

Steam Sterilization & Integrity Testing Procedures

Steam sterilization of filtration assemblies is a common operation in aseptic processing with stainless steel systems. The goal of this tech note is to describe a procedure for steam sterilization and integrity testing of a filter assembly. Because steam sterilization is the most common source of damaging a filter, it is recommended to test the integrity of sterilizing-grade filters after sterilization, before the filtration process.^{1,2,3} Therefore, the procedure describes both the Steaming-In-Place (SIP) and the post-SIP, pre-use integrity testing of the vent and product filters.

The operations should be performed in the following sequence:

1. SIP of the hydrophobic vent filter assembly
2. Post-SIP, pre-use integrity testing of the vent filter.
3. SIP of the hydrophilic sterilizing-grade filter assembly (product filter).
4. Post-SIP, pre-use integrity testing of the product filter.

The recommended post-SIP, pre-use filter integrity test for hydrophobic vent filters is the HydroCorr™ test. As this test is performed at the upstream side of the vent filter and no downstream manipulation is required, it is an easy way to confirm that the SIP procedure has not damaged the vent filter.

Common post-SIP, pre-use filter integrity tests for hydrophilic filters are bubble point, enhanced bubble point and diffusion.

Steam-In-Place Procedure for a Sterile Tank Equipped with a Vent Filter

The manual operations that are described in this procedure should be performed in the order outlined below with the following assumptions:

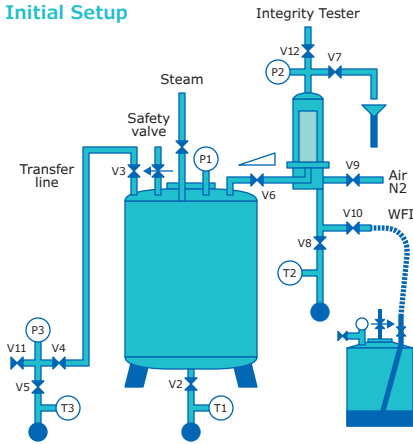
- Tank and adjacent piping is clean (e.g. by means of CIP) and empty
- Filter housing is installed and the correct vent filter is put in place
- All connections are checked for proper fitting
- System has been checked for leak-tightness by means of a pressure hold test
- All valves are closed and silicone tubing are attached to bleed valves and directed to a condensate drain
- Use caution to avoid contact with steam or hot stainless steel surfaces
- Wear protective glasses at all times and heat resistant protective gloves when necessary

Procedure

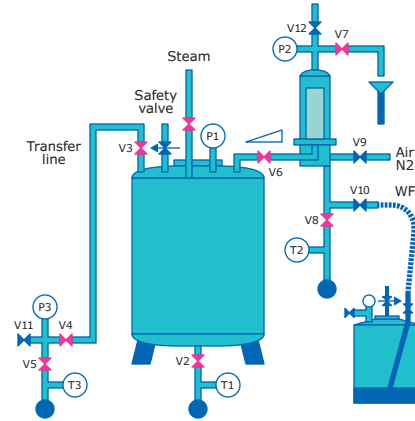
1. Check that the steam supply and compressed gas pressures are set up at the required values.
2. Respectively open V2, V3, V4, V5, and V1 to introduce steam to the system and to purge air from the system.
3. Partially close bleed valves V2 and V5 to build tank pressure to at least 0.5 bar and wait for the temperature gauges T1 and T3 to indicate more than 100 °C.

