Using Hydrophobic Air Filters as Bidirectional Protection between an Integrity Tester and a Filter Housing
Background
Integrity testing of sterilizing-grade filters is necessary in order to unambiguously rule out potential damage to these sterile barriers during the production of biopharmaceuticals. The principle of filter integrity testing is based on using an integrity test unit to pressurize the filter cartridge housing and to measure the pressure drop that occurs when gas molecules diffuse through a wetted filter membrane. The pneumatic connection required for integrity testing between the test unit and the filter to be tested entails a certain risk that liquid, aerosols or even microorganisms can be drawn back into the test unit. Vice versa, sterile test gas is not used during integrity testing; i.e., the interior of the test unit is not sterile. This, in turn, poses the risk that particles or microbes might be carried into the filter cartridge housing during the test procedure. Although the membrane of the filter under test ultimately constitutes a sterile barrier, it is still advisable to work meticulously in order to prevent contamination from occurring on the upstream side.

This Application Note describes the use of hydrophobic air filters (Sartofluor®, Midisart®) as reliable sterilizing-grade filters and liquid barriers between the integrity tester and the filter to be tested. Theoretical considerations and test data show that even a small, Midisart-type air filter installed in the test line will not affect the measurement results in any way.

Why use a hydrophobic air filter between the integrity tester and the filter cartridge housing?
It is a fact that integrity testing of sterilizing-grade filters is not performed under sterile conditions, ultimately due to the pneumatic connection between the non-sterile integrity test unit and the filter cartridge under test. The integrity test itself is performed on the unsterile, or upstream, side. As a result, the membrane to be tested, if intact, will retain its function as a sterile barrier for protection of a downstream product. Nevertheless, in many cases, it might be desirable to perform integrity tests under virtually sterile conditions. As the internal pneumatic components of the integrity testers used cannot be sterilized, installation of sterilizing-grade air filters (pore size of 0.2 µm) in the test line is ideal for ensuring sterile conditions. These filters reliably retain any potential particulate and microbial contaminants, stopping them from reaching the filter membrane of the cartridge to be tested. The air filters presented in this report have been validated as sterilizing-grade filters with respect to their microbial retention in compliance with the regulations generally valid throughout the industry.
Air filters feature hydrophobic filter membranes. This hydrophobicity is required to prevent blockage of the membrane caused by moisture present in air. The polytetrafluoroethylene (PTFE) material used in Midisart® and Sartofluor® filters is extremely hydrophobic, which not only provides protection against microbial contamination, but also simultaneously prevents the passage of water or aqueous solutions. Installed as bidirectional protection in the test line, these filters at the same time prevent liquid from the filter cartridge housing from flowing back into the integrity tester used. Only at the so-called water penetration pressure can water pass through a hydrophobic PTFE membrane. For the Midisart® membrane, this pressure is above 4.5 bar (65.3 psi), however, so the effectiveness of this filter as a water barrier is guaranteed up to this pressure.

Ultimately, it must be ensured that such a filter installed in a test line will not have any adverse effect on the integrity test performed. This is to be explained in the following:

**Is the filter area of Midisart® sufficient?**
The Midisart-type air filter has an effective filter area of only 20 cm² (~3.1 square inches). This raises the question as to whether this small filter area is sufficient and will not unacceptably limit the air flow rate during the integrity test. If so, this would then automatically have an unsuitable impact on the results of an integrity test performed.

During a filter integrity test, the highest air flow rate must be measured during the phases of pressurization and venting. In these phases, the filter cartridge housing under test is filled up to the programmed test pressure and, at the end of the test, vented so that the pressure inside returns to the normal atmospheric level. A 10” filter cartridge housing has an upstream volume of approximately one liter. At a differential pressure of 100 mbar (~1.5 psi), Midisart® 2000 delivers an air flow rate of around five liters per minute. This clearly indicates that the air flow rate of Midisart® filters does not limit flow in any case during the test phase, not even in multi-cartridge housings.

