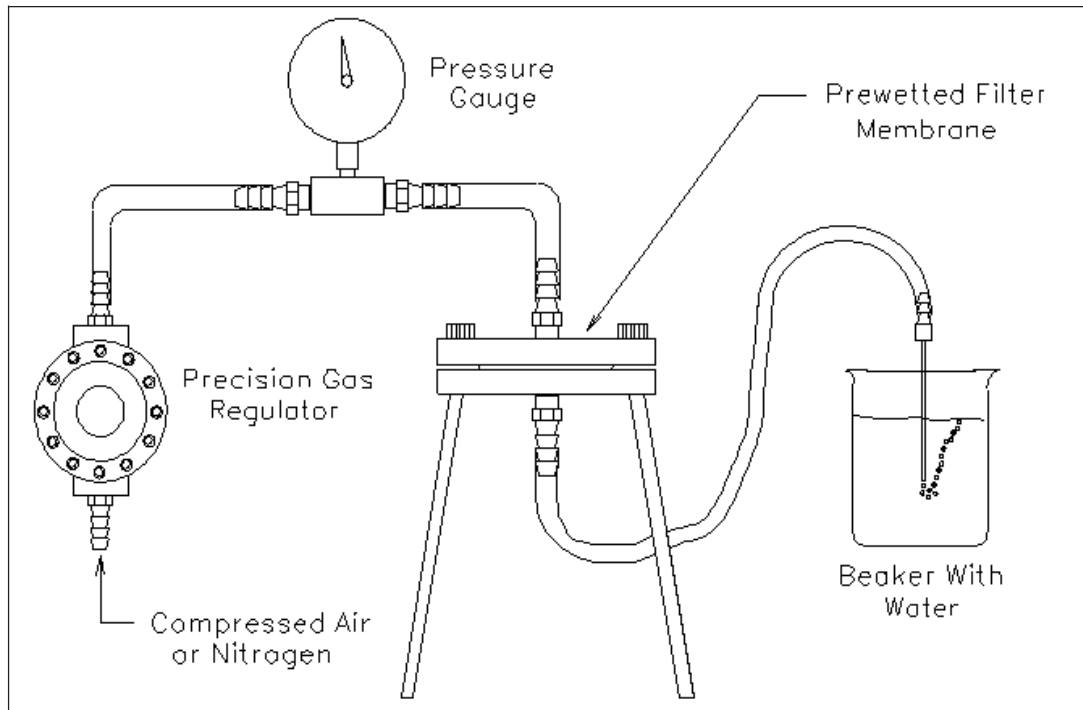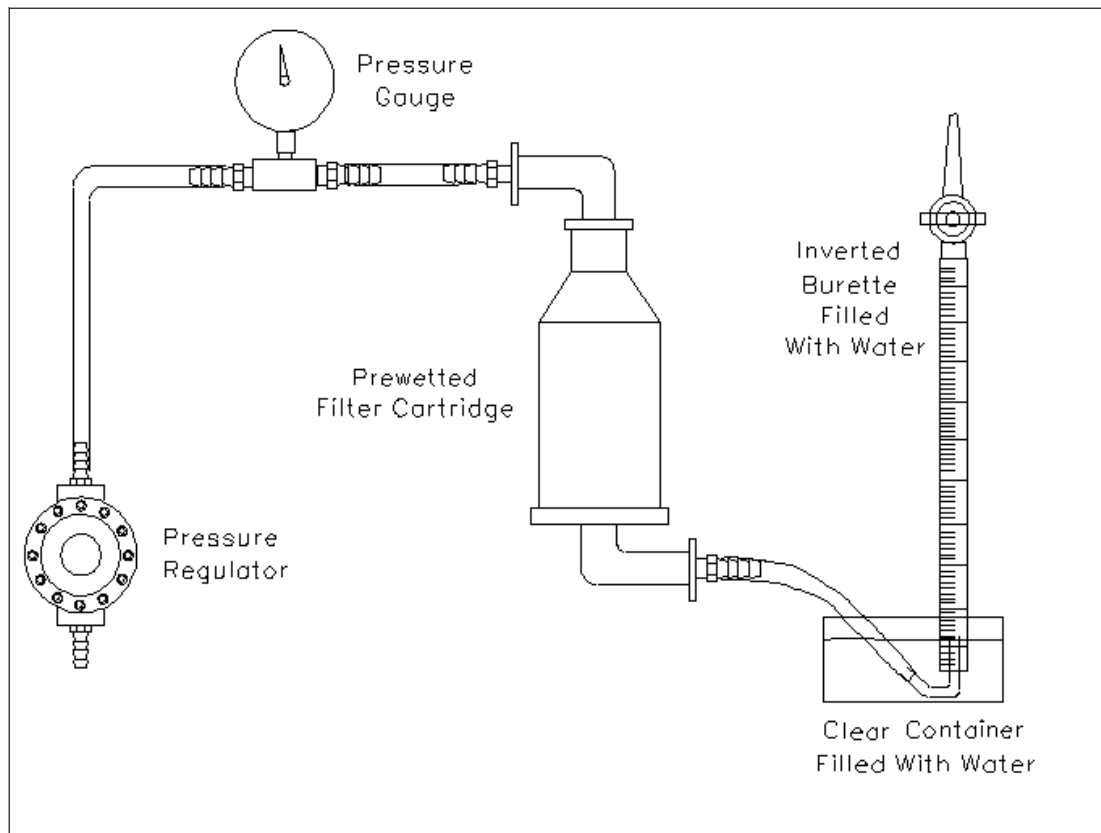
Figure 8: Typical Manual Bubble Point Test Setup.

