Introduction to the Integrity Testing of Membrane Filters

With Particular Regard to the Sartocheck® Product Family

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1. Introduction

During the biopharmaceutical manufacturing process, it is essential that the final product is sterile (keyword – patient safety). Owing to the thermal instability of certain agents (e.g. vaccines), and the toxic properties of remaining cells (keyword pyrogen), a thermal sterilization process is not normally an option. For this reason sterile filters are used, whose narrow-pored membranes (normally termed 0,2 µm pore diameter) retain bacteria reliably (EU Guidelines to GMP). Thus, the filtrate is sterile, provided that the integrity (intactness) of the filter is guaranteed. And this precise point is not trivial because potential damage of the filter is not usually visible to the naked eye. This means that a sensitive measurement method is required that, using non-destructive techniques, facilitates a statement in respect of filter integrity.

This manual on filter integrity testing should provide a brief overview of recognized methods, their theoretical background and practical application. The general principles are generally applicable. Information is also provided on the special operation of devices from the current Sartocheck family. The subject of filter integrity testing could be studied in greater depth. The bibliography in the appendix provides reference to appropriate further reading.

2. Why Filter Integrity Testing?

Integrity testing of sterile filters is a relatively time-consuming but essential quality assurance measure. Even if the relevant filters are 100% tested following production by the manufacturer, potential transport damage, damage through improper handling or through steam sterilization, for example, can never be completely excluded. It is even possible that the filter could be damaged during filtration. For this reason, integrity testing is prescribed as mandatory in biopharmaceutical production processes. A position is adopted on this in numerous national and international regulations. Here is a brief overview of the most important regulations and their statements (no claim is made to completeness). The original English text should be inserted here to avoid interpretations in translation.

2.1 FDA Guideline on Sterile Drug Products Produced by Aseptic Processing (FDA, 2004)

The American health authority FDA formulates requirements in respect of integrity testing as follows:

"Integrity testing of the filter(s) can be performed prior to processing, and should be routinely performed post-use. It is important that integrity testing be conducted after filtration to detect any filter leaks or perforations that might have occurred during the filtration."

This American regulation contains a different formulation in respect of a pre-use and post-use test. While the integrity test post-use is prescribed as mandatory ("should be performed"), the test before filtration is less rigidly prescribed ("can be performed"). This different formulation has previously been interpreted to the effect that the pre-use test is a 'can regulation' while the post-use test is a 'must regulation'.

The following is noted of the gas filter:

"We recommend that filters that serve as sterile boundaries or supply sterile gases that can affect product be integrity tested upon installation and periodically thereafter (e.g., end of use). Integrity tests are also recommended after activities that may damage the filter."

A test of the air filter is thus recommended here after installation and then periodically throughout the duration of use. Testing should also take place following incidents that jeopardize the filter integrity.

2.2 USP (United States Pharmacopoeia) 23 (1995)
The US Pharmacopoeia adopts the following position:

"A membrane filter assembly should be tested for initial integrity prior to use, provided that such a test does not impair the validity of the system, and should be tested after the filtration process is completed to demonstrate that the filter assembly maintained its integrity throughout the entire filtration procedure. Typical use tests are the bubble point test, the diffusive airflow test, the pressure hold test, and the forward flow test. These tests should be correlated with microorganism retention."

In this regulation, the selection of words makes no distinction between the pre-use test and post-use test. In this context, the final remark is important, stating that the tests should be correlated with microbiological tests.

2.3 EU Guidelines to Good Manufacturing Practice, Annex 1: Manufacture of Sterile Medicinal Products (2008)

This European regulation adopts a clear position on the question of testing before and/or after filtration with a clear difference from the above FDA Guideline:

113. The integrity of the sterilized filter should be verified before use and should be confirmed immediately after use by an appropriate method such as a bubble point, diffusive flow or pressure hold test. The time taken to filter a known volume of bulk solution and the pressure difference to be used across the filter should be determined during validation and any significant differences from this during routine manufacturing should be noted and investigated. Results of these checks should be included in the batch record. The integrity of critical gas and air vent filters should be confirmed after use. The integrity of other filters should be confirmed at appropriate intervals.

The English text makes no distinction in its formulation ("should") between the pre-use and post-use test. Interestingly, the emphasis is at the beginning on "the integrity of the sterilized filter". This means that, in addition to the mandatory test after filtration, a test should also be implemented before filtration and namely after sterilization.

At present, there is some uncertainty as to the interpretation of the word "should". Finally, there is the issue of what is concretely demanded by the requirements of regional authorities. The FDA Guidance for Industry - Process Validation: General Principles and Practices (2011) makes the following comment: "FDA's guidance documents, including this guidance, do not establish legally enforcable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations,

unless specific regulatory or statutory requirements are cited. The use of the word should in Agency guidandes means that something is suggested or recommended, but not required."
Following this statement, the integrity test before use, after sterilisation is only a recommendation. Based on a risk assessment the user has to decide if the pre-use test should be implemented.

From this regulation, it is clear that a test or test method will suffice ("by an appropriate method such as bubble point..."). There is no evident necessity to implement several tests (e.g., combined diffusion and bubble-point test).

2.4 PDA (Parenteral Drug Association), Technical Report No. 26 (2008)

This technical report from the PDA firstly has no legislative status in principle. However, the statements contained in the report are fundamentally recognized by official inspectors as guidelines. Below are the principal statements in respect of integrity testing:

- Where the claimed purpose of the filter is to sterilize pre- and post-filtration integrity tests should be performed.
- Regulatory expectations regarding pre-and post-filtration integrity testing may differ regionally.
- Pre-filtration integrity test may be performed prior to sterilization of the filter and, preferably, after sterilization.
- Steps should be taken to ensure that the downstream side of the system remains sterile when performing a post-sterilization integrity test.

Again, the important aspect here is the recommendation to test pre-use **and** post-use. Furthermore, the reference to regional differences regarding the necessity for a pre-use test is important. Fundamentally, there can be different interpretations and additional country-specific regulations in effect here.

2.5 Before or After? When Must Testing Occur?

The integrity testing of sterile filters ultimately ensures a sterile production or filling of pharmaceuticals. As a result, integrity testing after filtration is mandatory. The test after filtration documents that the filter is intact and shows by definition that the filter was also intact before and during filtration. It is desirable here that the test is conducted as soon as possible after filtration is complete, in order to avoid the adhesion of product residue to the membrane, for example.

A potential blocking of the filter during filtration has to be avoided in order to allow a reliable integrity test after filtration.

Finally, integrity testing pre-use substantiates that an undamaged filter with the required pore size was correctly installed. Even if the filters have been individually tested by the manufacturer after production, this procedure checks for potential transport damage or possible defective installation. The pre-use test can also avoid the use of damaged filters. This would be discovered by the test after filtration in any event. However, this procedure can prevent a situation where product lots cannot be released due to a defective filter (significant financial damages).

It can therefore be stated at this point that the post-use test is an essential quality assurance step. This step enables damage to the filter to be recognized that would result in potential insterility of the product.

A pre-use integrity test can prevent damaged filters from being used in the production process. This can prevent financial damages. However, it is important in this context that the pre-use test does not contribute to an improvement of product quality. All damage identified during a pre-use test would be detected by the post-use test in any case.

In future, it would be desirable that the cited regulations take an unequivocal position on the matter and leave less room for personal interpretation.

3. Methods of Integrity Testing

3.1 What Does Integrity Testing Mean?

In the strict sense of the term, integrity testing of sterile filters means microbiological evidence that a filter element provides a sterile filtrate when loaded with a certain quantity of bacteria ("bacterial challenge test", cf. Chapter 3.10). Only via this test can it be **directly** proven that the test organisms used are reliably (100 per cent!) retained by the filter. This test type is a so-called destructive test that renders the filters unusable for their actual application. It is therefore self-evident that such a procedure cannot form part of daily routine usage.

Rather, in the case of routine integrity testing of sterile filters, the objective is not to damage the filter properties via the test procedure ("non-destructive test"). This means by definition that indirect methods – without the use of microorganisms – must be used.

An essential requirement for the sensible application of indirect methods of integrity testing is the correlation with microbiological analyses (see Chapter 3.10). Accordingly, the methods described below are firstly, per se, merely to be regarded as neutral, physical measurements whose significance only becomes apparent after comparison with direct microbiological tests. Nobody, for example, would be able to make a statement as to whether a filter with a measured diffusion of 9 ml/min is performing sterile filtration or not. Only verification that a filter cartridge with this diffusion also provides a sterile filtrate in the microbiological test gives the test result significance.

The respective manufacturers of the filter cartridges are responsible for the correlation of the test values with microbiological analyses in the course of their product validation. In the course of this work, all limit values are defined for the various integrity test methods so that the end user does not have to be concerned with this.

During routine integrity testing, it is therefore merely a question of whether the relevant applicable limit values are observed or not. Thus the work is decisively simplified.

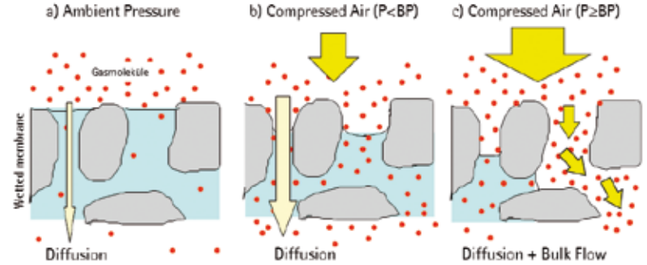
However, it is extremely advisable that the user should have at least a basic knowledge of the physical background to the test methods. Above all, this knowledge makes troubleshooting considerably easier when a test is not passed.

3.2 General Principle of Integrity Testing

All methods described below exploit a property of all filter membranes: they no longer allow free air flow when in a completely moistened state. The capillary forces of the narrow pores prevent the moistening fluid from being easily pressed back out.

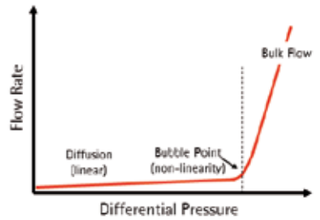
Loading one side with compressed air leads, with moderate pressures, to the dissolving of air molecules in the water-filled pores and to a net transport through the entire membrane (along the pressure and concentration gradients). With constant volumes on the unsterile ("upstream") side, the loss of air molecules automatically leads to a corresponding pressure drop that can be detected via pressure sensors. This pressure drop is measured by devices from the Sartocheck family and is the basis for all integrity test methods described below.

Figure 1: Schematic representation of the procedure of an integrity test: Where an overpressure is applied on the unsterile side of the filter and ambient pressure on the sterile side, there is a transfer of air molecules into the water-filled pores of the filter membrane. The loss of air molecules leads to a corresponding pressure drop in the system, which can be detected with relevant devices. If the applied pressure increases further, the forces will exceed the capillary forces inside the pores. The water is thus pressed out of the largest pores. This leads to a free passage of air and, thereby, to a disproportionately high pressure drop (= bubble point).



If the pressure on the inlet side of the filter is continually increased and the air flow through the membrane measured, the following behavior can be observed:

Figure 2: A graph of the air flow over the applied test pressure initially shows a linear trend (diffusion). From a certain pressure (= bubble point) there is a disproportionate increase resulting from pores being pressed completely clear.



In the lower pressure range, a linear relationship can be observed between the applied pressure and the air flow. With higher pressures, the air flow increases disproportionately because the pressure range is reached at which the moistening fluid is pressed from the pores. This allows most of the air to flow through unhindered and the volume of air flowing through is thereby considerably increased.

3.3 Important: Stabilization

With all of the test methods described below, it is important that a sufficiently long stabilization time is provided upstream of the actual test phase. As the term suggests, the system must be able to stabilize after pressurization. This has two principal reasons:

1. The compaction of the filter cartridges: To accommodate as much membrane surface area as possible in a small cartridge volume and thus optimize the filtration behavior, the membranes and non-woven rayon used are pleated (folded). This folding, however, is firstly also a three-dimensional structure which is pressed together (compacted) from one side during pressurization and then results in a volume change. This process is completed after a few minutes. If this is not fully completed owing to the selection of excessively short stabilization times, the compacted volume leads to a pressure drop during the test time, which the device then incorrectly interprets as diffusion. This may therefore lead to increased failures of integrity tests, even though the filters are de facto intact (i.e., false negative tests) and should therefore be absolutely avoided.

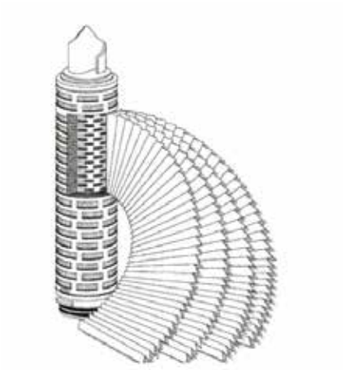
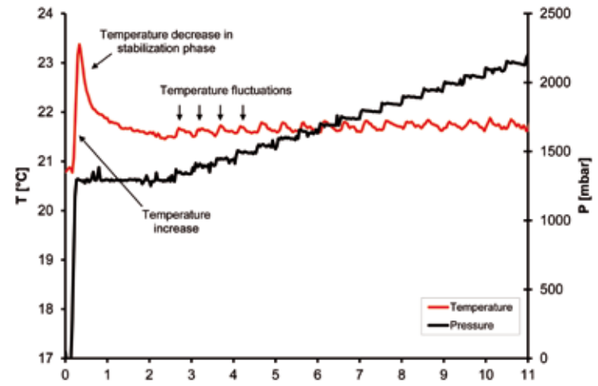


Figure 3: The three-dimensional architecture of the filter cartridges with their pleated (folded) membrane structures is compacted when pressure is applied. This leads to a pressure drop in the system which can be avoided by selecting a sufficiently long stabilization time.

