Best Practices for Critical Sterile Filter Operation
A Case Study
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A number of regulatory guidelines recommend preuse integrity testing of critical sterilizing liquid filters for aseptic processing (1–3). Before sterilization, a preuse test will confirm that a filter is installed properly and was not damaged during shipment or handling. Performing a preuse test after sterilization detects damage that may have occurred during the sterilization cycle. Testing after sterilization limits risk, so it is a practice applied based on risk assessment. Because it is perceived to reduce business loss risk, preuse post-sterilization integrity testing (PUPSIT) is a current industry practice especially in manufacturing products that will be marketed in the European Union (EU).

Unfortunately, it can be difficult to perform a PUPSIT without breaching system sterility. A number of methods have been developed for running PUPSIT and performing line conditioning without compromising sterility. Such methods use a flush bag, catch-can/flush bottle, or filter arrangement to create a sterile boundary on the downstream side of the product filter. Some applications use the downstream hold tank as a sterile boundary.

Here we describe a robust and versatile approach to PUPSIT using a self-venting, all-in-one sterile barrier membrane filter from EMD Millipore, the life science business of Merck KGaA, Darmstadt, Germany, which operates as MilliporeSigma in the United States and Canada. We also include filtration line design considerations for implementing barrier filters.

**Barrier Filters**
Millipak and Millidisk barrier filters are stacked-disc devices that combine hydrophilic and hydrophobic sterilizing-grade Durapore membranes, both on the top and bottom of each disc (Figure 1). Because of that unique combination of different membranes in parallel configuration, the devices can filter condensate, steam, wetting liquid, and gases without compromising the sterility of a steam-sterilized, autoclaved, or gamma-irradiated system.

Barrier filters can be used downstream of sterilizing-grade filters to maintain system sterility. Water can pass through the hydrophilic discs during a flushing sequence; air can pass through the hydrophobic discs during integrity-test and drying sequences. A barrier filter acts as an...
automatic vent during testing and drying phases. The volume of particle-free water and air that can pass through these filters is unlimited.

**Flushing and Testing Critical Product Filters**

Although filter rewetting and retesting should remain an optional activity when preparing a product final filter (sterilizing-grade filter) in line, the PDA Technical Report 26 suggests up to three repetitions (3). The number of retests should be considered when sizing for a flush bag. Barrier filters could provide a more versatile solution.

Filters that are not wetted efficiently the first time could give false failed test results. If rewetting volume is limited, end users might discard integral filters that only marginally failed because of improper wetting. Doing so could lead to unnecessary quality investigations as well as downtime associated with possibility of an integrity failure for the primary product filter (3). Each filter must be independently integrity-testable in compliance with the relevant regulations or guidelines. The step-by-step approaches in Figures 4 and 5 can be applied to the second product filter in a redundant filtration system.

**Sterility of product/filters**

*Sterile Boundary Attribute*

<table>
<thead>
<tr>
<th>Sterile Boundary Attribute</th>
<th>Barrier Filters</th>
<th>Catch Can or Flush Bottle</th>
<th>Flush Bag</th>
<th>Downstream Filters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method diagram</td>
<td><img src="image1" alt="Diagram" /></td>
<td><img src="image2" alt="Diagram" /></td>
<td><img src="image3" alt="Diagram" /></td>
<td><img src="image4" alt="Diagram" /></td>
</tr>
<tr>
<td>(All maintain sterility of product filter during preuse test.)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Restest</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Ability to dry the product filter</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Simple design</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
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</tbody>
</table>

**Filtration Line Configuration and Operation**

For critical filter applications such as final product-filling lines, barrier filters can be used to provide a sterile boundary while meeting regulatory requirements for preuse integrity testing. Typically, such filters are installed downstream of a product filter (Figure 3).

Figures 4 and 5 illustrate stepwise use of barrier filters to provide a sterile boundary in a stainless steel and a single-use filtration system with a single product filter. Regulators recommend redundant filtration as a risk-mitigation strategy for critical filtration applications. Redundant filtration is a type of serial filtration in which a second product filter is used as a back-up to protect against the possibility of an integrity failure for the primary product filter (3). Each filter must be independently integrity-testable in compliance with the relevant regulations or guidelines. The step-by-step approaches in Figures 4 and 5 can be applied to the second product filter in a redundant filtration system.