Recommended SIP Process for a Sterile Tank Equipped with a Vent Filter

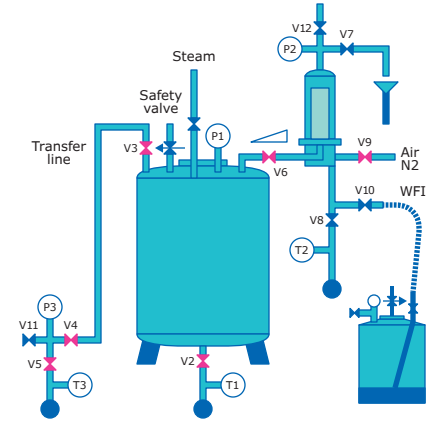
Initial Setup



SIP Cycle



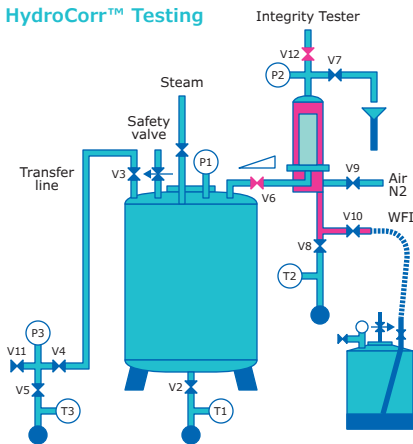
Cooling & Drying



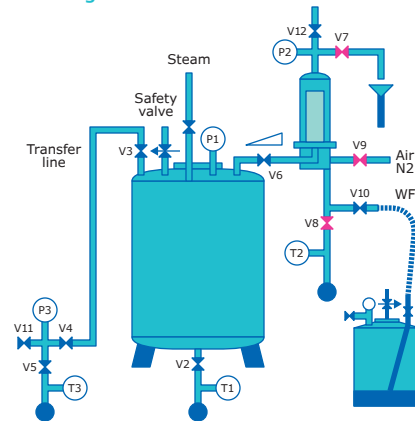
➤ Open Valve ➤ Closed Valve

Recommended Post SIP Pre-use Filter Integrity Test for Hydrophobic Vent Filters

HydroCorr™ Testing



Draining



➤ Open Valve ➤ Closed Valve

- Slowly open V6 to introduce steam to the vent filter. Crack open bleed valves V7 and V8 to establish a steady flow of steam and allow for condensate drainage and air removal from the filter housing.

Note: It is important to control the difference between pressure gauges P1 and P2 and keep the delta-P over the filter to a maximum of 100 mbar. For reverse direction SIP, use an Optiseal® filter or a code 7* filter. Do not use a code 0** filter.

- Ensure all air and condensate are effectively removed by keeping V2, V5, V7 and V8 cracked open so that a 15 cm wisp of steam and a continuous drip of water can be seen exiting.
- When the temperature throughout the system reaches over 121.1 °C, as measured by the temperature gauges T1, T2 and T3, the timer is started. Sterilization time should be at least 30 minutes or longer dependent on results of

validation. During the sterilization both pressure and temperature should be recorded regularly.

- When the required sterilization time has been achieved, close the steam supply valve V1 and slowly open V9 to introduce sterile compressed gas into the system.

CAUTION: Make sure that the system remains under positive pressure (as indicated by pressure gauges P1, P2 and P3) and control that the delta-P over the filter does not exceed 350 mbar.

- Allow for steam purge from all bleed valves and close valves V7 and V8 to increase the flow of sterile gas through the system. Maintain the gas flow to cool down the system until the temperature gauges T1, T2 and T3 indicate approximately 40 °C.
- Respectively close valves V5, V2 and V4, and keep V6 and V9 open to maintain a positive pressure into the sterile system while it is not in use.

* 2-226 O-ring locking outlet with spear assembly

** 2-222 O-ring outlet

Post SIP, Pre-Use Vent Filter Integrity Test Procedure

1. Close the compressed gas valve V9, keep V6 open, and open V7 to vent the system. Wait for the pressure as measured by P1 and P2 to drop to atmospheric pressure.
2. Fill the pressure vessel with clean pure water and attach the inlet of the vessel to a compressed gas supply at 1 bar. Attach the outlet tubing of the pressure vessel to V10.
3. Slowly open V10 to have water entering the filter housing. Ensure that the filling pressure does not exceed 1 bar and that air cannot enter the housing (e.g. empty pressure vessel). Continue filling until water is seen exiting the hose attached to V7.

Note: Should the filter housing be installed on top of a rather tall tank, it may prove useful to increase the pressure to adjust for gravity influence while the filling operation commences.