2. Temperature equalization: The loading of the filter cartridge housing with compressed air represents a considerable energy input into the system. This means that we have to do it here using a thermodynamic process which, as a consequence, causes a temperature increase in the system. The problem is that the temperature will subsequently align with the ambient temperature. Since the volume in the housing is constant, a temperature drop is accompanied by a pressure drop. However, the device cannot differentiate between a pressure drop caused by temperature fluctuations and one caused by diffusion. This means, in turn, that failure to observe a sufficiently long stabilization time can result in the failure of an actually intact filter cartridge and should therefore be avoided.

Figure 4: Temperature changes at the filter cartridges during a bubble-point test. In a bubble-point test, the test pressure is varied in a step-like pattern. With a temperature sensor inside the filter housing, the temperature was measured directly at the filter cartridge. Temperature fluctuations can be clearly seen with every pressure increase. The stabilization time must be selected such that the temperature can fall completely to the baseline level again to avoid the effects of temperature on the measurement result.



During the stabilization time, the integrity test attempts to create stable conditions. This means that the target value (programmed test pressure) is compared with the actual value (measured pressure) and, in the event of a discrepancy, more compressed air is added accordingly to reach the target test pressure. If the volume of compressed air added exceeds a determined limit value, the test is abandoned and an appropriate error message displayed (stabilization not possible, possible leak).

As soon as the stabilization time is successfully complete, the device transfers into the test phase. In the test phase no further compressed air is added, but rather the corresponding pressure drop within the programmed test time is measured and converted into diffusion or water intrusion accordingly.

In all of these integrity tests it is important that the sterile side of the filter is not closed. If, for example, a valve on the outlet side of the housing is closed, the pressure on the sterile side increases through diffusion, whereby

the differential pressure (sterile vs. unsterile side) can be considerably lower. However, since the differential pressure is the driving force for the diffusion, lower diffusion rates would be measured in this situation than are actually real. Thus there is an increased risk of false positive test results. This is critical and must be avoided in all cases. To avoid such a pressure increase on the sterile side, a sufficiently large volume must be applied on this side.

3.4 Pressure-Hold Test

The pressure-hold test represents a simple integrity test method that is, however, rarely used for testing filters in the biopharmaceutical industry. The test is frequently implemented in the food sector. The pressure-hold test is also suitable for leak-tightness tests.

The measured pressure drop can be used directly as a measure of the integrity of the filter, provided that the system net volume is constant or known. In general, the following relationship applies:

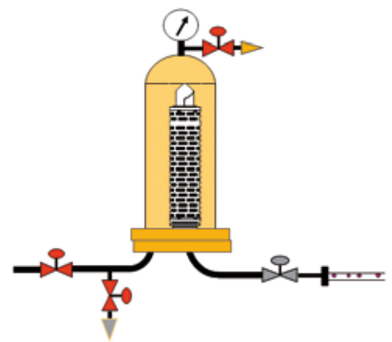
$\Delta p = (D \cdot t \cdot P0)/V_N$

$\Delta p =$ Pressure drop in the test time [mbar]
$D =$ Gas diffusion [ml/min]
$t =$ Test time [min]
$p_0 =$ Atmospheric pressure [mbar]
$V_N =$ System net volume [mL]

When checking a vessel for leak-tightness, for example, a corresponding test pressure and test time can be programmed. In an enclosed system, there should therefore be no considerable pressure drop. A previously defined limit value may not be exceeded in order to pass a test successfully.

When testing a filter cartridge, this must be uniformly moistened beforehand. After applying a specific test pressure, low volumes of air will move along the pressure and concentration gradients through the membrane owing to the solubility in the moistening fluid. This then results in a net loss of air molecules in the test housing. Since the volume of the housing used is constant, such a loss of air will be accompanied by a corresponding pressure drop.

Figure 5: Schematic design of a manual pressure drop test. The unsterile (upstream) side of the filter cartridge is loaded in the housing with a certain test pressure. A connected manometer can measure the pressure drop caused via diffusion. It is important that the outlet on the sterile side (downstream) is not closed to avoid an unwanted pressure increase.

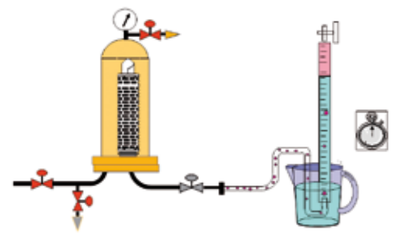


The disadvantage of the pressure drop test is the dependency on volume of the measured values obtained. This is why filter manufacturers cannot establish any universal limit values. It is more appropriate to implement a diffusion test as the gas diffusion through the membrane represents the basis for the measured pressure drop in any case.

3.5 Diffusion Test

The diffusion test is implemented similarly to the pressure-hold test in terms of the practical procedure. While the pressure-hold test measures the pressure loss occurring over a specific timeframe, the diffusion test returns information on the diffusive flow through the membrane. The means ultimately that a diffusion is obtained in a unit of ml/min.

Figure 6: Schematic design of a manual pressure diffusion test. Similarly to the pressure drop test, a constant test pressure is applied. The air on the sterile side that passes over via diffusion can be collected and the flow volume (ml/min.) can be quantified. It is also important in the diffusion test that the outlet on the sterile side (downstream) is not closed to avoid an unwanted pressure increase there.



However, since the Sartocheck devices with their integrated pressure sensor can only measure the pressure drop and not flow rates, the occurring diffusion must be calculated using the actual pressure drop, the available net volume (the upstream volume of the housing with mounted filter cartridge, see Chapter 3.9) and the test time.

In general, the following relationship can be established:

$$\frac{N}{t} = \frac{D \cdot L \cdot Dp \cdot F}{d}$$

N/T= Gas flow over time (mol/s)
D = Diffusion coefficient (liquid gas system)
L = Solubility coefficient (liquid gas system)
Dp = applied differential pressure
F = Gas/liquid interface
D = Thickness of interface

The diffusion is dependent on the type of test gas.
e.g., correction factor for nitrogen as a test gas:

$Diff_{N2} = Diff_{air}$

N/T= Gas flow over time (mol/s)
D = Diffusion coefficient
L = Solubility coefficient
Dp = applied differential pressure
F = Gas/liquid interface
d = Thickness of interface

When considering the formula, it is clear that the membrane surface area (F) as well as its thickness (d) are important parameters. This means that the diffusion increases with increasing membrane surface area and decreasing thickness. This relationship ultimately also limits the use of the diffusion test for very small filters. The occurring pressure drop with very small filter surface areas is, within acceptable time limits, too low (resolution of the sensor used), so that no sensible statement is possible. It is therefore recommended only to implement the diffusion test with filter surface areas from ≥ 150 cm².

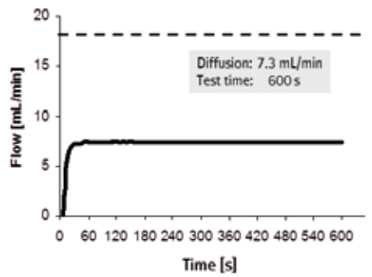


Figure 7: Typical curve of a diffusion test. The curve shows the flow through the membrane over time. This is measured at a constant test pressure. It is important that the maximum permitted limit value is not exceeded at the end of the test time.

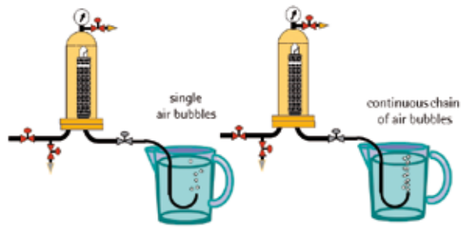
The following properties of the diffusion test should be noted here again:

- The diffusive gas volume is proportional to the differential pressure and filter surface area.
- The test pressure to be established must be below the bubble point (70-80% of the bubble point).
- Suitable for filter surface areas from 150 cm²; not suitable for very small filter units, such as syringe filters.
- The test provides information about the average pore size distribution of the entire membrane.

3.6 Bubble Point Test

The principal setup for implementing the bubble point (BP) test is no different from that for the diffusion test. However, the procedure for implementing the test is fundamentally different: whereas in the diffusion test, a fixed test pressure is worked with and the diffusion is measured over a defined timeframe, the test pressure in the BP test is increased incrementally until the so-called bubble point is detected.

Figure 8: Schematic design of a manual bubble point test. In contrast to the diffusion test, the test is not carried out under constant test pressure. Rather, the pressure is increased in a stepped manner until a disproportionate passage of air can be measured. In a manual test, this can be identified by an erratic increase in air bubbles if a drain tube is hung in a water bath.



As stated above, in the diffusion test, there is a flow of gas molecules through the water-filled pores. If the applied test pressure is increased, the diffusion rate increases in a linear manner with increasing pressure. Then at some point, the pressure is reached at which the capillary forces (holding the water in the pores) are exceeded and the water is thus actively pressed out of the largest pores. Air can then flow unhindered through the opened pores, meaning that a disproportionate pressure drop can be measured. If we plot the relationship between the gas flow through the membrane and the applied test pressure, then an almost ideal straight line (linear relationship between diffusion and test pressure) can initially be seen, which then begins to deviate at a certain point (disproportionate increase). And this precise point is known as the bubble point (see Figure 2).

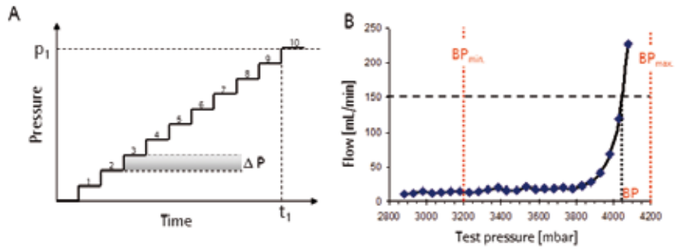


Figure 9: Typical procedure of a bubble point test. A) The test pressure is increased slowly and incrementally (Delta P) and at each level, the resulting pressure drop or diffusion is measured. B) With the BP curve, the diffusion rate is displayed over the test pressure. The typical BP curve shows a slow, mostly linear increase before deviating significantly once a certain pressure is reached (non-linearity through opened pores). As a test criterion, the measured BP must be greater than the specified limit value (minimum BP). The maximum BP has no effect on the test evaluation. Rather, it represents an upper test pressure at which the test is aborted (test passed).

By considering the underlying formula for the bubble point, it can be immediately seen that the radius of the pores is an important factor. The membrane surface area, on the other hand, is not a component of the pore. Thus it becomes clear that the bubble point test can be implemented across a broad range – from very small flat filter membranes (e.g., Minisart syringe filters) to large filter cartridges.

$$p_{BP} = \frac{2\,\sigma \cos \vartheta}{r} \cdot K$$

P_{BP} = Bubble point
σ = Surface tension
ϑ = Wetting angle
r = Pore radius
K = Correction factor (dependent on structure)

From the formula, it can be seen that the bubble point of a filter is dependent both on the filter material (wetting angle) as well as in particular the surface tension of the moistening fluid. Low surface tensions, such as those with organic solvents, emulsifiers and detergents, lead to a lower bubble point. Since the surface tension is, in turn, dependent on temperature, different BP values can be recorded at different media temperatures. The limit values defined by the filter manufacturers can, therefore, only be valid for defined test conditions (temperature, surface tension of the wetting medium).

The bubble point test, therefore, provides a statement about the largest pores within a membrane. And this precisely is important information because the largest pores also naturally present the largest risk for a potential passage of microorganisms.

The following properties of the bubble point test should be noted here again:

- The test pressure is increased incrementally until the device detects a disproportionate air flow as a measurement for the BP.
- The bubble point is the pressure at which the moistening medium is pressed from the largest pores.
- The bubble point is independent of the membrane size and, rather, is a measurement for the largest pore diameter in the membrane.
- Universally suitable for small and large filter surface areas.

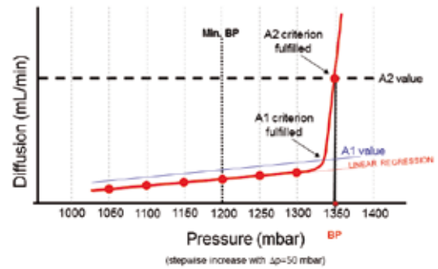
Automatic Detection of the Bubble Point

The test algorithms stored in modern test devices ultimately serve the objective of detecting the start of non-linearity in the pressure-diffusion curve. The following should explain in detail how this unfolds in the devices of the Sartocheck family:

As mentioned above, the test pressure in BP test is increased incrementally. To obtain a sensible resolution, it is recommended that measurements are taken in pressure levels of 50 mbar. Since the test time naturally increases with the number of pressure levels required, it is not sensible to start from zero. Rather, it is normal to start with a pressure of 90% of the programmed minimum BP value (or 4 pressure levels) (e.g., starting pressure of 1800 mbar at BPmin of 2000 mbar).

