Filter Integrity
Test Methods
This booklet is designed to provide a basic understanding of methods for testing the integrity of Millipore membrane filters and filter systems. Sterilizing-grade filters (0.22 µm pore size) are used as examples because they must meet the most exacting requirements. Test methods, however, can be applied to all Millipore membrane filters and final filter systems in general.

The ultimate measure of a sterilizing filter’s performance is bacterial retention. In the Millipore bacterial retention test, 0.22 µm discs and devices are challenged with a solution of culture medium containing bacteria *Brevundimonas diminuta* ATCC 19146) in the range of $10^7$ per cm² (just short of clogging the filter). The effluent is then passed through a second 0.45 µm filter disc which is placed on an agar plate and incubated. This test is conducted on accordance with HIMA methodology.

A section of a typical second filter is shown below left. No colonies appear, demonstrating that no bacteria passed through the first filter. The section shown below right is from a typical second filter used in a similar test except that the first filter was 0.45 µm in pore size. The colonies on the filter surface confirm that the test bacteria were smaller than 0.45 µm since they passed through the first filter.
Integrity testing sterilizing filters is a fundamental requirement of critical process filtration applications in the pharmaceutical industry. FDA Guidelines require integrity testing of filters used in the processing of sterile solutions such as large volume parenterals (LVPs) and small volume parenterals (SVPs). The FDA also requires corresponding testing documentation be included with batch product records.

Two classifications of integrity testing are destructive and non-destructive. Millipore’s practice is to perform destructive testing as a lot release criteria on samples from each manufacturing lot of all fabricated sterilizing-grade filter products, and nondestructive testing on each sterilizing-grade filter prior to sale to insure its integrity.

**Destructive Testing**

Millipore performs destructive bacterial challenge testing in accordance with ASTM F838-83 methodology. Destructive challenge testing is the best way to determine a sterilizing filter’s ability to retain bacteria. Bacterial challenge testing provides assurance that the membrane and fabricated device meet the critical performance criteria of a sterilizing filter. The test is performed on a statistical sample of each lot of membrane and fabricated devices produced.

During the Millipore bacterial retention test, 0.22 µm filter discs and devices are challenged with a solution of culture medium containing bacteria (*Brevundimonas diminuta* ATCC 19146) at a minimum challenge of $10^7$ per cm$^2$. The effluent is then passed through a second 0.45 µm assay filter disc that is placed on an agar plate and incubated.

**Non-Destructive Testing**

Non-destructive testing may be done on filters before and after use. Integrity testing sterilizing filters before use monitors filter integrity prior to batch processing, preventing use of a non-integral filter. Integrity testing sterilizing filters after a batch has been filtered can detect if the integrity of the filter has been compromised during the process. Detecting a failed filter alerts operators to a problem immediately after batch processing, eliminating delay and allowing rapid reprocessing.

There are three types of non-destructive testing – the bubble point test, the diffusion test, and the waterflow integrity test for hydrophobic filters (HydroCorr™ Test). The pressure hold, forward flow, and pressure decay tests are variations of the diffusion test. The stringent requirements of the pharmaceutical industry dictate that nondestructive filter integrity testing must be performed in each sterilizing application.

To be able to use an in-process non-destructive integrity test, physical tests were developed that correlate to the bacterial challenge test. A specification for the physical test correlates directly to the bacterial challenge test. Once this correlation is established, it is determined that a cartridge passing the physical test is an integral sterilizing filter.

**Bubble Point Test**

The most widely used non-destructive integrity test is the bubble point test (see box). Bubble point is based on the fact that liquid is held in the pores of the filter by surface tension and capillary forces. The minimum pressure required to force liquid out of the pores is a measure of the pore diameter.

\[
P = 4k \cos \theta \frac{\sigma}{d}
\]

- $P$ = bubble point pressure
- $d$ = pore diameter
- $k$ = shape correction factor
- $\theta$ = liquid-solid contact angle
- $\sigma$ = surface tension