Figure 9: Typical Test Setup for Downstream Manual Diffusive/Forward Flow Test.

sure, and any gas required to maintain the test pressure will pass through and be monitored by the mass flow (or other gas flow) sensor. After a suitable stabilization time, the amount of gas required to maintain the test pressure is equal to the total amount of gas passing through the wet filter.

The interpretation of the test result is similar to the downstream diffusive flow test discussed above. A passing result indicates that the retention capability of the filter tested has not been compromised.

In this test, all of the necessary manipulations are made on the non-sterile, upstream side of the filter, eliminating the risk of contamination of the sterile downstream side. Another feature of this test is that an upstream leak will lead to erroneous rejection of an integral filter. This type of test also lends itself to *in situ* testing.

Points to consider when performing direct upstream diffusive flow readings:

- Maintain a stable test pressure and temperature throughout the test.

- Flow sensor range must be commensurate with expected flow reading.

Contamination of the mass flow sensor with liquids will cause erratic readings.

Attributes and shortcomings:

- The test is relatively easy to perform and does not require manipulations of the sterile downstream side.

- Mass flow readings are independent of upstream volume and limited only by the sensitivity of the pressure regulator.

8.1.3 Manual Pressure Hold/Pressure Decay Test

The Manual Pressure Hold (or Pressure Decay) Test is an indirect method of determining the gas flow through a wet filter element (Trotter and Meltzer, 1998). In this approach, the wetted filter is pressurized to the specified Diffusive/Forward Flow Test pressure. After an appropriate stabilization time, the housing is isolated from the gas supply by means of a shut-off valve in the typical arrangement, as shown in Figure 11. As the test gas escapes by diffusion through the wetted filter element, the pressure in the housing slowly decays at a rate that can be measured quantitatively with a timer and a suitably sensitive pressure sensor.

The rate of pressure decay depends on the actual gas flow rate as well as the upstream volume of the filter housing. Because the upstream volume is not the same for all filter housings, the acceptable pressure decay limit is specific for each filter/housing combination. Using the known upstream volume, the pressure decay limit can be calculated using the following equation:
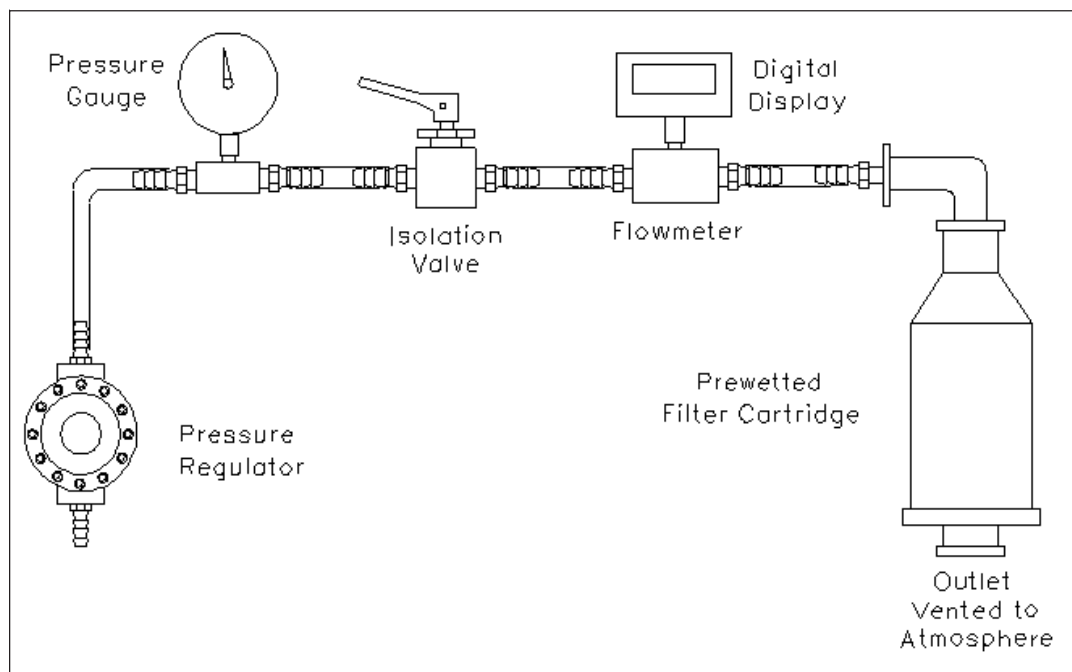

Figure 10: Typical Test Setup for Upstream Diffusive/Forward Flow Test

$$\text{Pressure Decay Limit} = \text{Test Pressure}\left[1 - \exp\left[\frac{-\text{Test Time} * \text{Diffusive}/\text{Forward Flow Limit} * \text{Standard Pressure}}{\text{Upstream Volume} * \text{Test Pressure}}\right]\right]$$

<div align="right">(1)</div>

If the pressure decay observed in the test is below the allowable decay limit, the filter passes the physical integrity test. Because the flow can be calculated from the rate of pressure decay, meeting the pressure decay limit will also assure the retention capability of the filter tested. An excessive pressure decay rate may be indicative of a defective filter and should be investigated further (see Section 8.6).

Like the upstream diffusive flow measurement discussed above, the pressure hold test does not require manipulations of the sterile downstream side of the filter, and also enables upstream leak detection and *in situ* testing.

Points to consider when performing the Pressure Hold/Pressure Decay Test:

- Check for and repair leaks in the system.