The applied pressure is held at each level for a certain time and the occurring diffusion rate calculated. Beneath the bubble point there is, as explained above, a linear relationship between differential pressure and diffusion rate. Now it is a question of detecting a disproportionate increase in the diffusion-pressure relationship at the subsequent pressure levels, i.e., determining the precise point at which the curve begins to deviate. Thus, the objective is to identify a significant deviation of the straight line (linearity). As soon as the diffusion increases disproportionately (A1 criterion) and a minimum diffusion value (A2 criterion) is exceeded, the bubble point is deemed to be identified.

Figure 10: Criteria for automatic detection of the bubble point. The devices of the Sartocheck range use the A1 value (criterion for disproportionality) and the A2 value (criterion for minimum diffusion) to detect the bubble point. Only if both criteria are fulfilled can a bubble point be found.



For this, it is necessary to give the device sensible parameters in order to use clear and, above all, objective criteria. When programming a standard BP test, the selectable parameters are limited. This is particularly true of the test category selection which should be made based upon the filter size:

	Which Filter?	A1-Value	A2-Value
Small system:	Flat filter, mini-cartridges, capsules up to 5"	5 ml/min	50 ml/min
Standard:	10"–20" filter cartridges and capsules	15 ml/min	150 ml/min
Special system:	30" filter cartridges | capsules and smaller multi-branch systems	30 ml/min	240 ml/min

For larger multiple housing, a customer-specific BP test should be used. Please contact Sartorius Stedim Biotech if specific information is required.

Based upon the test category selection, the A1 and A2 values decisive for the BP detection are automatically established. These values determine how sensitively a disproportionate increase can be determined and should always be adapted to the filter size to be tested. The test category selection has no influence on the size of the pressure levels, which are pre-defined at 50 mbar for a standard BP test.

The **A1 value** represents the permitted distribution of diffusion values with increasing test pressures. As previously explained, until the BP is reached, there is a linear relationship between the increasing pressure and the occurring diffusion. The measured values can, however, deviate slightly from the ideal straight line, without departing from the linear progression. In order to be able to detect an erratic non-linearity, the A1 value must be exceeded from one pressure level to the next. Only then is the first criterion fulfilled.

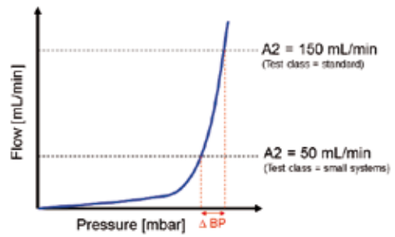
The **A2 value** represents the required minimum diffusion that is realistic when the BP is exceeded. In contrast to the A1 value, this limit value does not have to be reached from one pressure level to the next, but can build up over several pressure levels.

Only when both criteria – A1 and A2 – are fulfilled, is the bubble point deemed to be detected. Consequently, the pressure at which the A2 value is exceeded is defined as the bubble point.
The use of two criteria is necessary to be able to differentiate between a monotone increase (then A2 would be fulfilled but not A1) and a non-linear, disproportionate increase. If only the A2 criterion is fulfilled, but not A1, the test is aborted with an appropriate error message.

Figure 11: With a relatively linear increase in the diffusion-pressure ratio the A2-criterion can be fulfilled without the A1-criterion being fulfilled. Thus the bubble point is not recognized despite the minimum diffusion being reached and, instead, a corresponding error message is displayed.

The sensible selection of the test category (and thus precisely of A1 and A2) has a direct influence on the detection of the bubble point. The A2 value represents the minimum diffusion at which the BP is then deemed as identified (if A1 is also simultaneously fulfilled). Since the curve has a certain incline, this means that, according to the test category (thus A2 value), the BP will be identified sooner or later.

Figure 12: The selection of the A2-value can have a significant effect on the detected bubble point. Rather, a lower minimum diffusion (A2) is reached and leads to a lower bubble point. Thus, it becomes clear that the recommendations in respect of the A2-value should be adhered to or should be sensibly adapted in special cases.



Thus, it is clear that an incorrect selection of test category can lead to significantly lower or higher BP values. The classification specified in the table should therefore be observed.
When implementing a combined test (diffusion + BP), the test category is automatically defined for the BP test based upon the measured diffusion.

The Customer-Specific Bubble Point Test

The devices of the current Sartocheck family also enable programming of a so-called **customer-specific BP test**. This uses, in principle, the same algorithms as the standard BP test, but allows greater freedom in the selection of different parameters. Thus, for example, the A1 and A2 values can be freely selected and the size of the pressure levels modified. However, this procedure has direct effects on the identification of the BP and test precision. The values should, therefore, be established or altered with care and, wherever possible, after discussions with the application specialists of Sartorius Stedim Biotech GmbH.

3.7 Diffusion Test vs. Bubble Point Test – Which Test is Appropriate for Me?

As explained in detail above, both the diffusion test and bubble point test are suitable for testing filter cartridges for integrity. The obvious question is which test type is more suitable or whether both tests should be implemented to increase reliability. The following can be established:

Because diffusion is dependent on surface area, it is only sensible to implement the diffusion test from membrane surface areas of 150 cm^2. This means by definition that, for smaller filter surface areas, the bubble point test is the automatic selection.

Standard filter cartridges, however, have larger filter surface areas and can, in principle, be measured by both methods. Owing to the different measuring methodology, however, there are different points of focus: while the bubble point test detects the largest pores in a membrane (mostly independent of surface area), the diffusion test places its emphasis on the entire membrane surface area. A short example is given for clarification:

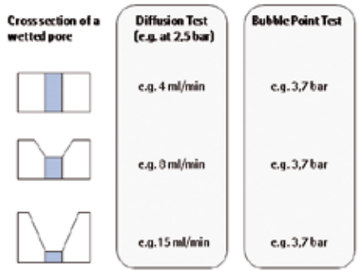
As explained above, the formulae for the diffusion and bubble point tests differ principally in that, in one instance, the entire surface area of the filter is considered and the pore size is, therefore, of no importance. In the bubble point test, the radius of the pore is input into the formula and there is no consideration of the membrane surface area.

As the pore size distribution around a static average moves with a certain spread, the diffusion test provides a better picture of the total pore distribution in the membrane. However, there is no emphasis here on the largest pore diameter.

The bubble point test could theoretically detect a single excessively large pore. However, the information regarding the average pore size distribution is lost here.

Thus, it becomes clear that the two test types differ completely in their informative value. It is therefore also conceivable that there are unfavorable configurations in which the same filter cartridge may pass one test type successfully but fail another test type. However, this should clearly be the exception and does not justify implementing both test types. Each test type is clearly correlated with the bacterial challenge test and is reliable in itself.

Figure 13: Theoretical consideration of the effect of the pore shape or moistening status on the results of the diffusion and BP test. Owing to the surface-dependent diffusion, the diffusion test reacts considerably more sensitively when wetting is incomplete.



Theoretical consideration assumes ideal pores, i.e., cylindrical structures, that can be better formulated mathematically than irregularly-shaped pores. Taking the three cases shown above, in case a) the pore is an ideal cylinder with identical pore diameter through the entire depth; case b) is an example where the pore is not ideally cylindrical but rather crater-shaped on one side and case c) shows a larger and deeper crater. However, in these cases, only the narrower parts of the pores are moistened.

If these example pores are tested by both methods, the following picture emerges: the bubble point test calculates the same bubble point for all 3 scenarios. The reason for this is that the narrowest pore diameter is identical in all 3 cases. This means that equal capillary forces are also effective within these segments and these forces must be exceeded in the BP test. The thickness of the layer or thickness of the water column within the pores is not relevant here.

In the diffusion test, in contrast, the layer thickness is also a component of the formula (see Page 14). The diffusive gas transport through the thinner water layer is considerably faster, leading to a larger diffusion being measured.

This albeit exaggerated theoretical example shows, therefore, that the test results of the diffusion test and bubble point test are not comparable in every case. This example also shows that, as a result, the diffusion test reacts considerably more sensitively to wetting problems.

At this point, it is re-iterated that the regulations to be applied only prescribe one suitable integrity test. It is not necessary to implement a combined test (consisting of a diffusion and BP test) and the time required to implement a combined test generally precludes such a practice.

3.8. Testing of Hydrophobic Membranes

3.8.1 The IPA Test

The test types described above, such as diffusion test and bubble point test are applicable for hydrophilic (= can be wet with water) membranes. For the area of air and gas filtration, it is essential for various reasons, however, that hydrophobic (= water-repellent) membrane material is used. As a result, it is per se not possible to implement the above tests as these require the complete wetting of the membrane with water.

However, there is a trick that can be used to still make this membrane testable: by moistening with a mixture of alcohol and water (typically 60% IPA/water) the membrane can be artificially hydrophilized and reliably moistened with this mixture. In this moistened condition, the standard tests can then be implemented. It must be ensured that the moistening mixture used by the filter cartridge manufacturer is used. The use of 99% ethanol, for example, instead of 60% IPA will displace the detected bubble point significantly. In such a case, specially adapted limit values should be used.

Generally, the IPA-based integrity test of air filters has a number of disadvantages:

- Implementation only possible before sterilization
- IPA residue must be reliably removed after the integrity test
- The degree of hydrophobicity of the membrane is not tested
- The correct installation of the filter cartridge is not tested
- The test takes place on the membrane in a different condition (artificially hydrophilic)

These disadvantages can be avoided by implementing a so-called water intrusion test instead of the IPA-based test.

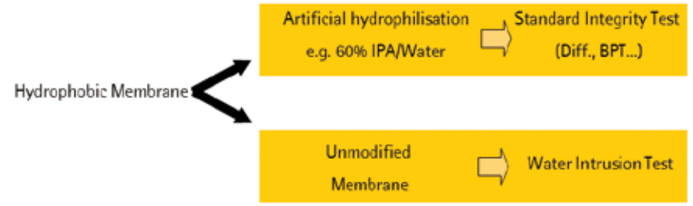


Figure 14: For testing hydrophobic filter materials (such as PTFE), there are two different procedures: either the membrane can be artificially wet with polar solvents (e.g., isopropyl alcohol) and then tested accordingly with a bubble point or diffusion test; or the membrane remains unaltered and is checked for integrity with the so-called water intrusion test (WIT).

3.8.2 The Water Intrusion Test

The water intrusion test (WIT) was developed by Sartorius in 1991 and represents a milestone in filter integrity testing.

The principle of the test is relatively simple and shows similarities with the diffusion test on hydrophilic filter cartridges. In contrast with the diffusion test, it is not the air flow through the water-filled pores that is measured, but the water entry (= intrusion) into the hydrophobic, air-filled pores. The hydrophobic membrane (e.g., PTFE) cannot be naturally moistened, so there is no chance of water leaking through the tested membrane when it is loaded with water. Upon pressurization, the forces act against the hydrophobic forces and small (!) amounts of water will enter into the pore structure (important: no water exits on the sterile side). At a constant volume (filter housing) there is, similarly to the diffusion test, a pressure drop which can be detected through appropriate sensors.

The practical procedure of the WIT is that the filter housing is filled with water from the unsterile side (upstream), so that the complete filter cartridge and/or its membrane is covered with a water column. The sterile side of the filter is unaffected throughout the entire test.

If even the smallest areas of the membrane are not covered with water, the WIT is no longer possible because the applied compressed air will immediately escape through the uncovered pores (= air filter). In addition, it must be considered that the water level can drop due to the compacting of the filter cartridge (cf. Chapter on Stabilization) and may become too low during the test phase so that the test gas can leave via the PTFE membrane.

A sufficiently large air bubble must remain at the tip of the housing dome in which the pressure sensor can detect a corresponding pressure drop.

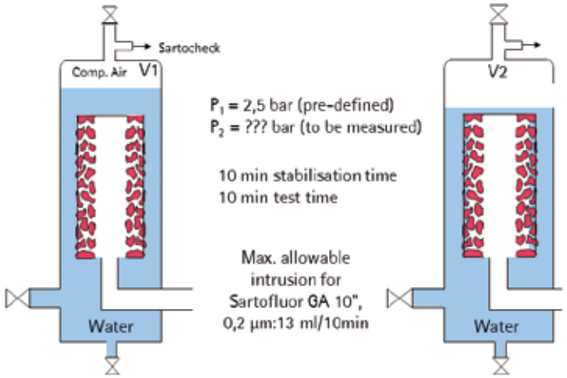


Figure 15: Principal setup for implementing a water intrusion test. The filter cartridge in the housing must be completely filled with water from the sterile side. The hydrophobicity of the PTFE membrane prevents the passage of water onto the sterile side. The pressure drop in the remaining air volume can be used to measure the penetration (intrusion) of water into the pore structure of the membrane.

The water intrusion is calculated in accordance with the following formula:

$$WIT = \frac{\Delta P \cdot V_m}{P_0 \cdot t}$$

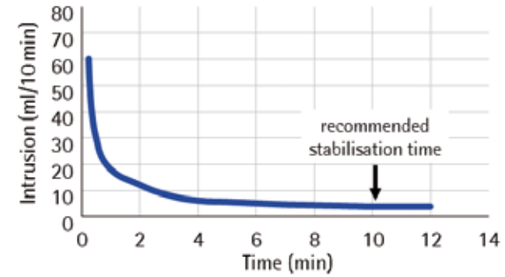
WIT = Water intrusion rate
DP = Pressure drop (mbar)
Vm = Inlet-side gas volume (ml)
Po = Atmospheric pressure ...
t = Test time ...

The limit values for intrusion correlate directly with the bacteria challenge test (HIMA).