The purpose of the following test procedure was to verify our theoretical assumptions outlined above: Sartopore® 2 sterilizing-grade filters (0.2 µm) with and without Midisart® installed in the test line were tested. The small Midisart® filter was used in the “worst case” test scenario. As larger Sartofluor® air filter capsules have considerably more filter area than does Midisart®, these can be considered to have a negligible impact on flow rate, if it can be demonstrated that Midisart® itself does not have any limiting effect. Following successful wetting of the Sartopore® 2 capsules to be tested, they were integrity tested using the Sartocheck® 4 plus tester set to standard test parameters.

An integrity test is comprised of the phases of pressurization, stabilization, testing and venting. The pressurization and venting phases are necessary, but initially do not have any influence on the test results. For the test phase, however, it must be absolutely ensured that the Midisart® filter used does not have any impact on the test results. In this phase, only drops in pressure are measured, which are caused by diffusion of the test gas through a wetted membrane. In this case, tests can be performed to verify that the flow rates (diffusion rates) are very low and the Midisart® filter connected between the test unit and the filter housing has no restrictive effect on these rates. For instance, a 10” Sartopore® 2 cartridge (0.2 µm) has a diffusion limit of merely 18 ml/min. This clearly indicates that the air flow rate of Midisart® filters does not limit flow in any case during the test phase, not even in multi-cartridge housings.

Three diffusion tests and three bubble point tests were performed on each Sartopore® 2 MidiCaps, using different Midisart® filters in each case. The test results are given in Table 1:

<table>
<thead>
<tr>
<th>Test No.</th>
<th>Diffusion Test Results [ml/min]</th>
<th>Bubble Point Test Results [mbar]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.6 3.7</td>
<td>3928 3934</td>
</tr>
<tr>
<td>2</td>
<td>3.4 3.5</td>
<td>3977 3933</td>
</tr>
<tr>
<td>3</td>
<td>3.4 3.4</td>
<td>3978 3983</td>
</tr>
</tbody>
</table>

**Table 1:** Test results of filter integrity tests of a Sartopore® 2 MidiCaps filter (0.2 µm, size 9). The test with a Midisart® filter in the test line has no significant impact on the test result.
These results show that there are no significant differences in the outcomes of the tests performed with and without a Midisart® 2000 filter installed in the test line. Nearly identical results were obtained both for the diffusion tests and for the bubble point tests. Therefore, these results prove that the use of a Midisart® filter in the test line is safe and reliable.

![Effect of Midisart 2000 on Bubble Point Test of Sartopore 2 MidiCaps (5445307Hf–SS)](image)

Figure 2: Bubble Point Tests without (black curve) and with Midisart® filter (red curve) in the test line. Each curve represents the average curve of three bubble point tests. Both curves with and without protective filter are almost congruent, i.e. no effect of the additional Midisart® 2000 filter in the test line.

Generally, it must be noted that the flow rates given apply only to dry filters. Should a film of liquid partially or completely cover the hydrophobic PTFE membrane of the Midisart® filter, this will reduce the flow rate accordingly and may even lead to complete blockage of the membrane. In this respect, users need to ensure at all times that any liquid that collects inside the integrity test tubing will not affect Midisart®.

Direction in which the air filter is installed
An additional question to answer is in which direction the air filter needs to be installed between the integrity test unit and the filter cartridge housing. Basically, air filters can be used in either direction. However, regarding their design, the filters are not symmetrical. As a result, the maximum differential pressure for an air filter installed in the direction of filtration will differ from that for a filter installed in the direction opposite to filtration, for instance. Therefore, the direction in which a filter is installed would play a critical role only in situations in which this maximum pressure tolerance would be exceeded during integrity testing. The PTFE membrane used in Midisart® is highly permeable and features high flow rate performance, among other characteristics. Therefore, no substantial pressure differences will occur across the filter membrane during the pressurization and venting phases. In all integrity test phases, there are thus no limiting differences in pressure. In this respect, the direction in which the air filter installed would not matter.