- Keep the upstream volume to a minimum to increase the sensitivity and shorten the stabilization time.

- Be sure the downstream of the filter is vented to atmospheric pressure.

- Use a pressure gauge with the proper degree of accuracy.

- Define conditions for the test, including the temperature and the test gas used.

- Maintain a steady temperature within the manufacturer's specified range throughout the duration of the test.

Attributes and shortcomings:

- The test is relatively easy to perform.

- No downstream manipulations are required.

- Test results can be correlated to bacterial retention, but the upstream volume must be determined to establish the maximum allowable pressure drop rate.

- Long test times may be needed, particularly for low rates of pressure decay.

- The test resolution is limited by the resolution of the pressure sensor used and decreases with increasing upstream volume of the system.

- The test is sensitive to temperature fluctuations in the upstream volume (temperature should not change by more than ± 1 °C during the test).
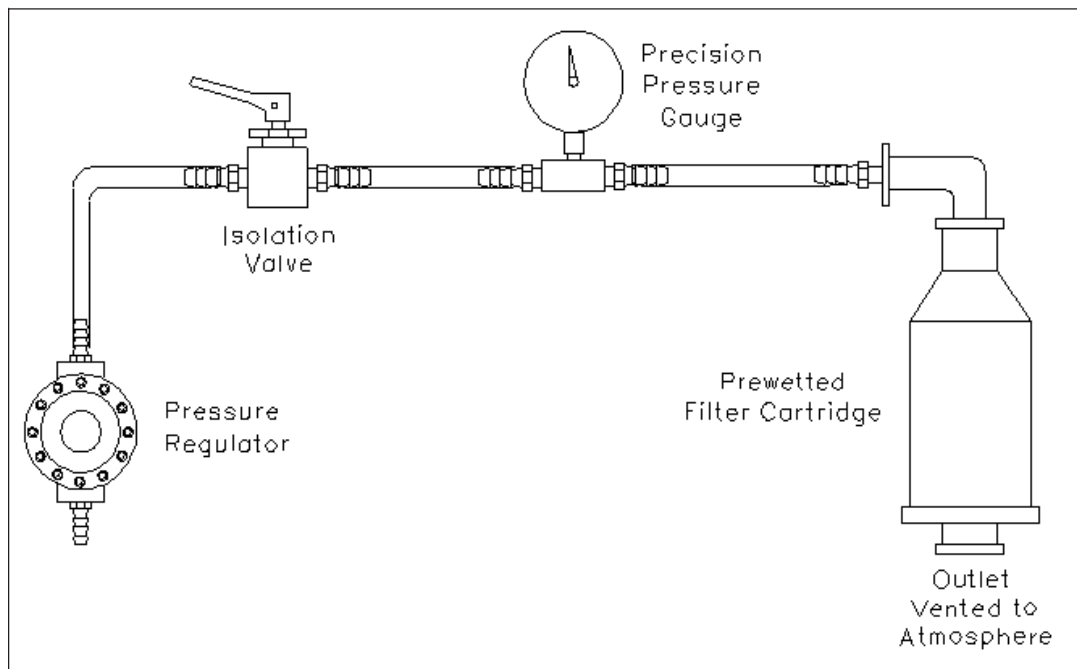

Figure 11: Typical Test Setup for the Manual Pressure Hold/Pressure Decay Test.

8.2 Water Intrusion Integrity Test Approach

The principle behind water intrusion testing is that dry hydrophobic membrane structures will not allow passage of water at low test pressures. At pressures below the breakthrough pressure (pressure required to force water through the pores), a small but measurable flow of water takes place, analogous to the diffusive flow of test gas in traditional wetted membrane integrity tests (Dosmar et al., 1992; Jaenchen et al., 1997). This flow rate is similarly proportional to the surface area and the porosity of the membrane tested. This is discussed in more detail in Appendix D.

In practice, the Water Intrusion Test is performed by flooding the upstream side of the hydrophobic filter element with water and observing the flow of water at a specific test pressure. Much as a gas diffusion-based test is performed at a gas pressure below the bubble point of the filter, which is wetted with an alcohol solution, the Water Intrusion Test is performed at a test pressure below the water penetration pressure. A manual approach is very impractical due to the low water flow rates involved and the need for a downstream flow measurement. Thus, the test is typically performed using an automated setup similar to the one shown in Figure 12. After completion of the test cycle, the water is drained from the housing. If moisture interferes with the intended application, the filter assembly can be dried by blowing down or flushing with dry gas.

The automated test instrument measures the flow of water indirectly by monitoring the amount of test gas that displaces the water at the set test pressure. The interpretation of the test result is also similar to that of the diffusive flow test, but it should be noted that some instruments report (gas) flow corrected to standard or atmospheric conditions. Because water is essentially incompressible, 1 mL of water displaced in the test will equal 1 cc of gas at a test pressure (e.g., 3 bars, or about 45 psig), but it corresponds to about 4 cc at atmospheric conditions.

If the rate of water intrusion observed in a given test is as low as or lower than the maximum allowable value at the specified test pressure, the filter is deemed integral and can be placed in service. A passing result indicates that the retention capability of the filter tested has not been compromised. A flow rate exceeding the maximum allowable limit may indicate a defective filter and should be investigated further (see Section 8.6).

The Water Intrusion Test offers distinct advantages over traditional solvent wet filter integrity tests. The need for introducing potentially flammable and contaminating solvents is eliminated. In addition, the test does not require manipulations of the downstream side, lending itself to *in situ* testing. The most significant advantage for many applications is that in water intrusion testing the membrane stays dry, thus shortening the blow-down time required to get the filter ready for use after the integrity test.