From this, it can be seen that, with a known net volume (the volume of the air bubble), the measured pressured drop can be used to calculate the water intrusion mathematically .

In principle, only a low intrusion of water into the PTFE membrane is found, which consequently leads to only low pressure drops. This means, on the one hand, that only filters with a membrane surface area of at least 1000 cm² can be sensibly tested. Smaller surface areas are on the periphery of, or even below, the resolution of the pressure sensor. On the other hand, this means particularly that the water intrusion test reacts sensitively to excessively short stabilization times. It is known that the compaction of the filter cartridge is only complete after approx. 6 minutes. After this time, no more major effects on the test result are to be expected. Thus, for the WIT, we recommend a stabilization time of no less than 10 minutes.

Figure 16: Pressure drop in the initial phase of the WIT. Owing to the compacting of the filter cartridge, a pressure drop is recorded directly after pressurization. This pressure drop has nothing to do with the water intrusion, but rather reflects mechanical effects on the pleated filter structure. The integrity tester evaluates any pressure drops as water intrusion. In this respect, it should be ensured that the stabilization time is sufficiently long (recommendation 10 min.).



The following advantages of the WIT compared with the IPA test can be cited:

- Testing of the hydrophobic filter in installed condition
- Testing of the filter cartridge possible after steam sterilization
- The hydrophobic properties are tested
- The complete system (filter cartridge plus housing and seals) is tested
- No manipulation is required on the sterile side
- No drying of the filter cartridge necessary after the test

The following requirements must be fulfilled:

- Surface tension of the water at least 70 mN/m
- No temperature difference between test water and the air
- No temperature drift during measurement
- The filter membrane must be completely hydrophobic
- No product residue on the membrane that could influence hydrophobicity

3.8.3 Requirements for the Filter Housing and Installation

As explained above, in the WIT, the housing is filled with water, which must be completely removed again after the test is completed. As a result, there are special requirements for WIT-capable air filter housing:

1. Facility to connect the test hose and/or external pressure sensor to the top of the housing (quick coupling nipple, e.g., type Stäubli RBE03)

2. Facility to connect the hose package from the WIT trolley to the underside of the housing (automatic filling and draining) and/or valve for manual draining

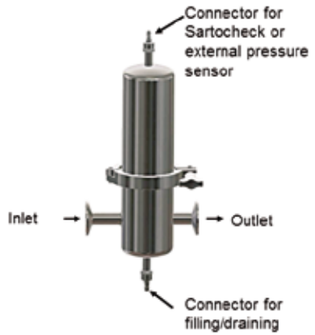


Figure 17: Typical WIT-suited air filter housing. In addition to the connection for the test hose/pressure sensor on the top side, there is a self-closing connection on the underside of the housing. This connection is used for the automatic filling and emptying for the water intrusion test.

In addition, when installing the housing it must be ensured that there is a facility to block the supply line (as close as possible to the housing) and that there are no spaces remaining in the supply line which remain filled with air during filling. Such enclosed air bubbles can lead to problems when determining the net volume. Furthermore, these air bubbles expand when the housing is depressurized and can push out water which may cause moisture to run back into the tester.

3.8.4 Water Intrusion Test vs. Water Flow Test

Various technologies for the implementation of filter integrity tests have established themselves in the market. The devices of the Sartocheck family use the pressure drop method to mathematically calculate the diffusion or water intrusion under known parameters. Other suppliers use flow meters to obtain the corresponding measuring results.

The important aspect in this context is obviously the necessity that the result obtained must be independent of the method used! This means that a defective filter cartridge must be reliably detected and an intact cartridge recognized as intact, regardless of which device and which underlying methodology is used by the different suppliers.

In the past, however, the different methods have resulted in water intrusion values being specified on the one hand and so-called water-flow values on the other. The devices of the Sartocheck family can implement both test types in principle. The practical test implementation is identical with only a slightly modified formula used for the calculation:

$$Intrusion = \frac{\Delta p \cdot V_n}{t \cdot P_0} \cdot \frac{ml}{t} \qquad WaterFlow = \frac{\Delta p \cdot V_n}{t \cdot P_{abs}} \cdot \frac{ml}{t}$$

ΔP = Pressure drop
Vn = Net volume (water flow corrected)
T = Test time
P_0 = Standard atmospheric pressure (1,000 mbar)
P_{abs} = Absolute test pressure

The difference lies merely in the fact that one (WIT) uses the standard atmospheric pressure (1,000 mbar) and the other uses the absolute test pressure (atmospheric pressure plus test pressure). This produces the following relationship:

$$\frac{\textbf{\textit{Intrusion value}}}{\textbf{\textit{Water flow value}}} = \frac{\Delta P \cdot V_n}{t \cdot P_0} \cdot \frac{t \cdot P_{abs}}{\Delta p \cdot V_n}$$

$$\textbf{\textit{Intrusion value}} = \frac{3500 \cdot \textbf{\textit{Water flow value}}}{1000}$$

$$\textbf{\textit{Intrusion value}} = 3{,}5 \cdot \textbf{\textit{Water flow value}}$$

Thus, it is clear that there is a factor of 3.5 between the WIT and WFT values. Before the test, one should therefore check whether the filter cartridge manufacturer has defined the limit value for the WIT or WFT in order to select the appropriate method.

Unfortunately, the terms WIT and WFT are not used uniformly in the market. This means that a supplier specifies WIT limit values for its filter cartridges when these are actually WFT values. This again underlines the importance of clearly understanding the significance of the limit value.

3.8.5 Automation of Implementation

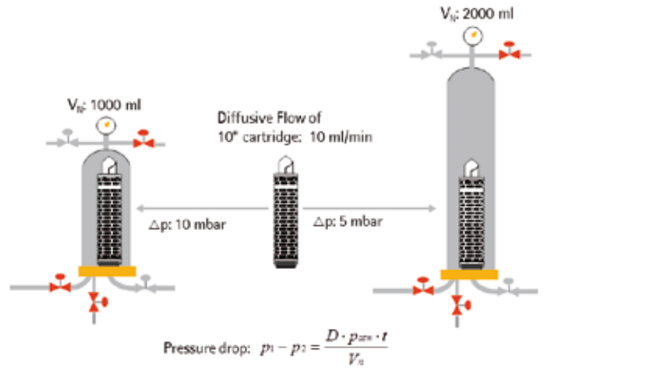
As described above, the implementation of a WIT requires the filter cartridge housing to be filled with water. After the end of the actual test, the water must be removed from the housing. In addition, it must be ensured that the water temperature does not deviate from the ambient temperature. These steps can be automated with the use of a so-called WIT trolley. This trolley is controlled via a central Sartocheck 4 plus. The trolley holds a sufficiently large collection container to store the water used for filling. A temperature sensor monitors the correct water temperature. At the start of a WIT, the filter cartridge housing is automatically started and the test sequence started. After completion of the test, the run-off valve opens automatically and the water flows back out of the test housing. The trolley can also be used to activate a cooling cycle. This allows hot stainless steel housing (e.g., after steam sterilization) to be quickly cooled back down to room temperature so that the process of integrity testing runs considerably more efficiently.

Figure 18: The WIT trolley is used for the automatic implementation of the water intrusion test. The test is monitored by a Sartocheck 4 plus, which is placed on the device. An internal water tank in the device, special sensor technology and automatic valve control result in highly efficient implementation of the WIT.

3.9 Determining the Net Volume

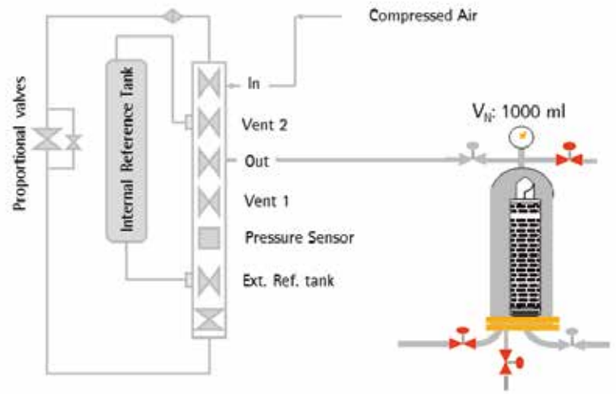
The Sartocheck test devices use the measured pressure drop to calculate the diffusion or water intrusion. However, the size of the pressure drop is highly dependent on the size of the volume in which the measurement is taking place:

Figure 19: Since the test is implemented using the pressure drop method, it is important to know the precise net volume. An identical diffusion rate with double the net volume will reduce the pressure drop by half. This really must be observed when implementing the test. Otherwise the test results will be erroneous.



Thus, it can be seen that it is important either to know the net volume or to determine this during the test. Sartocheck offers the facility to automatically determine the net volume before the actual integrity test. At this point, we should describe briefly how this occurs: Sartocheck uses a 1L stainless steel reference tank in the device, the volume of which is precisely measured and the value stored in the device.

Figure 20: Schematic representation of the internal pneumatics of the Sartocheck 4 plus. With automatic net volume calculation, intelligent valve control in the device with use of the internal reference volume results in an automatic measurement of unknown volumes.



During automatic net volume determination, the housing is at first pressurized with the programmed test pressure and briefly stabilized. Housing is then depressurized before a valve then opens to the internal reference tank so that the pressure is equalized. Using the measured pressure drop, the unknown (net) volume of the filter cartridge housing can be determined.

$$p_1 \cdot V_r = p_2 \cdot V_g \qquad V_g = V_r + V_n$$

$$V_n = \frac{p_1 \cdot V_r}{p_2} - V_r$$

V_n = Net volume in ml
V_r = Reference volume in ml
V_9 = Total volume
P_1 = Test pressure in the reference tank
P_2 = Pressure in total volume according to t

This means that, by using the internal reference tank, an unknown volume can be measured automatically. The internal reference volume is suitable for measuring volumes up to 14 liters. In order to avoid unacceptable inaccurancies, a larger external reference tank (standard = 10 liters) must be connected for greater volumes. This can be used to measure containers with a net volume of up to 150 liters.

If the net volume is too large, this is automatically identified by the device and a corrsponding error message is displayed and printed out.

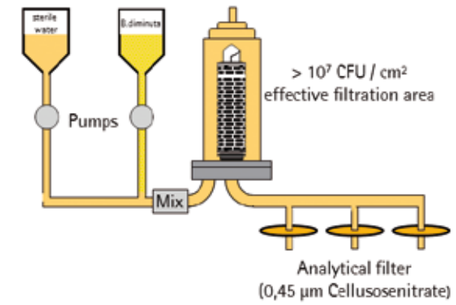
3.10 Significance of the Test Results – Correlation with the Bacterial Challenge Test

At this point, it should be expressly re-iterated that the results of the different integrity test methods do not make any statement regarding sterile filtration properties. A diffusion value of 8 ml/min, for example, cannot be sensibly evaluated by anybody without necessary background information.

So that this is possible, the filter cartridge manufacturers validate concrete limit values for the individual filter types for the different integrity tests. The end user must then simply decide whether the established criteria are adhered to or not.
To establish the limit values, bacterial challenge tests are implemented. In accordance with ASTM Guideline (ASTM F 838-05, 2005) a filter may only be classified as a sterile filter if it delivers a sterile filtrate after loading with 10^7 test bacteria, type *Brevundimonas diminuta* per square centimetre of filter surface area. This test with the extremely high density of bacteria is a worst-case scenario and substantiates the high reliability of the filters used.

To implement the bacterial challenge test, the bacterial solution is filtered using the filter cartridge to be tested and the filtrate is, in turn, passed through a special analysis filter. This is subsequently rinsed (to remove any product residue), placed on an agar plate and incubated under optimal growth conditions. If the filtrate is sterile, not a single bacteria colony should be found after incubation. These particular tests are implemented using filter cartridges for which integrity test values have been previously determined

Figure 21: Test design for implementing a bacterial challenge test (BCT). An aqueous bacterial solution is filtered through the filter cartridge to be tested. The filtrate is, in turn, filtered through analysis filters, which are subsequently incubated on agar plates under defined conditions. Only if no colonies of the test bacteria used (Brevundimonas diminuta) are detected is the Bacterial Challenge test successfully passed.



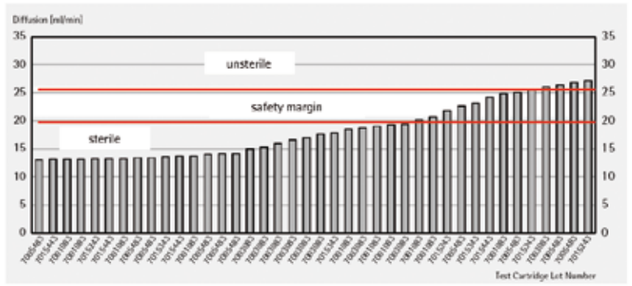


Figure 22: To calculate the limit values for an integrity test, the results of the bacterial challenge test (BCT) are correlated with the integrity test values. Each bar relates to the measurement of a filter cartridge. The bars are laid out with increasing diffusion values. The upper line defines the interface between the sterile and unsterile results in the BCT. The limit value is defined for the integrity test method (bottom line) with provison for a certain safety margin.

If, for example, increasing diffusion values for different filter cartridges are applied in turn, at some point there will be a transition between the cartridges that deliver a sterile filtrate and those that deliver an unsterile filtrate. The relevant limit value for the integrity test method is then established with provision for an additional safety margin. Details on this can be found in the relevant validation guides for filter cartridges.