4. Close V10 and bleed air from the pressure vessel by slowly opening the pressure relief valve on top of the vessel until atmospheric conditions are reached.
5. Close V7, open V12 and attach an automatic filter integrity tester to V12.
6. Double check that V6 is open and that V7, V8, V9 and V10 are all fully closed™ and run the HydroCorr™ test.
7. When the test is finished and a positive result (i.e. pass) is obtained, close V6 and V12 and detach the filter integrity tester.
8. Open V7 and V8 to drain water from the housing. The draining can be facilitated by carefully opening V9 and applying pressure until the system is empty.
9. Fully open V9 to allow for drying of the filter over a period of 30 min.
10. Close V7 and V8, open V6 and keep V9 open to build and maintain a positive pressure into the system while it is not in use.

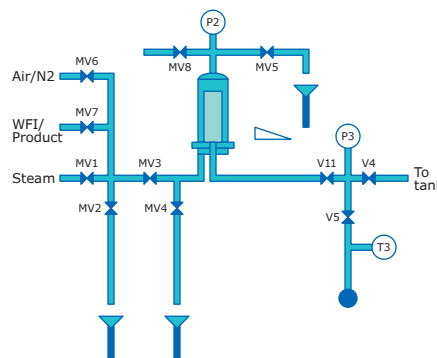
Steam-In-Place Procedure for Product Filter

The manual operations that are described in this procedure should be performed in order.

Prior to commencing the procedure, the following is assumed:

- Filter housing is installed and the correct product filter is put in place
- Product filter is dry
- All valves are closed and silicone tubing are attached to bleed valves and directed to a condensate drain
- Use caution to avoid contact with steam or hot stainless steel surfaces
- Wear protective glasses at all times and heat resistant protective gloves when necessary

Recommended SIP for the Product Filter



Procedure

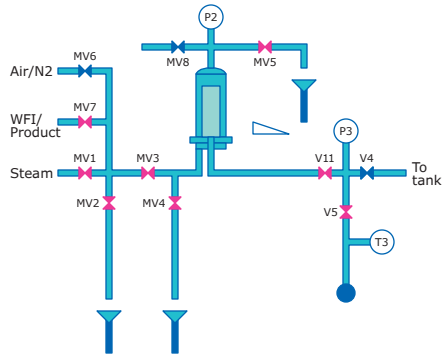
1. Check that the steam supply and compressed gas pressures are set up at the required values.
2. Open MV1 and MV2 and purge the steam line until complete absence of condensate.
3. Fully open MV4 and MV5 to allow for subsequent air and condensate evacuation.
4. Slowly open MV3 to progressively introduce steam and heat up the filter.
5. Partially close bleed valves MV2, MV4 and MV5 so that a wisp of steam and a continuous drip of water can be seen exiting.
6. Respectively open V11 and crack open bleed valve V5 to establish a steady flow of steam and allow for condensate drainage and air removal from the filter housing.

Note: It is of utmost importance to control the difference between pressure gauges P3 and P4 and keep the delta-P over the filter to a maximum of 350 mbar.
7. Ensure all air and condensate are effectively removed by keeping MV2, MV4, MV5, and V5 cracked open so that a 15 cm wisp of steam and a continuous drip of water can be seen exiting.
8. When the temperature downstream of the product filter, as measured by the temperature gauge T3, reaches over 121.1 °C, the timer is started. Sterilization time should be at least 30 minutes or longer, as established during validation. During the sterilization phase both pressure and temperature should be recorded regularly.
9. At completion of the sterilization cycle, close the steam supply valve MV1 and slowly open MV6 to introduce compressed gas into the system.

CAUTION: Make sure that the system remains under positive pressure (as indicated by pressure gauges P3 and P4) and control that the delta-P over the filter does not exceed 350 mbar.