Points to consider in water intrusion integrity testing:

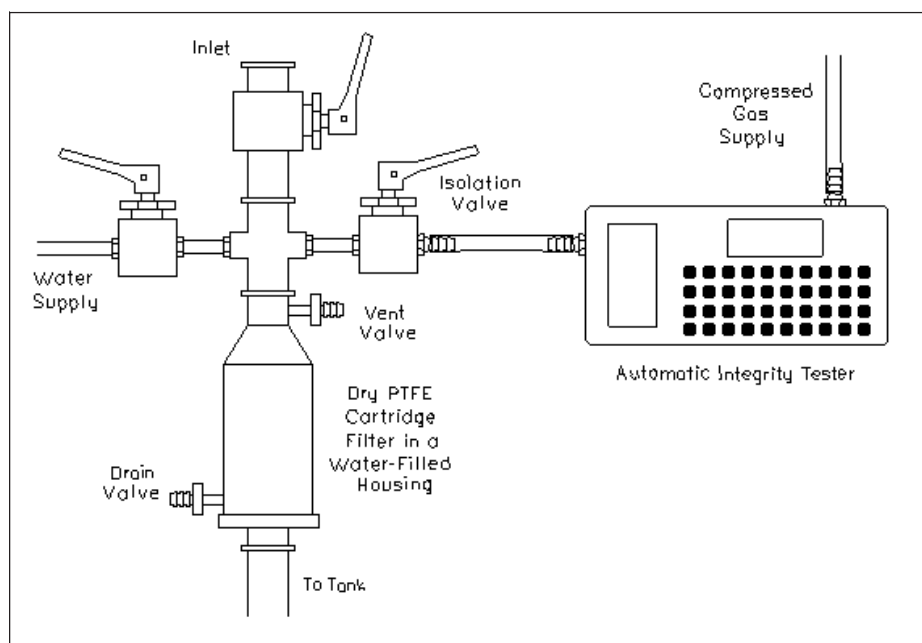• Check for and repair leaks in the system.

Figure 12: Water Intrusion Test

- Make sure filter is dry prior to initiating the test.

- Maintain a constant temperature throughout the duration of the test.

- Use high-quality water (water without contaminants that change the surface tension).

- Allow sufficient stabilization time, including time to saturate water used with test gas used by automated integrity tester.

- Use of a sensitive flow meter or pressure transducer is required for the detection/calculation of low flow rates, typically expressed in tenths of a milliliter per minute.

- When calculating flow values using upstream pressure measurements, precise quantification of the upstream volume is required for each test.

- Limits may need to be adjusted for different automated integrity testers.

Attributes and shortcomings:

- The test permits testing of hydrophobic membrane filters without use of flammable or contaminating solvents.

- The test has short blow-down and drying times after integrity testing.

- The results can be correlated to bacterial retention.

- The results may be adversely affected by incomplete drying after moist heat sterilization, resulting in false failures.

- The results may be adversely affected by contaminants that leave hydrophilic areas on the filter, resulting in false failures.

Note: The Water Intrusion Test is not suitable for small area filters (typically less than 0.5 ft^2) due to the extremely low flow rates involved, which will exceed the capabilities of the instrument. An alternate water-based integrity test for small-area filters is the Water Breakthrough/Penetration Test. In this test, lack of water flow at the water intrusion test pressures indicates that the water breakthrough point of the filter has not been exceeded. This test verifies proper installation of the filters and, more importantly, confirms absence of gross defects, but it is not considered a definitive test for critical applications.

8.3 Aerosol Integrity Test

The aerosol integrity test has been used historically within the pharmaceutical industry for detecting failures in systems containing HEPA- and ULPA-grade filters, from general HVAC systems to spray dryers and isolators (Johnston et al., 1995). It consists of challenging the filter with a high concentration of an aerosol in the particle size range of 0.2 to 0.3 microns, typically generated from a highly refined mineral oil. A downstream sensor detects aerosolized oil droplets penetrating the filter.

Although originally developed for depth filters, the aerosol integrity test is also applicable for checking the integrity of membrane gas filters that are qualified with an aerosol bacterial challenge. It is primarily used for filters providing sterile gas that does not come into contact with the final product, for example, in fermentor inlet air, or other non-critical applications. Sampling by laser particle counters is used to detect penetration during the test. This level of sensitivity in detection should ensure that penetration of the test aerosol is detected before a potential passage of bacteria.

The test should be correlated to an aerosol bacterial challenge at the predetermined challenge level, as outlined in section 7.2.

Performing the aerosol integrity test requires two entry points, one on the upstream side for the challenge aerosol and one on the downstream side for detection of aerosol penetrating the filter. The test consists of challenging the filter with approximately 10^7 particles (in the size range of 0.2–0.3 micron) per cm^2 of filter area. The test aerosol is typically generated from a highly refined mineral oil using compressed air.

The downstream filtered air is passed through a particle counter to determine the number of particles. Historically, light scattering photometers were used, but these had limited sensitivity. Current instruments utilize laser particle counters, which provide increased test sensitivity.

Points to consider when performing an Aerosol Integrity Test:

- Ensure the filter is dry. It is possible that the integrity test will not detect defects if the filter is wetted out.

- Always ensure that the downstream connection is completely dry and free from condensate, to protect the downstream particle counter.

Attributes and shortcomings:

- Test times are shorter than many liquid-based integrity tests.

- The results can be directly correlated to an aerosol bacterial and viral challenge.