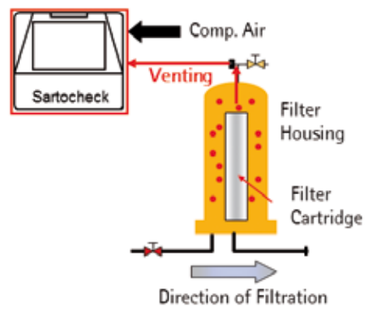
4. Filter Integrity Testing: Measures for Increasing Reliability of Testing

In principle, when performing integrity testing via customary methods, it is necessary to establish a pneumatic connection between the test device (e.g., Sartocheck) and test housing. This connection naturally also poses a potential risk via returning fluids and aerosols that are carried back. Since an integrity tester will be used numerous times over the years, in some cases daily, it is necessary at this point to provide ways of increasing reliability in relation to hygiene and avoiding potential cross-contamination.

4.1 Use of External Vent Valves

When implementing integrity tests, it is necessary that the filter cartridge is brought to a defined test pressure. This occurs by using a test hose to introduce compressed air via the test device. After the test is complete, the housing must be de-pressurized again. To achieve this, valves open inside the device and the compressed air flows back to the device and is released into the room via corresponding vent outlets or can be discharged in a more targeted manner via hoses. Depending on usage, it may be sensible not to let the compressed air pass back out of the housing through the test device since this prevents potential contamination of the device interior.

Figure 23: The pneumatic connection between test device and filter cartridge housing may allow potential contamination or aerosols to enter back inside the device. Protection against such a situation may be expedient.



A solution to this is the use of external valves. When using such a valve, the air is no longer returned through the device during the ventilation process but is released directly from the housing into the room or discharged from the housing via hoses.

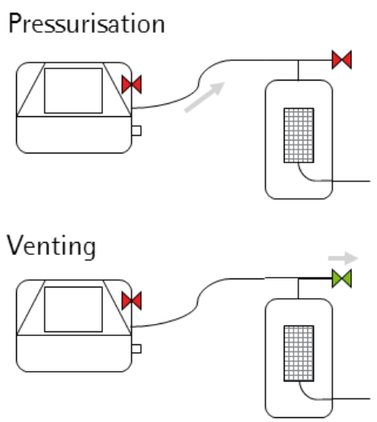
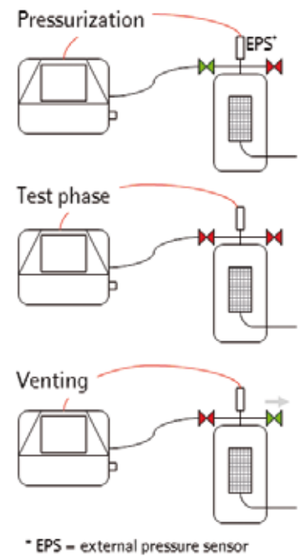


Figure 24: When using an external valve, this takes over the controlled removal of the compressed air during the ventilation procedure. The internal valve remains closed. This ensures that the compressed air and any particles/microbes carried with it do not enter back into the device.

When using an individual vent valve, the air is discharged via the vent valve but there is also an open connection during ventilation between the housing and test device. To increase reliability yet further, a second so-called isolation valve is installed between the test housing and test hose. This valve is closed during the ventilation process to guarantee maximum reliability.

Figure 25: Through the use of two external valves, and an external pressure sensor not only can air be removed in a controlled manner during ventilation but access from the pneumatic line to the test device can be directly blocked (isolation valve). This configuration results in even safer test implementation.



The isolation valve is only open during pressurization and is closed during the test phase and subsequent ventilation. This requires the use of an external pressure sensor because no pressure changes can be registered through the closed test hose by using the internal pressure sensor.

The Sartocheck 4 plus device has two electrical connection facilities on the right-hand side for the external valves and external sensor. Upon activation, the Sartocheck 4 plus automatically assumes the correct valve control, meaning that the user does not have to control or monitor anything else.

Figure 26: The Sartocheck 4 plus integrity tester has various connections on the side. One of these sockets is for the connection of external valves. After activation of the valves in the Sartocheck 4 plus menu, these are automatically controlled via the device.

4.2 Using a Hydrophobic Protective Filter

There are applications where product residues or aerosols repeatedly flow from the housing back into the test hose and may also enter the device. A simple protection against this is the use of a hydrophobic filter between the test hose and housing. The hydrophobic membrane allows air through with ease but repels fluids. As the moving air volume during integrity testing is very low, small vent filters (e.g., Midisart 2000) are sufficient here. Tests have shown that the intermediate filter has no effect on the results of the integrity test.

When installing the filter, however, it must be ensured that any residual fluid can run out and will not be deposited on the filter, which would lead to a blockage.

4.3 Cleaning the Internal Pneumatics

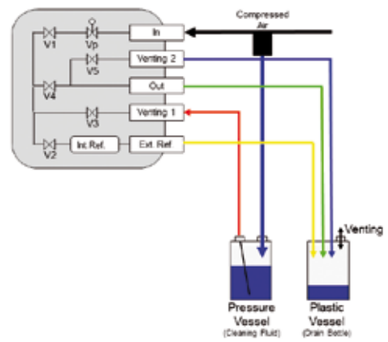
The measures cited above are intended for protection, i.e., appropriate measures should prevent the integrity tester from becoming contaminated through the test process. Given that the device can be used for several years and that most tests are implemented without the measures cited above, it is sensible and indeed necessary to ensure that the internal pneumatic parts can be reliably cleaned should the need arise. One impressive feature of the devices in the current Sartocheck family is that the internal pneumatic components are made exclusively from stainless steel and PTFE. This provides excellent chemical resistance, meaning that the parts can even be cleaned with aggressive cleaning agents (up to 1 M NaOH).

Against this background, Sartorius Stedim Biotech has developed a patented cleaning process, whereby the user can perform internal cleaning independently or have this performed by the Sartorius Service Department. The process requires a special cleaning set that is ultimately responsible for pumping the cleaning fluid. The cleaning itself is controlled via the device's standard software.

Figure 27: The cleaning set for Sartocheck consists of a plastic container for collecting the cleaning and rinsing liquid and various hoses with quick couplings. In addition, a stainless steel pressure tank is used to force the cleaning fluid into the device. The complete cleaning process is controlled via the device software.

In principle, the cleaning process has three stages: in the first stage, the cleaning solution (e.g., water or NaOH) is forced into the device via a pressure tank and left for a while. In the next stage, this cleaning solution is removed and in the third stage, the device is rinsed with clean water to remove any loosened dirt particles and cleaning fluid residue. In the final stage, the internal pneumatics is then dried with compressed air. Once drying is complete, the device is available for regular use again.

Figure 28: Schematic representation of the device cleaning design. Cleaning solution is fed from the pressure tank into the device using compressed air. A corresponding valve switching ensures that all internal, pneumatic components involved in the test implementation are reliably rinsed. The wastewater is collected in a plastic collection container via the drain tubing.



During validation tests, it has been shown clearly shown that the cleaning cycle even reliably removes artificial contamination (e.g., with BSA). It has also been shown that the rinsing process removes any sodium hydroxide used and that the drying phase runs efficiently.

4.4 Automatic Recognition of Non-Connected Filter Housings

Background Information
In most cases, self-closing quick couplings are used for the necessary connections between test device and filter housing. If, in error, a filter housing is not connected correctly, the self-closing quick coupling of the test hose will result in only the test hose itself being pressurized and not the filter housing to be tested. In such a situation, the connected integrity tester will neither be able to measure any significant diffusion nor any non-linearity in the air flow in order to recognize a bubble point (BP). However, it is the case that only a maximum diffusion rate and|or minimum BP are defined as test criteria. Both criteria (max. diffusion is not exceeded and the BP is above the min. BP) can thus be fulfilled if only the test hose is tested. Consequently, an integrity test without a correctly connected filter housing can result in a passed test which, fatally, means a false positive test result.

An experienced user could immediately identify the unrealistically flat curve progression of the diffusion or BP test. However, against a background of daily routine, there remains a reliability risk. At this point, we should describe a function of the Sartocheck 4 (from version 2.03) and the Sartocheck 4 plus, which guarantees to reliably identify such errors in test setup.

How Can False Positive Test Results Be Avoided?
Sartocheck 4 plus (and Sartocheck 4 Version 2.13) allows the definition of three additional, independent parameters. When these new parameters are used correctly, situations such as unconnected filter housings, defective quick couplings or non-removed protective caps are reliably identified.

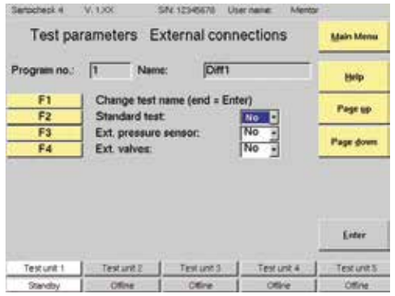


Figure 29: In order to activate the three reliability parameters, a non-standard test must be programmed.

To maintain the recognized procedure of test evaluation (differentiation between passed and non-passed test), the underlying criteria for this have not been altered. However, if an incorrect test setup is detected by the device, the following error message is shown on the display and marked in red on the printout.

Figure 30: Displayed warning message of the Sartocheck 4 | Sartocheck 4 plus when a defective test design is detected.

The following parameters can be defined by the user:

- Min. diffusion
(for diffusion test, WIT, WFT)
This parameter describes a realistic minimal diffusion rate to be expected when testing a certain filter. Each connected filter will lead to a significant diffusion.

- Min. net volume
(for all tests with automatic volume determination)
This parameter describes the minimum net volume of the housing connected to the test device (test hose plus upstream volume of filter housing|capsule). As soon as a filter is connected to the test hose of the Sartocheck, this results in an increase in the volume. An unconnected filter or filter housing results in an excessively small net volume of the system, which can be detected by programming this parameter.

- Min. flow with BP test (only for BP test)
This parameter describes the minimum diffusive flow shortly before reaching the max. BP. An unconnected filter will result in an extremely small flow and can thus be clearly detected.

The underlying concept between these three reliability parameters is the fact that a significant diffusion as well as a larger net volume must be present as soon as any filter is connected. To reliably cover all possible test scenarios, all three parameters are necessary.

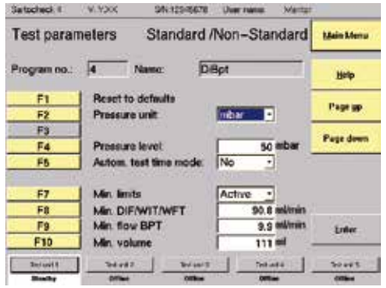


Figure 31: This screen view shows where in the menu of the device the additional reliability parameters are defined.

Programming the Parameters

The three parameters described above have been defined in order to detect incorrectly connected filters during the integrity test with a Sartocheck. Therefore, these parameters must be sensibly programmed so that a definite distinction can be made between the two states (filter connected v filter not connected). Since the occurring diffusion rates and net volumes are dependent on the individual test setup, no universal values can be recommended for the reliability parameters. However, the following procedure can be recommended:

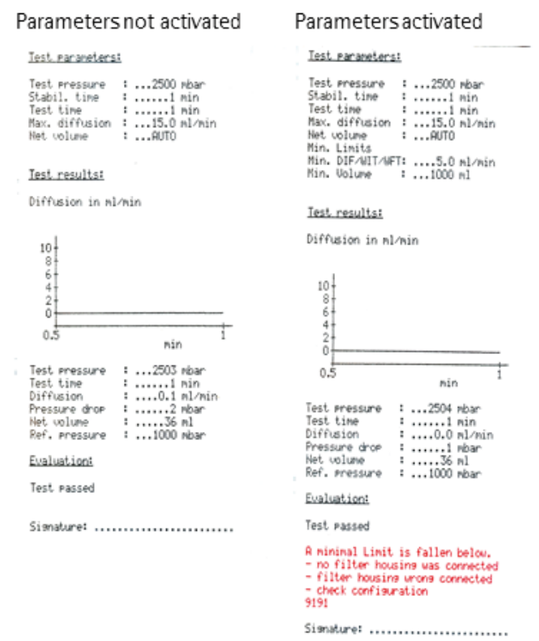
Minimum diffusion:
Establish the "blind diffusion" (in ml/min.) with no filter connected (typically 0 ml/min.). The established diffusion can be labeled as the minimum diffusion value. This value is always low (typically 3 or 4 ml/min.) and should never be too close to the actual test result in order to avoid false error messages.

Minimum flow in the BP test:
This parameter corresponds to the air flow directly before the maximum bubble point is reached. Thus, it is recommended that ½ max. BP, for example, is programmed for this parameter.

Minimum net volume:
This value should be programmed with approx. 80-90% of the actual volume with a correctly installed filter. Example: if the Sartocheck measures a volume of 1,000 mL with a correctly connected filter housing, then a minimum net volume of 800 mL, for example, can be programmed. The only condition for the programming is that the programmed value lies between the actual value and the value with no filter connected. This is typically a very wide range, making programming anywhere between the two extremes very easy.

The Sartocheck 4|Sartocheck 4 plus enables this reliability parameter to be activated and de-activated. However, an unconnected filter cartridge housing can only be reliably detected with the reliability parameter activated.

Figure 32: Printout from the Sartocheck 4 with deactivated (left) and activated (right) reliability parameters. A diffusion test has been implemented with a disconnected test hose. This status was only recognized by the device with activated reliability parameters. When one of the criteria is fulfilled, a corresponding warning message is displayed in red and printed.