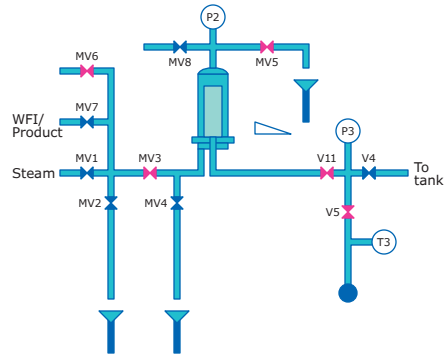
Recommended SIP and Cooling Process for Hydrophilic Product Filter

SIP Cycle



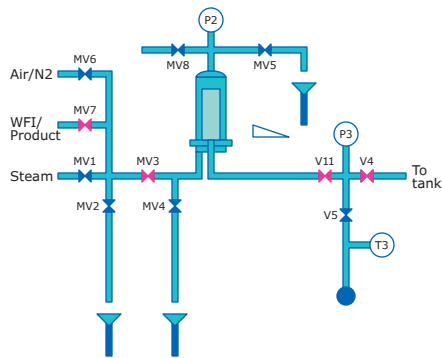
✂ Open Valve ◀ Closed Valve

Cooling Cycle



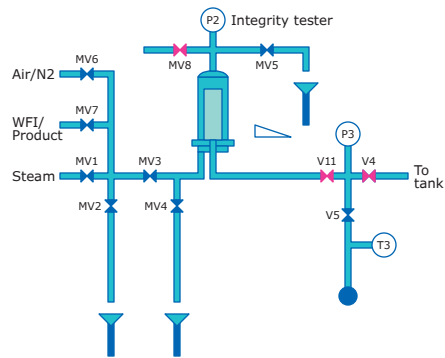
Recommended Post SIP Pre-use Filter Wetting and Integrity Test for Hydrophilic Product Filter

Wetting with Product



✂ Open Valve ◀ Closed Valve

Bubble Point Test



10. Allow for steam purge from all bleed valves and close valves MV2 and MV4 to increase the flow of gas through the system. Maintain the gas flow to cool down the system until the temperature gauge T3 indicates approximately 30 °C.
11. Respectively close valves V5, V11 and MV5, and keep MV6 and MV3 open to maintain a positive pressure into the sterile filter system while it is not in use.

Post SIP, Pre-Use Filter Integrity Test Procedure

1. Ensure that V3, V6 and V7 on the vent filter are open and that the sterile tank downstream of the product filter is vented at the atmospheric pressure.
2. Maintain MV3 open, close the compressed gas supply valve MV6, and open MV5 to vent the system. Wait for the pressure as measured by P4 to drop to atmospheric pressure.
3. If possible, set the inlet product pressure at 2.8 bar. Gradually open MV7 to fill the filter housing with product and vent the filter housing from MV5, until all upstream air has been released.
4. When product is seen exiting the hose attached to MV5, close the vent valve MV5 and continue to maintain the 2.8 bar pressure for at least one minute to dissolve any residual gas within the filter and ensure membrane wetting.
5. Fully open the downstream valve V11 and gradually open V4 to set the differential pressure (P4-P3) at approximately 200 mbar.
6. Continue to flow product through the filter to the sterile tank at appropriate pressure differential for at least five minutes.
7. Then close MV7 and MV3 to isolate the filter and fully open V4.
8. Open MV8 and attach an automatic filter integrity tester.

9. Double-check that MV8, V11, V4, V3, V6 and V7 are open and that MV3 and MV4 are all fully closed and run the enhanced bubble point test.
10. When the test is finished and a positive result (i.e. pass) is obtained close MV8 and detach the filter integrity tester.
11. Open MV7, MV3 and MV5 to restart the filtration of the product.
12. When product is seen through MV5, close MV5 and continue the filtration of the product.

References

1. EudraLex Volume 4, Annex 1. Manufacture of Sterile Medicinal Products. 2008.
2. FDA Guidance for Industry on Sterile Drug Products Produced by Aseptic Processing – CGMP, Center for Drugs and Biologics and Office of Regulatory Affairs. Food and Drug Administration, Bethesda, MD, 2004
3. Technical Report No. 26: Sterilizing Filtration of Liquids. Parenteral Drug Association: Bethesda, MD, 2008.

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