**Sterilizing:** The process begins with sterilization of a filtration system by SIP, autoclaving, or gamma-irradiation. If the chosen method of sterilization is SIP, then the barrier filter’s low-point vent on its upstream side will be kept open during the SIP cycle. Condensate, steam, and air will pass through the filter to drain on its outlet. When the cycle ends, the low-point vent will be closed. The system then cools down with application of compressed gas (to maintain positive pressure as well as sterility in the filtrations system).

**Wetting:** The second step ensures that a product filter is totally wetted with particle-free water for its integrity test. This step also flushes away extractable residues from a sterilized product filter element. To keep a filter train independent, the vent on the product filter is left open initially, and the isolation valve to downstream equipment is closed. The product filter vent is closed after air in its housing has been vented. Water is directed to the drains through the barrier filters. Isolating the flow path to the barrier filters can enhance wetting for increased applied pressure drop through a product filter.

**Testing:** PUP/SIT is either a diffusion or bubble-point test (depending on the product filter). In all cases, pressurized gas is applied on the product filter’s upstream side, which is isolated from the system elements both upstream and downstream. Only the drain line with the barrier filters remains open to allow the free flow of test gas through the hydrophobic portion of their membranes.

**Drying:** Before product is introduced into the filtration line, the product filter typically is blown down and dried to prevent dilution of the product stream. The associated gas is vented through the barrier filter.

**Barrier Filter Integrity Testing:** Millipak and Millidisk barrier filters are integrity tested offline using 70/30 isopropyl alcohol (IPA) as the wetting fluid.
Process: Once the integrity of the product filter is confirmed, the sterile filtration process can begin.

After Processing: After the sterile filtration process, product recovery through a sterilizing-grade filter can be achieved through air blow-down with application of a low differential pressure (air or nitrogen) of 5 psi to the filter. Users can apply a buffer chase, but product dilution must be accounted for. At the end of product recovery, sterilizing-grade filters are integrity tested with particle-free, water-based, alcohol (70/30 IPA/water), or product-based integrity test specifications. For particle-free-water-based or alcohol integrity-test specifications, a filter must be flushed adequately with the test liquid to remove residual product before testing. Product-based integrity-test specifications can be developed through support from filter vendors.

**Design Considerations**
Use of Millipak and Millidisk barrier filters in a filtration line is simple and straightforward (Figures 4 and 5). Similar to all critical applications, important process steps and conditions should be reviewed during system and process design to ensure successful implementation of this application. Verification testing should be performed before implementation of an assembly with barrier filters for product filtration.

**Sterilization of Filtration System:** Sterilization renders a system or equipment free of microorganisms and is a critical step, especially for aseptic manufacturing processes. Filters can be sterilized through SIP for Millidisk format (cartridge filters) and autoclaving or gamma irradiation for Millipak (disposable capsule) filters (Figure 6). Thermocouples, radiation dosimeters, and biological indicators serve as the worst-case positions within a filtration system and assembly for validation of sterilization.

**Flushing and Wetting Product Filters:** A filtration system may be flushed and wetted to remove extractables after sterilization as well as for product filter integrity testing. The flushing or wetting liquid passes though the barrier filters to a drain. Flushing/wetting conditions are derived from vendor recommendations (5,6) and can vary among product filters. Inlet pressures on barrier filters during flushing/wetting procedures should not exceed 0.7 bar. If enhanced wetting of a product filter is required, higher static-hold pressure can be implemented across it. However, the downstream section of that product filter (including the barrier filter) should be isolated during such a high-pressure hold step.

**Key Verification Point — Efficacy of Product-Filter Wetting:** The efficiency of wetting a product filter(s) through barrier filters can be verified by performing product filter integrity testing. Results can be compared with filter specifications and past trending.