- The test is applicable to sterile gas production under dry conditions.

- The test requires manipulations on the sterile downstream side of the filter.

- Small amounts of mineral oil will be deposited on the filter media.

8.4 Automated Integrity Test Instruments

As mentioned before, the Water Intrusion Test and the Aerosol Integrity Test must be performed with automated instruments. For traditional integrity tests based on wetted membranes, automated integrity test instruments offer advantages over manual methods.

Some manual integrity tests methods require downstream manipulations that could compromise the sterility of the system. To maintain sterility of the downstream side, any automated integrity test units must perform the integrity test from the upstream (non-sterile) side of the filters.

Automated integrity test instruments offer several advantages over manual tests. These include:

- Increased sensitivity through pressure transducer or mass flow meters

- Minimized operator variability and subjectivity

- Better reproducibility of results

- Hard-copy printout of test results

- Software security

- Assurance of system sterility (upstream connections only)

Automated integrity test units utilize either pressure transducers or mass flow meters to perform pressure decay (indirect) or gas flow (direct) measurements. These automated integrity test instruments can conduct Diffusive/ Forward Flow or Pressure Hold/Decay Tests, and Bubble Point Tests.

Diffusive/Forward Flow Test and Pressure Hold/Decay Test measurements may vary depending on the type of sensor selected, total upstream volume, temperature stability, and measurement time interval.

Bubble point measurements may vary depending on the algorithm used to determine where the discontinuity in the air/gas flow curve occurs during a series of pressure decay tests conducted at increasingly higher pressures. As the algorithms are proprietary to each instrument manufacturer, the endpoint criteria may vary among different instrument models (Sundaram et al., 2000a, 2000b).

Automatic integrity test equipment, both hardware and software, must be qualified. Users should contact the instrument manufacturer for validation documentation and information concerning the qualification of the particular instrument (Stanbury and Whitaker, 1989; Czermak and Capatano, 2003). If applicable, the requirements of Title 21 Code of Federal Regulations (21 CFR Part 11) "Electronic Records; Electronic Signatures" must be considered by users of such instrumentation.

Instrument qualification requirements are similar to those for other process test equipment, with similar Installation Qualification/Operational Qualification (IQ/OQ) and Performance Qualification (PQ) testing. These include:

- Programming evaluation: test parameters, test method, programming the unit and the test

- Unit sensitivity evaluation

- Unit start-up

- Unit calibration

- Performing the tests

- Integrity test performance evaluation: Bubble Point, Diffusive/Forward Flow, Pressure Hold/Decay, Water Intrusion Tests

- Testing other functions: volume determination, failure mode, rejecting invalid entries

- Test printout evaluation

- Computer software evaluation

- Password protection qualification

- Peripheral function evaluation: date/time clock, memory, cleaning

8.5 Considerations for Integrity Test Practices

An integrity test conducted prior to sterilization will only confirm that an integral filter of the correct grade has been properly installed. Integrity tests conducted prior to the filtration process are preferably performed after ster-

ilization. The post-sterilization test will detect whether or not the filter was damaged during sterilization. In such cases, steps must be taken to ensure that the downstream side of the system remains sterile. An upstream integrity test must be used when downstream aseptic conditions must be maintained.

Assurance of microbial retention throughout the critical filtration process must be confirmed by a post-filtration integrity test.

As a general rule, a filter should be integrity-tested prior to being placed into a critical application to ensure that it is capable of performing its stated function. For critical sterile applications (product or critical surface contact), the best practice is to test filters upon installation or *in situ*, and after use. For gas filters in extended use applications, or in less stringent applications, some filter users have specified an integrity test frequency based on factors such as historical process durability, time on line, or number of sterilization cycles. No single approach applies to all applications, and an appropriate testing frequency and rationale should be selected using risk analyses considering impact on product quality and on regulatory compliance.

For extended use applications, the following approaches are being used. They are listed in order of increasing risk:

- Install parallel filters so that while one filter is in use the other filter can be tested and prepared for use.

- Use redundant filters, i.e., two filters in series, in combination with periodic integrity testing and change-out.

- Use a combination of periodic integrity testing and change-out schedule.

- Test filters only after the first sterilization.

- Test filters only upon installation.

- Do not test filters, and base filter change out on historical data.

The risk associated with some of these practices is that any product produced since the last successful integrity test may not meet the expected microbial quality attributes if the filter fails to meet the required test criteria. This, in turn, will trigger the need for thorough investigation and may result in loss of product.

Pre-process (after sterilization) testing *in situ* requires the use of non-invasive testing procedures, most readily accomplished by using automated integrity test instruments. When the use of alcohol-based solvents in the manufacturing environment is of concern, the Water Intrusion Test becomes the only feasible test. It is important to note that any *in situ* integrity test verifies that the filter(s) has been properly installed.

8.6 Troubleshooting Integrity Test Failures

If a sterilizing filter fails an integrity test, the filter may be defective. However, there could be many causes for the apparent failure of an integrity test, primarily related to the wetting of hydrophobic membranes. Additional testing required due to an apparent filter failure must be incorporated in a written standard operating procedure. Any confirmed or true failures require an investigation.

To distinguish between filter damage and possible test problems or artifacts, the following steps may be taken:

- Confirm that the appropriate integrity test parameters were employed.

- Confirm that there are no leaks in the test system.

- Confirm that the test was conducted at the specified temperature.

- Confirm that the test equipment has been properly calibrated.

- Confirm that the test setup is properly assembled and functions properly.

- Confirm that the correct filter has been installed.