4.5 The Barcode Scanner: Additional Reliability and Higher Efficiency in Data Input

A barcode scanner is ultimately merely another option for data input into the device along with a keyboard and touch screen. Reading the information encoded in 1D barcodes or 2D data matrix codes offers the facility for fast and, above all, error-free reading of certain data. Today, all main suppliers of integrity testers offer the option to connect a barcode scanner.

The Sartocheck 4 plus integrity tester offers yet further options: the Sartorius Stedim Biotech filter cartridges are marked with a laser inscription near the top adapter. This contains information such as filter type, lot number and individual item number and pore size in plain text. In addition, there is a data matrix code on the filter cartridge that can be directly read using the bar-code scanner of the Sartocheck 4 plus. Upon reading, the device recognizes the code as a Sartorius Code and subsequently inserts the coded information automatically in the comment fields provided. This avoids reading and input errors, particularly with the lot number.

Intelligent processing also means that, when the relevant code is read, the appropriate test program(s) is/are automatically selected from the program database. In view of the fact that such a test device can store up to 250 different test programs, the advantages of automatic program selection are self-evident. Beside the time savings, reliability is the most significant aspect. Incorrect program selections can be reliably avoided via this procedure.

Figure 33: The Sartocheck 4 plus filter integrity tester provides a connection facility for a barcode scanner. This enables the coded information – e.g., data matrix code on the filter cartridges – to be read and automatically transferred to the test device. An intelligent data processing allows the automatic selection of the correct test program from the internal program database.

5. Time is Money: Options for Shortening the Test Time

However, performing filter integrity tests costs time. Indeed, it can take a good 20 to 30 minutes to perform a diffusion test, bubble point test or water intrusion test. For this purpose, it appears desirable to come up with rational options for shortening the test time provided that the proper test results can still be guaranteed.

5.1 What Factors Determine the Test Time?

An integrity test is made up of several different test phases. Generally speaking, a distinction can be made between a stabilization phase and the test phase. As the name suggests, the purpose of the stabilization phase is to achieve stable test conditions. Particularly when pressure is applied at the beginning of the test, e.g., when the tester is pressurized to a programmed test pressure of 2500 mbar, a thermodynamic process takes place: the filter cartridge housing to be tested fills with the test gas (e.g., compressed air) - a process that per se inputs energy into the system. This energy input can be quantified directly in that a temperature increase of as much as several degrees Celsius is measured. After pressurization, the temperature drops again or temperature compensation takes place between the filter cartridge housing and the environment. Since the volume in the filter cartridge housing remains constant, a drop in temperature in the gas-filled housing leads to a corresponding drop in pressure (pV=nRT). The integrity tester connected to this system cannot differentiate between a pressure drop caused by diffusion and a pressure drop caused by temperature fluctuations. As a result, a shortening of the recommended stabilization time is always associated with the risk of unstable conditions, which can significantly impair the test results. Therefore, it is fundamentally not recommended to substantially shorten the stabilization time just to save time.

In contrast to the stabilization phase, however, constant conditions prevail during the subsequent test phase. This creates several options for shortening the test time. These options will now be explained in greater detail.

5.2 Automatic Test Time Mode for Diffusion Test and Water Intrusion Test

During the diffusion test, the system is stabilized to a filter type-dependent test pressure and then the occurring diffusion is measured for a certain amount of time (typically 10 min) at an unchanged pressure level. Under very stable conditions, the measurement may not notably change throughout the entire test. Moreover, it has been found that the real test values obtained on filter cartridges are usually far within the permissible limit values. This fact begs the question as to whether it is really necessary to wait until the end of the test under stable conditions (i.e., stable measurements) when it is already clear much earlier that the measured filter cartridges will pass the test.

The following example serves to illustrate this point: a programmed diffusion test was used to measure a 10" filter cartridge, type Sartopore 2 (0.2 µm) at a test pressure of 2500 mbar. Just briefly after the test started, the diffusion stabilized to a mean of 7.3 ml/min (see Figure 1). The measured values showed a very low spread; the measurement was extremely stable right from the outset. Lastly, one had to wait for the 10-minute test phase to be over to obtain an already confirmed positive test result.

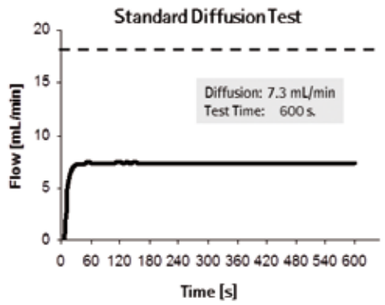


Figure 34: A diffusion test of a 10" Sartopore 2 (0.2 µm) filter cartridge with programmed standard parameters (10 min test time at 2,500 mbar) produces a result of 7.3 ml/min. Notably, the test result was already clear from the start of the test and was clearly within the tolerances for the limit value (max. diffusion). In such a situation, one must ask whether it is really necessary to wait for the entire 10 minutes test time.

By considering a logical stability criterion, however, the test time can be significantly shortened. The Sartocheck 4 plus features an option that allows the activation of this kind of stability criterion. When the automatic test time mode is activated, a test signal is registered as stable, when the mean variation of 10 consecutively measured values is within a defined band (±2%). Once this condition is met, the test terminates early and the device issues the test result "Test passed".

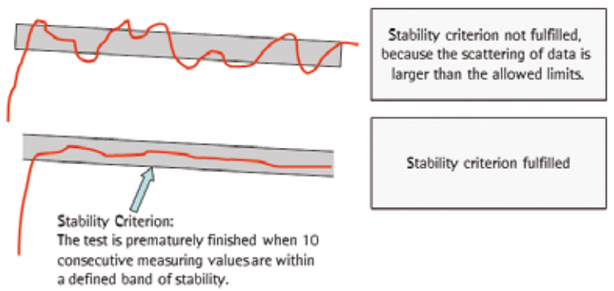
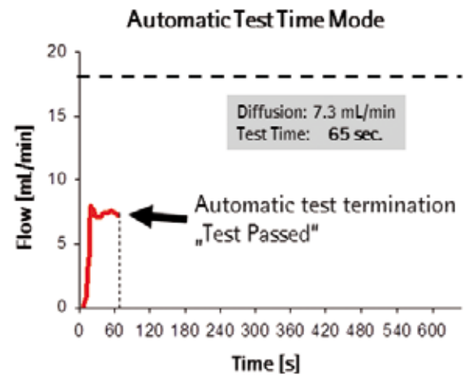


Figure 35: Schematic representation of how the stability criterion works in the automatic test time mode.

The following section describes how the diffusion test was repeated on the same filter cartridge using identical test parameters, but with the automatic test time mode activated. In the first trial run, the diffusion test took 10 minutes, whereas it was cancelled after just 65 seconds when run with the automatic test time mode activated (Figure 3). The test was evaluated as passed, also with a measured diffusion of 7.3 ml/min. This represents a shortening of the test time by 89 % with identical test results. However, the test result does not necessarily have to be identical. When the diffusion curve shows a decline, then obviously there will be a higher value at the beginning of the test than towards the end. The important thing in this regard – as mentioned above – is the large tolerance between the real test result and the relevant limit value. This fact demonstrates that shortening the test time still delivers accurate results.

Figure 36: Diffusion test performed on a Sartopore 2-type filter cartridge (identical cartridge as in Fig. 1) with the automatic test time mode activated. The test was terminated successfully after just 65 seconds. This is equivalent to shortening the test time by 89%.



The printout and/or the file with the results indicates that the automatic test time mode was activated, making it instantly apparent to the reader.
The automatic test time mode can be used for the diffusion test, water intrusion test and water flow test types.

5.3 Bubble Point Test

Performing the bubble point (BP) test can also be very time-consuming. The automatic test time mode function is not appropriate for the BP test because its test conditions per se are not really ever stable. Rather, the BP test involves elevating the pressure incrementally until the bubble point criterion has been reached (disproportionate increase in airflow rate). Despite the above, two different means can be employed when performing the BP test that likewise help to considerably reduce the test time.

5.3.1 Programming the Maximum Bubble Point

The most important parameter for evaluating the test is the minimum bubble point. The BP test counts as passed as soon as the pressure levels have reached this minimum pressure without the tester detecting the bubble point. As described above for the diffusion test, the real test value is usually far removed from the pre-defined limit value (min. BP). This means that sometimes many pressure levels are reached between the min. BP and the actual BP. Because every pressure level takes up additional time, one can ask whether this information about the actual bubble point is really so imperative. Whenever this is not the case, the test time can be significantly shortened by programming the maximum BP lower. The following example of the tested Sartopore 2 cartridge aims to provide a clear illustration of this:

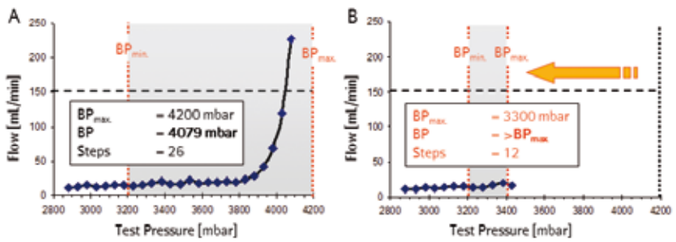


Figure 37: Shortening the test time on the bubble point test by programming lower values for the maximum bubble point. The diamonds represent the individual measured values (pressure levels).

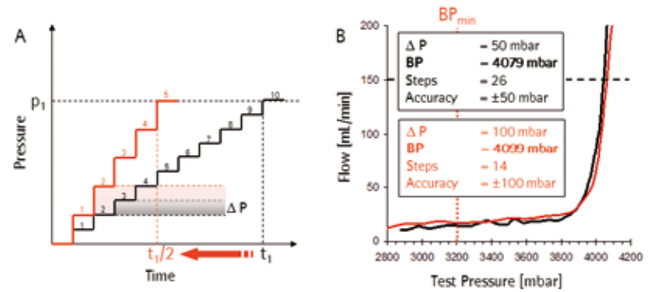
In the first case, a BP test was programmed with a BPmin of 3,200 mbar and a BPmax of 4,200 mbar. The bubble point was detected at 4,079 mbar, which required a total of 26 pressure levels. In the second case, the BPmax was set low, namely at 3,400 mbar. This means that the Sartocheck 4 plus terminates the test as soon as this BPmax is reached and displays the message "Test Passed, BP>BPmin". As explained above, the only criterion for evaluating the test is the minimum BP, whereas the maximum BP is absolutely irrelevant for the test evaluation. Ultimately, the maximum BP only reflects the maximum test pressure at which the test is terminated (successfully). In this example, only 12 pressure levels were required; this converts to a reduction in the required test time by over 50%.

It should be noted, however, that any information about the actual bubble point is lost using this method. Here, it is up to the users to decide whether this method is suitable to meet their individual needs or satisfy their internal operating procedures. At any rate, it is legitimate as far as the regulatory requirements are concerned.

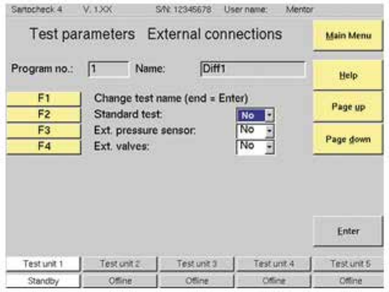
5.3.2 Changing the Pressure Levels

As mentioned at the beginning, during the BP test, the test pressure is elevated incrementally until the criterion for BP detection has been reached. In this context, it becomes obvious that it will take longer when the pressure is regulated in smaller increments than if larger increments were used. By doubling the pressure levels, it only takes half the test time to achieve the given target pressure.

Figure 38: Changing the pressure levels on the BP test. By doubling the pressure level increment from 50 to 100 mbar, the respectively required pressure is reached in half the test time. This fact was also confirmed by testing a Sartopore 2 cartridge. The effect of this change on the test result was negligible.



The standard pressure level setting programmed on Sartocheck testers is 50 mbar. If a non-standard test is selected when programming a test (BP test), the pressure level can be selected freely. Whenever that is the case, please note that the measuring accuracy of ±50 mbar stated in the unit's technical specifications for the BP test only applies to the pre-set pressure levels of 50 mbar. If, for example, the pressure levels are increased to 100 mbar, then the measuring accuracy is also reduced accordingly to ±100 mbar. As illustrated in the example above, this lower measuring accuracy is adequate in most of the cases. Here, with pressure levels of 50 mbar, the BP will be found at 4,079 mbar and, with pressure levels of 100 mbar, the BP will be found at 4,099. In both cases, this is far is far in excess of the permitted limit value and appears acceptable.



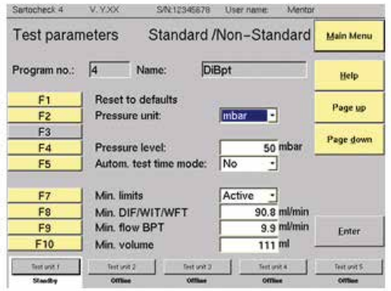


Figure 39: To activate the different methods for shortening the test time, i.e., using the automatic test time mode for the diffusion test and WIT or changing the pressure levels for the BP test, it is necessary to select a non-standard test. Next, a menu item appears in a window where both the automatic test time mode can be activated and the corresponding BP test pressure level can also be set.