**Key Verification Point — Gas Flow Rate of Barrier Filters After Flushing/Wetting Procedure:** Ensuring that the hydrophobic membrane in a barrier filter remains dry is critical. Such dryness can be verified in the filter following a flushing/wetting procedure.
during the qualification phase with a low-pressure bubble-stream test. This includes disconnecting barrier filters from their assembly and determining their gas flow-rate level at 100 mbar (1.5 psi) pressure, then comparing that to the nominal gas flow-rate level of a new filter unit wetted optimally at low pressure for five minutes. Considering the average hydrophobicity level of polyvinylidene fluoride (PVDF) discs, flow rates in the 30–100% range of nominal rate are characteristic of a “breathing” unit.

Key Verification Point — Using Wetting Medium Apart from Water for Injection (WFI): Compatibility and intrusion pressure/wettability for the hydrophobic filters within barrier filters should be verified before the filters are used. Wetting hydrophobic filters can reduce their air-flow capacity, leading to increased pressure drop across the filter assembly during integrity testing or product-filter blow-down. Millipak and Millidisk barrier filters validation guides provide chemical compatibility information summaries (7–9).

**Integrity Testing of Product Filter**

Filtration assembly designers should include strategies to minimize product hold by reducing piping or tubing length and to ensure maximum product recovery. The strategy for integrity testing product filters also should be well thought-out, especially for redundant filtration assemblies. In 2012, Felo and coworkers at MilliporeSigma provided an in-depth look at how product filters can be integrity tested in a single-use assembly (10). Their strategy can be applied to stainless steel systems as well.

**Interference on Product Integrity Testing from Barrier Filters:** Barrier filters are placed downstream of a product filter. To verify the absence of interference, the integrity test result of the product filter both with and without the barrier filters can be compared. Those results should fall within 70 mbar for a bubble-point test and 5% for diffusion flow.

**Failure Mode Test:** To simulate a worst-case scenario (failure-mode test), users can examine how a fully wet barrier filter gas flow rate compromises a product-filter integrity test. Millipak and Millidisk barrier filters can be fully wetted by flushing with WFI at 3 bar.

**Adaptation of Troubleshooting Decision Tree:** If a product filter fails its integrity test, users can apply a...
troubleshooting decision tree such as the example given in PDA’s Technical Report #26 (3).

**Drying of Filtration System**

To minimize product dilution or contact of product with the wetting liquid (either buffer or water) before filtration, the assembly may be blown down to remove wetting liquid. The current industry practice of blowing down a filtration system ranges from 30 minutes to three hours.

**Duration of Drying:** Exact drying times should be verified on site and determined during qualification by weight and visual checks. The same time taken to reach the “dry weight” of the assembly will be required for drying the assembly during operation.

**Acceptable Applied Pressures:**

Typical pressures applied for drying filtration assemblies are 0.5 bar higher than the bubble-point pressure of a product filter. Such pressures should not exceed the maximum allowable pressure of the “weakest link” in an assembly. That might be silicone tubing, a connector, or a barrier filter (4.1 bar for Millipak and Millidisk formats), for example. If the required pressure is greater than what the weakest link allows, then blow-down pressure should be reduced, and an extended drying time can be applied.

**Absence of Air-Flow Interference:**

Restriction of air flow through fittings, connectors, tubing, or piping used in an assembly should be minimized. As a product filter dries, the air-flow rate will increase and pressure drop across the product filter will decrease.

**Integrity Testing of Barrier Filters**

Barrier filters are integrity tested offline with 70/30 IPA/water as a wetting medium and bubble-point test specification of ≥1,280 mbar (18.5 psi). These filters can be wetted by dynamic flushing or static-soak methods. The wetting procedure of barrier filters can be found in a technical guide (5). A 15-minute static soak can be applied to either Millipak or Millidisk barrier filters.

For critical product applications in which resources are readily available, a barrier filter should be integrity tested after the product filter has passed integrity but before product filtration. This minimizes the risk of reprocessing product because of a poor installation or nonintegral barrier filter caused by mishandling. For situations in which resources are limited and product can be reprocessed, barrier filters can be integrity tested after product filtration.

**For Best Practices**

Barrier filters help enable best practices of aseptic filtration lines for flushing/wetting and preuse integrity testing of product filters. In particular, implementation of Millipak and Millidisk barrier filters is easy and provides flexibility and versatility to the filtration line.

**References**


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