Tests of bag systems: necessity of sterilization by gamma irradiation
As a result of the growing trend toward single-use products employed in the manufacture of biopharmaceuticals, pre-assembled filter capsules are increasingly being installed on single-use bag systems. To avoid compromising the sterility of the filter capsules connected to these systems even during integrity testing, a Midisart® BV filter (gamma-sterilized variant of Midisart® air filters) must be used, which has been presterilized by gamma irradiation together with the entire bag system. The tubing of the Sartocheck® unit is then connected by the appropriate coupling to the air filter, and the downstream filter capsule can then be tested.

Even in this test equipment setup, Midisart® BV will not affect the integrity test. Rather, it ensures that the required conditions for sterility are reliably met during the test. However, it must be considered that the properties of plastic polymer materials are altered by the gamma irradiation procedure used. This will result in lower housing burst pressure values, which may be critical for integrity tests under specific circumstances. While a maximum pressure of 3 bar (~44 psi) is permitted for Midisart® 2000, only 1.5 bar (~22 psi) maximum is allowed for Midisart® BV after gamma irradiation. Depending on the type of filter to be integrity tested, the test pressure applied may exceed the maximum permissible pressure for the air filter. For example, Sartopore® 2 Gamma MidiCaps with a pore size of 0.1 µm must be tested at a pressure of 4 bar (58 psi) using the diffusion method. Taken at face value, the maximum burst pressure specification for Midisart® BV would preclude its use.

As mentioned above, the housing burst pressure limits the maximum allowable operating pressure of an air filter. Yet this limitation can be overcome by using a suitable mechanical stabilizer for Midisart® BV, which restores the air filter’s resistance. As a result, test pressures higher than the maximum burst pressure can be used without compromising the integrity of Midisart® BV. The following test procedure is designed to demonstrate this:

![Mechanical stabilizer for Midisart® filters. The clamp stabilizer can be easily mounted and reliably protects the irradiadiated Midisart® filter from bursting during integrity testing of gamma capsules.](image)

Figure 3: Mechanical stabilizer for Midisart® filters. The clamp stabilizer can be easily mounted and reliably protects the irradiadiated Midisart® filter from bursting during integrity testing of gamma capsules.
The housing burst pressure of 10 Midisart® BV filters in unsterilized condition and of 10 Midisart® BV filters in gamma irradiated condition (50 kGy) was tested with and without a mechanical stabilizer (max. test pressure 10 bar = 145 psi).

### Table 2: Effect of the mechanical stabilizer on the housing burst pressure of Midisart® BV. The burst pressure can be increased to more than 10 bar even after gamma irradiation using this stabilizer. Therefore, this device can be used to enable the usage of Midisart® BV in the test line for testing gamma capsules.

<table>
<thead>
<tr>
<th></th>
<th>Without Tri-Clamp</th>
<th>With Tri-Clamp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsterilized</td>
<td>7.57±2.7</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Irradiated with 50 kGy</td>
<td>3.41±0.7</td>
<td>&gt;10</td>
</tr>
</tbody>
</table>

The results presented here show that the use of a ring-shaped stabilizer, a Tri-Clamp, on Midisart® BV enables burst pressure values of more than 10 bar to be measured. This means that Midisart® BV mounted inside a Tri-Clamp stabilizer is suitable as a sterile barrier for integrity testing of gamma-irradiated filter capsules. Even the high test pressure required for a 0.1 µm capsule is no longer a problem for this air filter. As a result, the limits specified for the maximum operating pressure of Midisart® BV do not apply.

### Summary

The tests described here show that Midisart® 2000 and Midisart® BV are suitable for use as protective filters between the test unit and the filter during integrity testing. The results of the filter integrity test are unaffected by the additional Midisart® filter. Furthermore, it could be demonstrated that when clamped inside a mechanical stabilizer, even gamma-irradiated air filters of the Midisart® BV type are appropriate for use as protective sterilizing-grade air filters during integrity testing. Their mechanical stability reduced by gamma irradiation is thus restored by the Tri-Clamp attached in place. The resulting maximum burst pressure values of these air filters are then much higher any of the test pressures relevant for the filters to be integrity tested.

The currently valid regulations do not require that such a protective filter be installed in the test line. In this regard, it is up to the user to decide whether the benefits of preventing upstream contamination will outweigh the risks and cost of filters that may otherwise become contaminated by integrity testing.