6. Special Cases in Integrity Testing

6.1 Problem of Testing Multiround Housings

The methods described above for filter integrity testing are excellently suited to testing individual filter cartridges or capsules. However, there is frequently a requirement to test multiround housing (e.g., 3 x 30" or larger). In this case, there is a fundamental problem with all test methods, namely the potential masking of defective filter cartridges by intact filter cartridges. This is explained briefly below.

By way of example, we have three filter cartridges installed in one housing. The specification allows a maximum diffusion of 20 ml/min per filter cartridge. A linear projection of this would produce a total limit value of 60 ml/min. However, it should be noted that the actual diffusion value is, in most cases, significantly different from the limit value (cf. Fig 22, BCT). As a result, it is quite possible that a filter cartridge that is only slightly defective (e.g., diffusion = 24 ml/min) will be masked by the other two cartridges (e.g., both 15 ml/min) (total diffusion = 24+15+15= 54 ml/min). This means that the filter housing with the three installed filter cartridges is successfully tested ("test passed"), even though one of the three cartridges is defective.

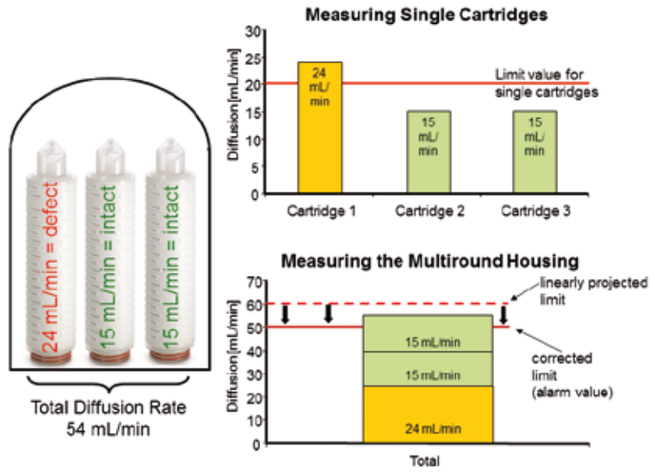


Figure 40: Schematic representation of the difficulties in the integrity testing of multiround housing. Owing to the difference between the actual test values and the maximum permitted limit value, there is a risk that slightly defective filter cartridges could be masked by correspondingly intact cartridges. With a corresponding multiplication of the individual limit values, the test is considered passed even though one individual cartridge exceeds the permitted limit value. This can be prevented in many cases through adapted limit values or the definition of so-called alarm values.

This example underlines that, when testing multiple housing, there is a risk that it will not be possible to detect a non-intact filter cartridge. There are a number of principle options to overcome this problem:

6.1.1 Separate Testing of the Individual Filters

The individual testing of the filters is clearly the most reliable method. Here, the individual filter cartridges must be removed from the multiple housing and installed in a separate individual housing. A standard integrity test is then implemented, observing the limit values defined by the manufacturers. However, separate testing is time-consuming and therefore not desirable in all cases.

6.1.2 Diffusion Test – Customizing Limit Values

For the purposes of risk minimization, specially customized limit values or alarm values can also be used instead of scaling up individual values in linear fashion. The following procedure is recommended when doing this:

It is necessary to determine the value for the average actual diffusion. This is normally significantly lower than the regular limit value (Diffmax.). With the proviso that one would also like to detect filter cartridges that lie slightly above the permitted limit, for one cartridge the maximum permitted diffusion should be observed and, for the remaining filter cartridges, the actual average diffusion should be observed. The result then represents the customized total limit value or alarm value of the multiple housing (or the contained filter cartridges).

$$\text{Diff}_{\text{max multiple}} = (n-1) \cdot \text{Diff}_{\text{actual}} + \text{Diff}_{\text{max}} \cdot \beta$$

n = Number of filter cartridges in the multiple housing (Example: 3)
$\text{Diff}_{\text{max multiple}}$ = Diffusion limit value for the total cartridge
$\text{Diff}_{\text{actual}}$ = Average, actual diffusion of the individual filter cartridges (Example: 15 ml/min)
Diff_{max} = Diffusion limit value for the individual filter cartridges (Example: 20 ml/min)
β = Reliability factor

The assumptions in the example above would produce the following:

$$\text{Diff}_{\text{max multiple}} = (2 \cdot 15 \text{ ml/min}) + 20 \text{ ml/min}$$
$$= 50 \text{ ml/min}$$

Thus, a customized limit value is produced of 50 ml/min instead of 60 ml/min. For the example shown above, this would mean that the test of the multiple housing would also not be passed (Fig.40). It is highly sensible that this customized limit value is regarded as an alarm value. When the alarm value is exceeded, individual testing of the filter cartridges can be implemented, for example, to ensure absolute reliability.

However, it is re-iterated at this point that this is a method of risk minimization. With customized limit values too, there will not be total reliability (100% detection of defective filters). Here, it is the responsibility of the user to create appropriate risk identification where required, and to use this to make a decision regarding individual testing vs. limit value customization.

Furthermore, when the criteria is set very narrow, the probability of false negative tests increases. This should naturally also be avoided and there must a sensible compromise between maximum reliability and minimal failure rate (false negative tests).

6.1.3 Customization for the Bubble Point Test

As described earlier, special parameters (e.g., A1, A2 values) are used to detect the bubble point unequivocally via a disproportionate increase in the pressure-diffusion curve. The Sartocheck test devices enable three different test categories to be set up, via which the A1 and A2 values can be ultimately adapted to the occurring total diffusion.

When programming the "special systems" test category, 30" filter cartridges can be used and smaller multi-branch systems tested. When testing larger systems, limitations are encountered. It is therefore recommended that a customer-specific BP test is selected that allows the A1 and A2 values to be freely programmed. One is not then limited to the three pre-defined test categories and can adapt the values to the filter surface in a targeted manner.

It addition, it can also be sensible to adapt additional parameters. However, such parameters should only be adapted by persons who can precisely evaluate the effects of doing so. Such parameters are therefore only accessible to Sartorius service personnel (special password). Please contact the Sartorius application specialists should this be required.

Also when implementing the bubble point test, please remember that reliability in terms of detecting defective filter cartridges diminishes with the number of filters. Owing to the different progression of the BP curves of the individual elements, when parallel testing several filters in a housing, curve progressions may be obtained that show a less clear or displaced disproportionate increase. As discussed for the Diffusion Test, also for the Bubble Point Test there is a certain risk for masking other defective filter elements when tested in a multiround housing.

6.2 Implementation of a Pre-Use Test after Completion of Sterilization

The regulatory requirements regarding implementation of pre-use and post-use integrity tests have already been discussed (see Chapter 2). It is important in this context to note that particularly the integrity test pre-use and after completing sterilization can lead to problems in practical implementation.

As thermal sterilization represents a considerable load for the plastic materials, integrity testing is required to some extent by regulations after sterilization. Where a test is to be carried out after sterilization has already been completed, it must naturally be ensured that sterility is not jeopardized by the test procedure. In principle, the integrity test is implemented on the upstream side of the filter. In this respect, the membrane filter is always available as a sterile barrier. Thus it is not absolutely necessary to work in completely sterile conditions. However, it is certainly advisable to keep the potential bacterial load as low as possible.

In addition, sterile filters can be inserted between the test line and filter housing so that sterility on the upstream side is not jeopardized by the integrity testing. The inserted air filters have no influence at all on the test results (cf. Chapter 4.2). The use of external vent valves (cf. Chapter 4.1) also contributes to further increases in reliability. Through the use of the special cleaning function of the Sartocheck devices (cf. Chapter 4.3), the device interior can also be reliably sanitized. Thus it can be avoided that particles are forced out of the device during integrity testing onto the filter which is to be tested.

Before the actual test, the filter must be reliably rinsed and wetted. The rinsing water used must be collected under sterile conditions. After wetting is complete, the actual integrity test is commenced. The test procedure uses a defined differential pressure applied via the membrane (upstream = test pressure, downstream = atmospheric pressure). This is why it is essential that the downstream side of the filter is not closed. Otherwise the pressure on the downstream side would increase during the test (owing to the gas diffusion through the membrane) and thus the differential pressure would reduce. This could give false positive results. The already complete sterilization also naturally prohibits direct connection with the atmosphere. Therefore, it must either be ensured that the downstream volume is sufficiently large to prevent a significant pressure increase. Or, if this is not possible, sterile air filters are used to enable a sterile connection with the atmosphere and prevent a pressure build-up.

6.3 Integrity Testing of Filters on Bags

The trend in the biopharmaceutical industry towards single-use products also has consequences in respect of the integrity testing of the filters used there. When using gamma-sterilized filter-bag assemblies, the sterile filters must be implemented without influencing the integrity test of the sterile single-use bags.

The difference begins as early as the preparation, with the moistening of the filter used. When rinsing, the wetting fluid is rinsed through the filter and must be collected separately in so-called rinsing bags. The volume of the rinsing bag must be designed such that a second rinsing/moistening can be carried out if for example a first integrity test failed. An additional vent bag can be used to collect the air/fluid upon opening the vent valves.

Upon testing, it is also important that there is no significant pressure increase on the downstream side of the filter. This can only be achieved with a sufficiently large bag volume or through the use of hydrophobic, gamma-sterilizable air filters.

An example of a possible solution is described below. Please note that this is just an example. Concrete cases may require different technical solutions.

The following components are necessary in principle for such a testable filter-bag assembly:

1. a gamma-sterilizable sterile filter for the filtration of the medium used ②
2. a gamma-sterilizable bag for holding the filtrate ⑩
3. a gamma-sterilizable bag for holding the rinsing solution used ⑨ and two vent filters ⑧ of the Midisart BV variety (to avoid overpressure)
4. a bag for holding air/fluid upon exit from the vent valves of the sterile filter capsules ⑤
5. a gamma-sterilizable air filter for the connection line to the integrity tester ④
6. Clamp connections to disconnect individual components from the system ①③⑥⑦

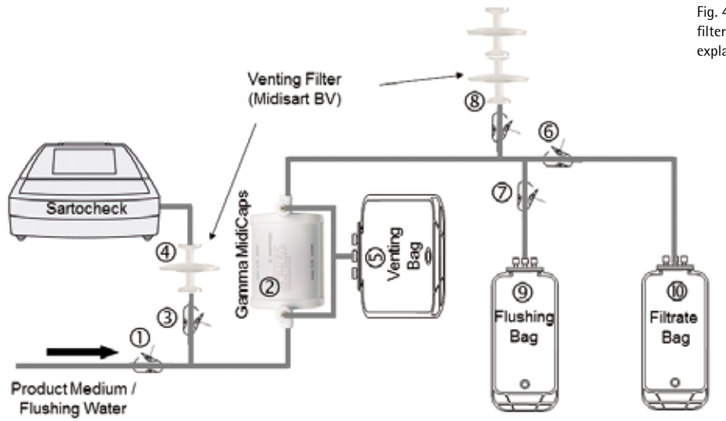


Fig. 41: Schematic design for testing of sterile filters installed on a bag system. More detailed explanations in the text.

An integrity test can now proceed as follows:

1. Rinsing the sterile filter capsule:
   - Collect the rinsing fluid in the rinsing bag ⑨.
   - The filtrate bag ⑩ and line to the integrity tester must be disconnected ③.
   - The connection to the rinsing bag must be open ⑦.
   - Remove the remaining air in the vent bag ⑤ from the capsule by briefly opening the vent valves.

2. Integrity testing of the sterile filter capsule:
   - Connect the test device to the Midisart BV ④ as a sterile barrier between the test hose and filter.
   - Close the connection to the filtrate bag ⑥.
   - Close the connection to the feed (product supply) ①.
   - Ensure that vent valves on capsule are completely closed.
   - Ensure that the connection ⑦ to the rinsing bag with vent filter is open.
   - Start the integrity test.

6.4 Product and Process-Specific Integrity Tests

In general, it must be ensured that the manufacturer specifications in respect of limit values for the different integrity test methods always relate to standard conditions. In this context, standard conditions always mean the...

- Use of water as a wetting medium
- Use of compressed air as a test gas
- Test implementation at room temperature (approx. 21°C.)

Differences may lead to significant alterations of the test results. Where these three basic conditions do not apply, there are two options. On the one hand, an attempt can be made to create standard conditions. However, there will always be cases where this is not possible.

If it is not possible to create standard conditions, separate limit values must be determined or validated. In a simple case, for example, nitrogen is used instead of compressed air as a test gas. Here, the correction factor is known ($Diff_{N2}$ = $Diff_{air}$ x 0.82).

It becomes more complicated when it is not possible to use water as a wetting medium. This can be the case when the product to be filtered can only be rinsed from the membrane with difficulty or not at all. In such a case, it must be assumed that this product residue could result in an adverse effect on the membrane (e.g., altered surface tension) and test results. Before very large volumes of rinsing water are used, unique so-called product-specific limit values can be determined. As a result, a newly defined limit value is obtained which is only valid when using that particular product for wetting. It is also necessary in such case that the limit value determined correlates with the results of the bacterial challenge test.

In principle, the procedure is as follows:
Three filters from different lots are wet with water, diffusion and bubble points tests are implemented and the results documented. The filters are then dried and wet with the product which is to be tested. The deviations between the water-based and product-based bubble point results can be used to correct the limit value accordingly. In addition, the test pressure to be applied for the diffusion test can be determined from the newly determined bubble point. Applying the determined test pressure, the maximum allowable diffusion rate is defined. For this approach the results are related to the water-based results and this ratio is used for the correction of the limit value.