**Bubble Point Procedure:**

1. Wet the filter with the appropriate fluid, typically water for hydrophilic membranes or an alcohol/water mixture for hydrophobic membranes.
2. Pressurize the system to about 80% of the expected bubble point pressure which is stated in the manufacturer’s literature.
3. Slowly increase the pressure until rapid continuous bubbling is observed at the outlet.
4. A bubble point value lower than the specification is an indication of one of the following:
   - fluid with different surface tension than the recommended test fluid
   - integral filter, but wrong pore size
   - high temperature
   - incompletely wetted membrane
   - non-integral membrane or seal
**Diffusion Test**

At differential gas pressures below the bubble point, gas molecules migrate through the water-filled pores of a wetted membrane following Fick's Law of Diffusion. The gas diffusional flow rate for a filter is proportional to the differential pressure and the total surface area of the filter. At a pressure approximately 80% of the minimum bubble point, the gas which diffuses through the membrane is measured to determine a filter's integrity. The flow of gas is very low in small area filters, but it is significant in large area filters. Maximum diffusional flow specifications have been determined for specific membranes and devices and are used to predict bacterial retention test results.

Where:

\[ K = \text{Diffusivity/Solubility coefficient} \]
\[ P_1, P_2 = \text{Pressure difference across the system} \]
\[ P = \text{Membrane porosity} \]
\[ L = \text{Effective path length} \]
\[ A = \text{Membrane area} \]
\[ DF = \text{Diffusional Flow} \]

\[ DF = K (P_1 - P_2) \frac{A}{P} \frac{L}{L} \]

**Pressure Hold Testing**

The Pressure Hold Test, also known as pressure decay or pressure drop test, is a variation of the diffusion test. In this test, a highly accurate gauge is used to monitor upstream pressure changes due to gas diffusion through the filter. Because there is no need to measure gas flow downstream of the filter, any risk to downstream sterility is eliminated.

The pressure hold value is dependent on the diffusional flow and upstream volume. It can be calculated using the following equation:

\[ \text{Pressure Hold} = \frac{D (T) (P_a)}{V_h} = \Delta P \]

**Diffusion Test Procedure**

1. Thoroughly wet the filter with appropriate test fluid, typically water for hydrophilic membranes or an alcohol/water mixture for hydrophobic membranes.
2. Slowly increase pressure on the upstream side of the filter to the recommended test pressure provided by the manufacturer, typically at least 80% of the minimum bubble point specification.
3. Allow the system to equilibrate.
4. Measure the gas flow at the outlet for one minute with an inverted graduated cylinder or a flow meter.
5. A diffusional flow reading higher than the specification is an indication of one of the following:
   - wrong pore size
   - temperature other than ambient
   - incompletely wetted membrane
   - non-integral membrane or seal
   - liquid/gas combination different than the recommended fluids
   - inadequate stabilization time

Pressure hold testing is the method employed by most automated integrity test systems, including the Millipore Integritest® II Plus system. The Integritest II Plus system is accurate to 0.28 mbar [0.004 psi] which makes it ideal for measuring the typically small 7 mbar/min [0.1 psi/min] changes in upstream pressure. The Integritest II Plus system can also determine bubble point by measuring changes in upstream pressure, and conduct a water flow integrity test on hydrophobic filters.
Because the bacterial retention test is destructive, a more practical, non-destructive test is used for confirming the integrity of sterilizing membrane filters and filter systems. Millipore filters are non-fibrous membranes containing millions of capillary pores. Although the pores are highly irregular and tortuous in shape configuration, they are carefully controlled for effective pore diameter and function as capillary tubes. In a non-destructive test, as in the bubble point test, the pores can be thought of as capillary tubes.

The Bubble Point Test is based on the fact that liquid is held in a capillary tube by surface tension. The minimum gas pressure required to force liquid out of the tube is a direct function of tube diameter.

The pressure required to force liquid out of a liquid-filled capillary must be sufficient to overcome surface tension and is a direct measure of effective tube diameter.
The bubble point test is a sensitive visual technique and is performed routinely as part of the Millipore Quality Assurance Program.

In the illustration above, a wet Millipore microporous membrane filter is placed in a fixture and flushed with water, thereby filling the pores. Compressed air enters beneath the filter and the pressure increased gradually to a point where water displaced from the pores with the largest effective diameter. When this occurs, a steady stream of bubbles appears in the water above the filter.

The bubble point test detects minor filter defects and out-of-size pores and correlates with the bacteria passage test.