For the Diffusive/Forward Flow or Bubble Point Tests:

- Confirm that the correct wetting fluid and wetting procedure were used.

- To rule out incomplete wetting, re-wet the filter according to the specified procedure, and repeat the test or use another qualified integrity test procedure.

- If wetting remains an issue, consider using an alternate wetting solution for which limits are available.

For the Water Intrusion Test:

- Dry the filter and retest it.

- Perform an alcohol-wet Diffusive/Forward Flow or Bubble Point Test. Water Intrusion Test results may be adversely affected by hydrophilic areas from pro-

cess residues (particularly post-use) on the filter, which can cause false failures. Performing the alcohol-wet tests can confirm that the filter is integral.

• In such cases involving compromised hydrophobicity, filters that are qualified solely on the basis of aerosol retention tests may not provide the expected retention efficiency if the filter is wetted in subsequent usage. A suitable solvent rinse procedure may restore such filters to useable condition. However, these concerns do not apply to filters qualified by liquid retention tests.

9. USER RESPONSIBILITIES FOR THE VALIDATION OF CRITICAL APPLICATIONS

Traditionally, the FDA has held individual pharmaceutical companies responsible for validating their critical processes. However, in the area of sterile filtration the agency acknowledges the fact that "when the more complex filter validation tests go beyond the capabilities of the filter user, tests are often conducted by outside laboratories or by filter manufacturers" (FDA Guidance for Industry – Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice, 2004). Whether carried out in-house or through filter vendors or contract laboratories, it remains the drug manufacturer's ultimate responsibility to ensure that worst-case formulation and processing parameters are adequately studied, evaluated, and documented.

PDA Technical Report No. 26: *Sterilizing Filtration of Liquids* proposes that filter validation responsibilities be shared between the filter user and the filter manufacturer. Appendix C lists the tests commonly performed by the filter users and filter manufacturers, based on general industry practice.

For liquid sterilizing-grade filters, the filter manufacturer is expected to present a relationship between the integrity test results and the results of a generic retention protocol for purposes of qualifying the filter as sterilizing grade. For hydrophobic filters, the equivalent would be the bacterial or viral challenge tests described in Section 7. The filter manufacturer typically provides this information in a validation guide. In addition to making available lot release test results for integrity, bacterial retention, and extractables, the filter manufacturer is also expected to provide qualification data for toxicity, durability, compatibility, and recommendations for integrity test parameters.

9.1 Bacterial or Viral Retention

The FDA accepts product-specific retention validation data for sterile filtration of liquids generated by filter manufacturers. Ultimately, the filter user is responsible for the applicability of the data presented. In contrast to sterile filtration of liquids, there is little, if any, evidence about the influence of the carrier gas in applications involving sterile filtration of gases. Most applications involve air or nitrogen, and the filtration efficiency for smaller particles is greatly enhanced in gas filtration, as discussed in Section 3.2. For these reasons, a process- and product-specific retention test is generally not required for air/gas filtration applications.

However, the filter manufacturer's qualification data should be evaluated carefully to justify the applicability to a given specific process. In special cases, filter users may want (or need) to complement generic qualification data with their own tests to ensure that the retention efficiency of the filter for the contaminants of concern is adequate.

In most cases, the user's specific process conditions, such as flow rates, differential pressures, temperature, and duration, will likely differ from those used by the vendor in its qualification testing. This in itself should not necessitate user-specific retention testing, especially for filters rated by a liquid bacterial challenge. Liquid bacterial challenge testing represents a worst-case condition for sterilizing gas filters because the retention efficiency in liquids is much lower than in gases. However, the compatibility of the filter under the user's specific process conditions must be established. Physical integrity testing, as discussed further in Section 9.3, is a determinant of filter compatibility.

9.2 Integrity Testing

Physical integrity tests, as described in Section 8, are used to confirm that the filter meets its retention capability claims. Any integrity test is meaningful only when it can be correlated to specific microbial retention characteristics. Such data are usually published in the filter validation guide, and should be evaluated carefully by filter users to ensure that the correlation is sound and based on physicochemical considerations.

Recommendations on integrity testing practices can be found in Section 8.5.

9.3 Compatibility and Service Life

Qualification data should be provided by the filter manufacturer to support maximum operating conditions, such as differential pressures and temperature, sterilization modes, and number of sterilization cycles. However, the

laboratory test conditions used by the vendor for qualification testing will not match the user's operating conditions. Compatibility of the filter under actual use conditions should be demonstrated. Compatibility may be demonstrated by integrity testing the filter before and after exposure to the conditions expected in the process.

In most applications, filters may be either left in continuous service or reused multiple times after sterilizing between uses. Service life should not be based solely on vendor data under typical conditions, and should be established by integrity testing the filters at periodic intervals, either in-process or in a separate validation study. If filters are replaced at specified time intervals, it is not necessary to establish the failure point, but data should be generated to demonstrate that the filters retain integrity for the duration of their use.

Appendix A:

Theoretical Aspects of Retention Mechanisms in Air

The diffusional interception retention mechanism applies to very small particles at low face velocities. Air molecules are in a state of constant, random motion, and they strike particles suspended in the air. When faster-moving air molecules strike small airborne particles, typically smaller than 0.3 μm, these particles can be displaced by this molecular bombardment. The random movement of particles resulting from these molecular collisions is known as Brownian motion. This phenomenon increases the probability that a small airborne particle will collide with a fiber in the filter material.