As one of its services, the Sartorius Stedim Biotech validation service can help the user by determining product-specific integrity test limit values for them.

7. General Requirements for Automatic Filter Integrity Testers

7.1 21CFR Part 11 – What Is Relevant Here?

The regulation 21CFR Part 11 (part 11 of Title 21 of the Code of Federal 19 Regulations; Electronic Records; Electronic Signatures, 1997) describes requirements of electronic equipment with regard to the compilation and management of electronic files. The manufacturers of such equipment must take appropriate measures to ensure that subsequent manipulation of such electronic files is practically prevented. In this context, it must also be ensured that unauthorized access to the system is excluded. This can be implemented via password-protected access to the equipment, for example, and through the allocation of specific user rights.

Without going into the details of the individual requirements, it should be noted that this regulation only applies to equipment with an electronic result memory. Currently, it is still permitted to operate equipment that does not fulfill the requirements of 21CFR11. However, this only applies to equipment with no such memory.

The Sartocheck range of equipment features purely paper-based models (Sartocheck 3 plus). As previously mentioned, these devices do not fulfill the requirements of 21CFR11. However, it is permitted to operate these devices because no test results are stored electronically. Instead, the test results are documented via paper printout and archived.

The Sartocheck 4 plus premium model, on the other hand, uses an electronic result memory and fulfills all relevant requirements of 21CFR11.

7.2 GAMP – What Is Behind this?

The GAMP (**G**ood **A**utomated **M**anufacturing **P**ractice) guidelines were published in 1995 by the British Pharmaceutical Industry Computer Validation Forum. The guidelines regulate the validation of computer-supported systems in the pharmaceutical sector (manufacturers and suppliers). A number of versions have been published over the years, the latest version being GAMP 5 which was published in 2008. It should be noted that these are recognized guidelines with no legal obligation.

Computer-supported systems used in the pharmaceutical sector are now normally developed in accordance with the GAMP version valid at the time of development in a validated environment. This is ultimately a quality feature and ensures the principal suitability (in terms of stability and data security) of the equipment for use in the pharmaceutical sector. The devices in the current Sartocheck range have been developed and validated in accordance with current GAMP guidelines.

8. Most Frequent Sources of Error in Integrity Testing

The user has to interpret and investigate results when there are problems with the integrity testing. Failing an integrity test is always a problem because this is often an important release criteria for the product lot and, therefore, may well result in significant financial damages.

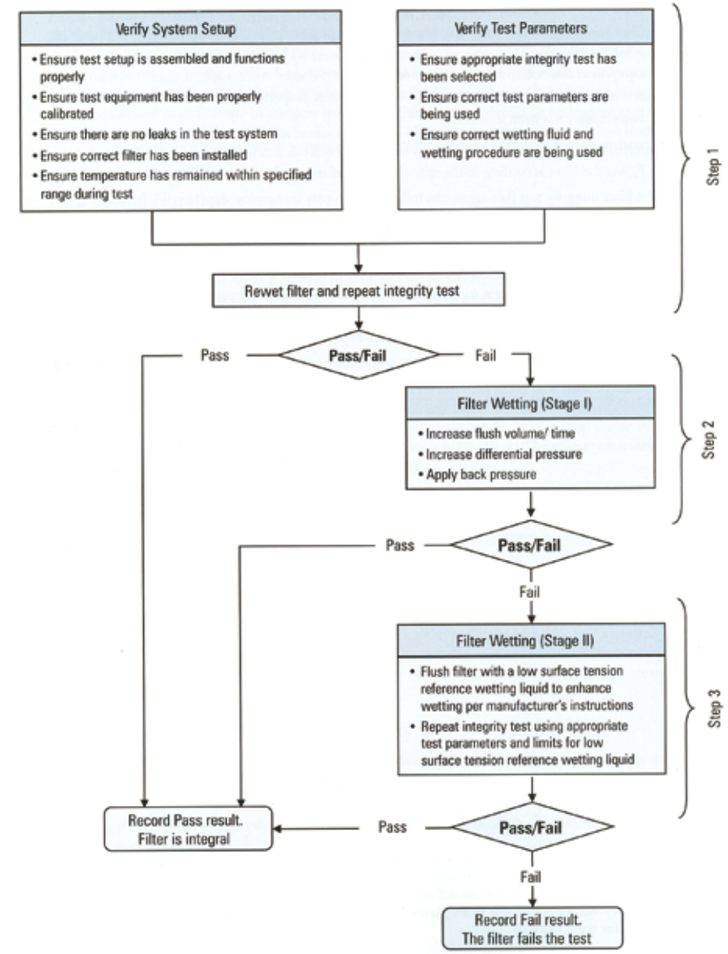
In the worst case, the filter is actually defective meaning that a sterile filtrate cannot be guaranteed. In such a case, the filtration can be repeated if the valid SOPs and process validation allow this procedure. If this is not possible, the entire lot in question must be rejected.

However, it should be noted that in most cases, the filter itself is intact. It is far more likely that a change in conditions or other reasons are responsible for a negative test result. The main causes are listed below and corresponding methods of resolution outlined.

False negative results can be immediately identified (i.e., filter cartridges that are actually intact but still fail the test). Far more dangerous and far more significant are false positive tests, i.e., defective filter cartridges that still obtain a positive test result. This is a major safety risk and must be avoided in all circumstances.

Technical Report No. 26 of the PDA gives a clear operating procedure for when failed integrity tests occur.

Figure 42: Schematic decision tree for non-passing test results. This representation illustrates the steps to be taken for error detection. (From PDA Technical Report No. 26)



The most frequent and important causes of error are discussed below.

8.1 Wetting Problems

Most of the of problems are attributable to wetting difficulties. This means that the pores are not completely filled with water. Pores which are not completely filled allow considerably more air to diffuse through them than completely wetted ones. This results in the diffusion test exceeding the permitted limit value and the measured bubble point possibly being lower than the minimum BP.

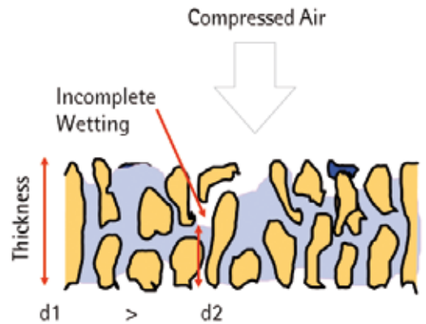


Figure 43: An incomplete wetting of the filter membrane can result in a considerably increased diffusion rate. The reason for this is that the layer thickness in the area of the incomplete wetting can be considerably thinner. This means that considerably more air molecules can pass through the membrane at a time. Accordingly, integrity tests can fail even though the membrane in itself is intact.

Due to the frequency of wetting problems, when an integrity test is failed, wetting should always be the first step. The wetting conditions recommended by the manufacturers should be observed.

8.2 Temperature Problems

A basic requirement for the reliable implementation of integrity tests is the presence of stable environmental conditions. Constant temperature is particularly important because temperature fluctuations result in corresponding pressure fluctuations in the filter housing owing to the relationship between temperature, pressure and volume. This is then falsely evaluated as diffusion or water intrusion. The general gas law applies:

$p \cdot V = n \cdot R \cdot T$

p = Gas pressure
V= Gas volume
n = Mole number of the gas
R = Gas-specific constants
T = Temperature in Kelvin

The problem becomes even more complex because, for example, the surface tension is also dependent on the wetting fluid, so a potential temperature change can have several effects.

Depending on the direction of the temperature change, it may be that the test results turn out to be too high or too low. One scenario (temperature drop) results in larger diffusion values, causing the tests to fail (false negative test results). This should naturally be avoided because resources are needlessly wasted (repeated test etc.). The situation is more critical, however, with temperature increases during the test. An increasing temperature will lead to a pressure increase in the housing and, as a result, can mask high diffusion values (defective filter cartridge). This can also result in a defective filter cartridge actually passing a test successfully. Evidently, this must absolutely be avoided for safety reasons. The current Sartocheck devices detect a pressure increase and issue a corresponding error message. However, small changes can still partially offset the diffusion and influence the test result.

In principle, it can be established that integrity tests can also be implemented at higher or lower temperatures. However, stable conditions are essential, i.e., no temperature fluctuations during the measuring phase. When measuring under lower or higher temperatures, however, the limit values must be adapted accordingly (calculation of process-related integrity test values).

Particularly with integrity testing after steam sterilization of the filter cartridges, it is essential to ensure that the steam-sterilized material has been restored to ambient temperature.

8.3 Product Residues on the Membrane

In practice, there are repeatedly cases where, after filtration is complete, product residues are not completely rinsed from the membrane. In such cases it is worth determining **product-specific integrity test limit values**. In such a case, the filter is not rinsed with water and wet after filtration. Instead, the original product is used for this. Depending on the properties of the product, the integrity test values can alter significantly from water meaning that a new limit value must be calculated. Technical Report No. 26 clearly recommends that product-specific values are determined using corresponding tests and not merely calculated. The Sartorius Stedim Biotech validation service would be happy to assist you with determining such product or process-specific limit values.
Product residues can also cause problems with the water intrusion test. It is quite possible that surface-active substances (e.g. Tween 80) make contact with the membrane surface. Depending on the substance, this can influence the hydrophobicity of the PTFE membrane. If a water intrusion test is then

implemented, the water intrusion can be too great, which then results in a failed integrity test. In extreme cases, this results in water actually passing through the hydrophobic PTFE membrane and water exiting onto the sterile side of the filter. This means that the WIT test is not passed, even though the filter membrane is intact. In some cases, it is completely impossible to rinse certain product residues from the membrane. In such cases, the WIT is not the correct method. In this situation, it is therefore recommended to implement an IPA-based test (see Chapter 3.8.1).

8.4 Leaks

All integrity methods discussed above are based upon the pressure-drop method with devices from the Sartocheck family. This means of course that any leaks in the system cause corresponding pressure losses which the device then evaluates as increased diffusion | water intrusion, resulting in an increase in negative test results.

When negative test results are obtained, a possible leak should always therefore be considered as a cause. Searching for a leak can be difficult. This should always, therefore, be conducted systematically to find the solution as quickly as possible:

a) Leaks on the test device
The valves and couplings on the test device or quick couplings on the test hose used can have leaks. To check this, it is best to start a pressure-drop test with a test hose connected (without filter). The quick coupling used is self-closing, meaning that the pressure-drop test checks the device pneumatics plus the connected hose. With a test pressure of 1,000 mbar, for example, there should be no relevant pressure drop with a test time of 1 or 2 minutes ($\Delta P \leq ½$ mbar/min). If the test is passed successfully, the device and test hose can be excluded as causes of the problem.

b) Leaks on the housing
The quickest way to differentiate between a possible defective filter cartridge and a leak on the housing is to use a blind plug instead of a filter cartridge. Again, a pressure-drop test is recommended, which should find no pressure drop in a leak-tight system. If, however, there is a pressure loss, all possible causes must be checked (seal rings, valves, quick couplings, etc.). If the housing is seal-tight, the filter cartridge itself is the likely cause (i.e., defective filter).

8.5 Too Small or Too Great a Net Volume

Test devices such as the Sartocheck 4 plus can be used for the integrity testing of multiple housings down to syringe filters. As described above, the pressure change which is measured results from diffusion at the membrane. The pressure change can be too small if, for example, a very large net volume is used with a very small membrane. This can result in realistic diffusion values causing a pressure drop, which is on the detection limit of the sensor used. In such a case, the volume must be reduced accordingly.

If, conversely, the volume is too small, relatively low diffusion rates cause large pressure changes which can then be difficult to regulate. In such a case, the net volume must be increased somewhat in order to obtain sensible measure values.

Sartocheck enables either the input of known volume values or the automatic measurement of an unknown net volume. Using an incorrect volume value during test programming can have significant effects on the test result. One should therefore check that the net volume entered is correct.

8.6 Other Test Gas

All limit values specified always relate to standard conditions, i.e., a temperature of 20° Celsius, pure water as a wetting medium and compressed air as a test gas. It is quite possible to use another gas (e.g., nitrogen) for integrity testing. In such cases, it must be ensured that the limit value for compressed air is adjusted for the diffusion test according to the test gas:

$Diff_{N2} = 0.82 \cdot Diff_{air}$

The bubble point test and water intrusion test are independent of the test gas used. In the bubble point test, the pressure is measured at which the water is completely pressed out from the first pores and the measurement curve takes a non-linear progression (disproportionate increase). This does not alter even if another test gas is used.

In the water intrusion test, the pressure drop in the gas compartment is used to measure the water volume entering the PTFE membrane. Here again, the test gas is not directly involved and, thus, has no effect on the test result.

9. Final Remark

The information compiled in this manual illustrates that the integrity testing of membrane filters is very simple in terms of the underlying principles. Ultimately, however, the interaction of several factors makes the matter more complex and makes troubleshooting far from trivial.

The principles explained in further detail on this subject and the solutions proposed can offer valuable basic approaches in individual cases. However, it is difficult or impossible to cover all possible causes of error. The objective of this manual is to highlight the most frequent avoidable errors, enabling the user to conduct daily routine work more reliably and without problems. The manufacturing process, technology used and type of installation can be so different that, in particular cases, concrete solutions can only be found on site. The Sartorius Stedim Biotech technical specialists are experienced in these matters and can help directly on site, so that the critical stage of integrity testing can be performed in a skilled and error-free manner.

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