The above illustration depicts a membrane that was manufactured out of spec. When the air pressure is increased to 30 psi, bubbles begin to appear in the water above the defective area. This is a clear indication of a defect since the minimum acceptable bubble point pressure for this particular membrane is 50 psi as correlated to bacterial retention.
An in-process bubble point test will detect damaged membranes, ineffective seals, system leaks and distinguish filter pore sizes. The illustration shows a bubble point test run with a Durapore® 0.22 µm filter.

For an in-process bubble point test, pressure from a nitrogen source forces liquid from a pressure vessel to fully wet the membrane filter.

Once all the liquid has been passed through the membrane filter, gas is in contact with the filter surface. The pores and downstream tubing are still full of water.

When the applied pressure reaches the bubble point pressure of the filter, liquid is displaced from the filter pores.
The three tests below are easy to use and have proved reliable in many thousands of tests to confirm the integrity of low volume or low surface area devices. Bubble point testing is the basis of two standard ASTM methods for determining filter pore size characteristics.

Bubble point test of low volume systems where bubbles can be seen in the receiving vessel. The bubble point pressure is the pressure at which bubbles first appear from a submerged inlet tube in the receiving vessel.

Bubble point test of low volume system where bubbles cannot be seen in the receiving vessel. The bubble point pressure at which a steady stream of bubbles is seen passing through the transparent section of tubing.

Bubble point test of low volume system where bubbles cannot be seen in fixed plumbing. The bubble point pressure is the pressure at which a steady stream of bubbles first appears from a submerged inlet tube in the container. This type of measurement technique is difficult to do without compromising sterility.
In high volume systems with final filter surface areas of 0.19 m² (2 ft²) or greater, a Diffusion Test is recommended.

In a wetted membrane filter under pressure, gas molecules migrate through the water-filled pores at differential pressures below the bubble point pressure of the filter by a diffusion process following Fick's Law of Diffusion. The overall rate of diffusion is proportional to the surface area of the membrane in the filter. In small area filters, this flow of air is very low, but in the large area filters used in high volume systems, it is significant and can be measured to perform a sensitive filter integrity test.

The diffusion test is based on the fact that gas will diffuse through the pores of a fully wetted filter. This diffusion rate is proportional to different pressure and surface area. When the pressure begins to exceed the bubble point of the filter, bulk gas flow results. There are orders of magnitude of difference between diffusional flow and bulk flow.

The flow of gas is limited to diffusion through water-filled pores below the bubble point pressure of the filter under test. At the bubble point pressure, the water in the pores is forced out and bulk gas flows freely through the filter.
In the diffusion test, pressure is typically applied at 80% of the bubble point pressure of the filter under test. When there is liquid downstream of the filter, the volume of gas flow is determined by measuring the flow rate of displaced water.

Displaced water is collected in a graduated cylinder and by using a stopwatch, the rate of diffusion is measured in milliliters of water per minute. This rate is then compared to a standard established for the particular filter system.

The rate of diffusion can also be measured by a gas flow meter.

A more sensitive gas flow measurement technique for the diffusion test than illustrated in the previous figure is show above. In this example, the downstream side is drained prior to the measurement, which is done with a sensitive flow meter.
In industrial settings, the flow rate is often measured on the upstream side of the filter with an automated instrument. Upstream Measurements do not require a tap into the sterile downstream side.

The measurement technique used by many automated devices is pressure decay. After that gas on the upstream side is pressurized to the desired test pressure, the device isolates the filter from the gas source and measures the rate of pressure decay resulting from gas leaving the upstream side of the filter housing. This decay rate can be converted to the standard volumetric flow rate expression for the diffusion rate via the ideal gas law.

Applying pressure at 80% of the filter bubble point pressure validates the Durapore filter’s integrity. There would be a dramatic increase in gas (and water) flow at this pressure if these were damaged membranes, wrong pore size filters, ineffective seals or systems leaks.
To Place an Order or Receive Technical Assistance
For information on test pressures and bubble points for specific filters, consult the manufacturer’s specification. For Millipore filters, call our Technical Services Department at one of the numbers listed below.
Our Application Specialists will provide technical assistance in setting up filter test methods tailored to specific systems and process conditions.

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