This probability, or the diffusion parameter, D, can be expressed as:

$$D = \frac{1}{v \bullet d_f} \times \frac{C \bullet k \bullet T}{3 \bullet N_y \bullet d_p} \qquad \text{(A-1)}$$

where $(C \bullet k \bullet T)/(3 \bullet N_y \bullet d_p)$ is the diffusion coefficient, C is the Cunningham correction factor, k the Boltzmann constant, T the absolute temperature of the air in °Kelvin, N_y the viscosity of air, d_p the particle diameter, v the velocity of gas, and d_f the fiber diameter.

As shown above, diffusional interception is inversely proportional to the gas velocity, particle diameter, and viscosity of the gas. Thus, the higher the velocity, the lower the retention effectiveness at small particle sizes. In addition, humidity can also affect the effective diameter of the particle and the viscosity of gas, which in turn lowers diffusional interception.

In the inertial impaction retention mechanism, heavier and larger particles can deviate from the streamline and collide with a fiber in the filter material due to their inertia. As the face velocity and mass of the particle increase, the probability of inertial impaction increases. This separation mechanism is most effective for larger particle sizes, typically above 1 μm. The inertia parameter, M_y, can be expressed as:

$$My = \frac{C \bullet r \bullet v \bullet d_p^2}{18 \bullet N_y \bullet d_f} \qquad \text{(A-2)}$$

where C is the Cunningham correction factor, r is the density of the particle, v is the velocity of air, d_p is the particle diameter, N_y is the viscosity of air, and d_f is the fiber diameter.

Gravitational settling is another removal mechanism that enhances the probability of contact with the filter material and removes particles from air streams. This mechanism is more effective at low face velocities and is more pronounced for larger particles of high density. As expressed in the following formula, it is highly influenced by the viscosity and the velocity of the air. The settling parameter, G, can be calculated from

$$G = \frac{u}{v} = \frac{C \bullet r \bullet g \bullet d_p^2}{18 \bullet N_y \bullet v} \qquad \text{(A-3)}$$

where u is the settling velocity of the particle, v is the air velocity, C is the Cunningham correction factor, r is the density of the particle, g is the gravitational constant, d_p is the particle diameter, and N_y is the viscosity of air.

Appendix B:

Maintenance, Preparation, and Characterization of *Brevundimonas diminuta* Challenge Suspensions

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.

Two standard methods have been recognized as suitable for preparation and maintenance of *B. diminuta* for challenge testing. 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 (American Society for Testing and Materials, 1983; Fennington and Howard, 1997; Leahy and Sullivan, 1978).

B. diminuta to be used for challenge testing may be confirmed to be ~0.3 μm–0.4 μm in diameter by ~0.6 μm–1.0 μm in length, via an optical or scanning electron microscope equipped with an appropriate measuring device. For each challenge performed, the size of the challenge organism must be confirmed by demonstrating passage through a 0.45-μm-rated membrane as a positive control (Bowman et al., 1967).

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 min. The cavitating action of the bath is effective in disaggregating bacterial cells without loss of colony-forming ability. This effect should be confirmed by optical microscopy and viable count (Fennington and Howard, 1997). Absence of significant aggregation is also confirmed by demonstrating penetration of 0.45-μm-rated membranes as a positive control for each challenge test.

Viability of the *B. diminuta* suspension should be confirmed using a suitable medium, such as Tryptic Soy or Mueller 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. Unless the use of a different culture medium has been shown to be equivalent, the same culture medium used for assessing viability should be used to determine any recovery of *B. diminuta* during the challenge test.

Appendix C:

Filter Validation Recommendations

Tests Commonly Performed by Filter Users and the Filter Manufacturers—General Industry Practices

Criteria	Filter User	Filter Manufacturer	
	Filter Device	Membrane Disc	Device
Bacteria Retention/ Integrity Test Relationship Data	(E)	(Q)	(Q)
Integrity Test		(Q/R/L)	(Q/R/L)
Integrity Test Methodology and Selection	(E)	(R)	(R)
Microbial/Viral Retention (Liquid/Aerosol)	(E)	(Q/L)	(Q/L)
Compatibility/ Service Life	E/V	(Q/R)	(Q/R)
Toxicity Testing		(Q)	(Q)
Effects of Sterilization Methods on Filter Integrity	(E/V)	(Q)	(Q)

Q = Qualification Testing

V = Validation Testing—Process-Specific

E = Evaluate Applicability to Process

R = Recommendation for Validation

L = Filter Lot-Specific Release Criteria

Appendix D:

Theoretical Aspects of Integrity Testing

The practical aspects of integrity testing of hydrophobic membrane filters are discussed in Section 8 of this report. Theoretical aspects of this topic have been extensively reported in the technical literature (Hoffman, 1984; Washburn, 1921; Waibel, 1996; Schroeder, 2003), and a brief summary is presented below.

Principle of Water-Based Integrity Tests

Water-based integrity tests rely on the principle that dry hydrophobic membrane filters will not allow passage of water at low differential pressures due to the cohesive surface tension of the water. The theory behind the Water Breakthrough Test is similar to that of the Bubble Point Test. Rather than overcoming the capillary force that holds the liquid within a hydrophilic pore, as in the Bubble Point Test, the pressure that must be applied to force the water into and through the hydrophobic pore structure is a function of the cohesive force. This is, in turn, a function of the surface tension, the degree of hydrophobicity, and the pore size and geometry. For a given filter type, the water breakthrough pressure can be described by the expression

$$P \ = \ (K \bullet \gamma) \, / \, d \qquad \text{(D-1)}$$

where g is the surface tension, d is the pore diameter, and K is a constant that encompasses the hydrophobicity of the material and the pore morphology.

In an integral filter, at pressures below the breakthrough pressure, a small but measurable flow of water (presumably in the form of water vapor) takes place, analogous to the diffusive flow of test gas in traditional wetted membrane integrity tests (Jaenchen et al., 1997; Meltzer et al., 1994). This is the flow that is measured during a Water Intrusion Test. This flow rate is similarly proportional to the surface area and the porosity of the membrane tested. The flow rate varies directly with the temperature of the water, suggesting that the driving force is the vapor pressure of the water.

At room temperature, a water intrusion profile as a function of pressure is generally quite low at low pressures, particularly if the downstream volume is saturated with moisture. As the test pressure is increased, water is first forced through the larger pores and then progressively through the smaller pores, resulting in the profile depicted in Figure D-1.

Principle of Integrity Tests that are Based on a Wetted Membrane

The following is reproduced from Section 7.1 of PDA Technical Report No. 26: *"Sterilizing Filtration of Liquids"*.

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. Additionally, the integrity test will help establish the similarity of the test filter to the filters validated to retain a bacterial challenge under process-related conditions. 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
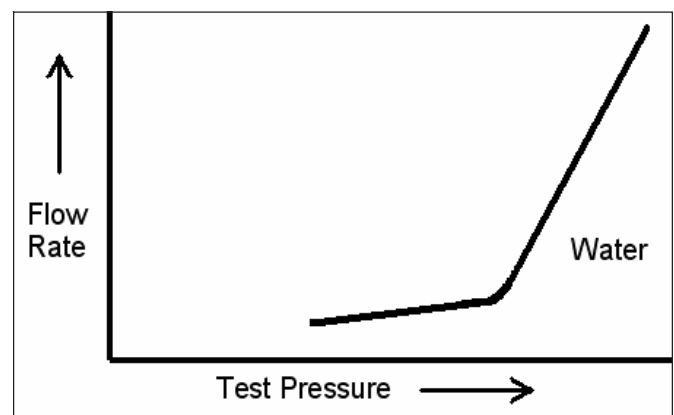

Figure D-1: Idealized Water Intrusion Test Profile of a Hydrophobic Filter Cartridge

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.
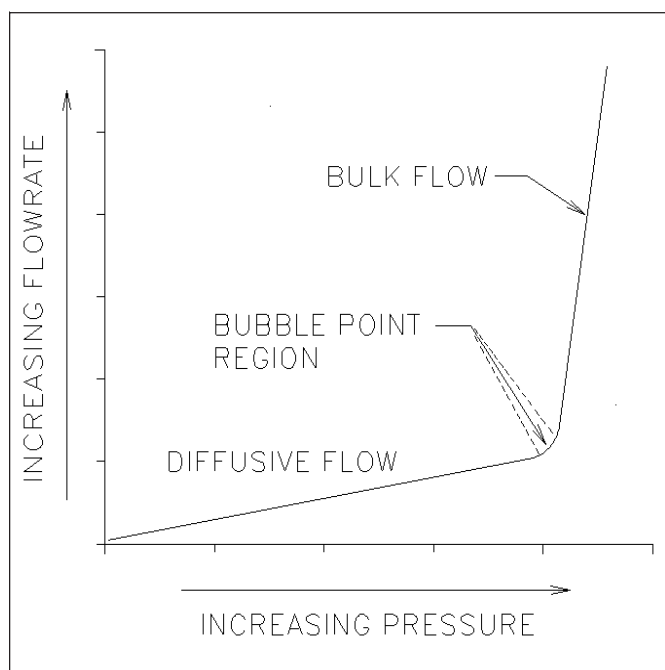
Figure D-2: Measured Airflow Downstream of Wetted Filter Membranes

Figure D-2 describes the relationship between measured airflow 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. The following is adapted from PDA Technical Report No. 26.

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

$$N = \frac{DHP}{L}\varphi$$
(D-2)

Where:

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 applied differential pressure (or gauge pressure if collected at atmospheric conditions)

f = 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

$$F = K_1 P$$
(D-3)

Where:

F = the volumetric diffusive flow

K_1 = a proportionality constant

P = the applied differential pressure (or gauge pressure if collected at atmospheric conditions)

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, eq D-3 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 of the largest pore(s) 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.

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

Where:

Q = the volumetric flow rate of the test gas

DP = 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."

$$P = \frac{4 k \gamma \cos \Theta}{d}$$
(D-5)

Where:

P = the differential pressure at which a given pore will open

k = correction factor for the shape of the largest pores

g = the surface tension of the wetting liquid

cos Q = the contact ("wetting") angle between the liquid and the membrane

d = the diameter of the largest pores

To demonstrate the dependence on the liquid used to wet the pores and its interaction with the filter material, eq. D-5 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

$$d = \frac{K_2}{P}$$
(D-6)

Where:

d = the diameter of the largest pore

K_2 = a proportionality constant

P = the differential pressure at which a given pore will open

Where K_2 is a correction factor accounting for shape of the largest pores 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 D-2, which depicts 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 eq. D-2 or D-3, 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 eq D-5.

NOTE: The Sterile Gas Filtration Task Force would like to emphasize that a precise pore size measurement as determined by the bubble point method is extremely theoretical. In actual application, it is, at best, an estimation of an indeterminate set of the largest pores, not just the largest pore, in the membrane. Practically, the correlation of any integrity test to a successful microbial retention test is the key determinant of the efficacy of the filter in its application.

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NOTES

PDA Journal of
Pharmaceutical Science and Technology

EDITOR: Lee Kirsch