Trouble Shooting Guide – Integrity Testing of Hydrophobic Filters

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1. Introduction

1.1. Scope

This technical troubleshooting document is applicable for Sartorius Sartofluor® GA, Sartofluor® LG and Sartopore® Air filters which all employ sterilizing-grade hydrophobic membranes.

1.2. Purpose of this document

This document is designed for trouble shooting after a filter integrity test has failed. The procedures described herein can be used for writing and improving SOPs for filter integrity tests. The document can also be used for training purposes.

1.3. Types of filter integrity tests

For hydrophobic membrane filters, three different integrity test methods are typically used to ensure filter integrity:

- WIT (Water Intrusion Test)
- Diffusion Test (after wetting with IPA/water 60/40 or 70/30 Vol. %)
- Bubble-Point Test (after wetting with IPA/water 60/40 or 70/30 Vol. %)

The choice of one of these filter integrity test methods depends largely upon what was processed with this filter prior to testing. The WIT only can be used if the membrane is clean and free of any hydrophilic spots or areas. Traces of oils, solvents or other contaminants on the membrane surface may lead to a false failed WIT.

If possible, the WIT should be used preferred, as this method is most sensitive and additionally verifies the hydrophobicity of the membrane. This is an important feature for reliable long-term retention in gas filtration e.g. for virus and phage retention.

The alcohol-wetted Diffusion and Bubble-Point Tests are much more robust for post-use testing because these tests do not rely upon the hydrophobicity of the membrane to yield a passing result.

2. General trouble shooting

Most of the filter integrity test failures apply to all the different types of integrity tests. The following addresses the common failure modes and how they can be troubleshot.

2.1. Leak-tightness of the test system

Filter integrity tests are developed to confirm the integrity of membrane filters. However, if there is air leakage somewhere within the system, the integrity test might fail, even though the filter membrane is integral. Essentially, leakage can manifest itself as an inordinately high rate of diffusional flow or water intrusion, because the filter integrity tester is not capable of differentiating between air leakages upstream of the membrane and air or water flow through the membrane itself. The following items should be checked to identify possible leakage:

- damaged seal(s) or gasket(s) within the filter, housing and all connections
- open valve(s) that should be completely closed during integrity testing
- leak-tightness of the tubing that connects the filter integrity tester to the filter being tested
- vent valves on filter of filter housing
- water level for WIT (full water envelopment of the filter membrane)

✓ Check the complete inlet side of the filtration system for leakages.
Additionally, inspect the tubing from the filter integrity tester to the filter housing.

Perform a pressure drop test on the Sartocheck® Filter Tester's inner pneumatics, tubing(s) and valves:
Connect the Sartocheck® outlet to the test tube. Create the following program using the following parameters (example: Sartocheck® 5 and 5 Plus Filter Tester):

- Diffusion test
- Number of samples: 1
- Advanced: no
- Volume determination: Measure
- Test pressure: acc. to the max. pressure resistance of piece to be tested, max. 6 bar
- Max. diffusion/sample: 0.5 mL/min.
- Stabilization time: 10 min.; Measurement time: 3 min.

Run the Sartocheck® program e.g. on the test tubing, housing with blind plug etc. Then try to close any identified leak.

2.2. Wrong test program or wrong filter

The test parameters must be entered correctly in the filter test program. In addition, the correct filter test program must be selected for the specific filter being tested.

Check if the correct filter test program was used?
Check if the correct filter was mounted for filtration?
Confirm if proper test parameters were used for the filter being tested.

Preventive: Use Sartocheck's® barcode scanner for confirmation of the correct filter and for selection of the corresponding filter test program. For product or filter-specific testing, a unique custom barcode can be created and linked to a specific filter test program.

2.3. Temperature influence

Typically, the integrity test limits are only valid within the range of 20 to 25°C. As all test methods and subsequent measurements are derived from pressure decay and pressure is directly influenced by temperature, the temperature of the filter system, the wetting fluids and the test gas itself must remain consistent throughout the test. Temperature shifts while performing an integrity test can have a major impact on the integrity test result. The recommendation is to keep the temperature of all contributing factors within the range of 22 ± 2°C.

Check if the ambient temperature is between 20 to 25°C?
Check if temperatures are ambient for the

- filtration system
- wetting liquid
- test gas

Check if there are other temperature sources which have an influence on the test:

- steam pipes
- direct sun light
- air conditioning systems

Keep the temperature during a re-test as stable as possible, the target is to limit the deviation within ± 2°C for Diffusion and Bubble Point testing and ± 1°C for WIT for all components involved in the integrity test.

2.4. Membrane test solutions (wetting liquids)

The integrity test values may vary if a different test solution is used. For test solutions which are mixtures comprising volatile solvents, i.e. IPA/water 60/40 Vol. %, the solvent content of the test solution potentially can change if the mixture is re-used and the volatile component is evaporating.
Also, the test solution used to wet the membrane can evaporate over time, resulting in a thinner wetting layer and correspondingly higher diffusion.
The time, in which the solvent evaporates, is mainly dependent on the nature (vapor pressure) of the wetting solution. Additionally, the temperature of the filter, the size of the filters up- and downstream volumes as well as the diameter of the opening from the downstream side to the environment have an influence.

Check the specification of the test solution (e.g. IPA 60:40 Vol.%).

Exchange the test solution in appropriate intervals. Solvents can remove product residues from membrane filters and be contaminated after use.

Re-wet the filter, esp. if there has been too much elapsed time between the initial wetting and the test itself, depending of the volatility of the solvent used.

2.5. Product residues on filter membrane

This applies only for post-use testing a filter element with possible residual substances on the membrane.

Typical causes of contamination of hydrophobic filters used for air and gas filtration:

- aerosolized product that migrates to the filter.
- cells, cell debris and the target molecule (e.g. mAb) transported by the gas flow to the membrane.
- condensate and oil within piping that migrates downstream to the filter
- disinfectants from sanitizing (e.g. originating from gloves or surfaces)

Typical contamination on hydrophobic filters used for liquid filtration (not applicable for Sartopore® Air):

- Residues of organic solvents
- Residues of oils
- Hydrophobic contaminants from a product stream
- Surfactants from the product stream

Check if volatile solvent is not evaporated after wetting.

Flush with IPA for cleaning the membrane prior to integrity testing. If IPA is not effective, use of a suitable compatible solvent.
Autoclave or SIP (cartridges only) the filter for removing product residues prior to integrity testing. Be aware this procedure causes thermal stress on the filter element.

Preventive: physically distance filters from possible sources of contamination or use a pre-filter to retain the contamination in front of the filter to be tested.

2.6. Volume measurement

Sartocheck® filter integrity testers use the measurement of pressure decay in conjunction with the net upstream volume to derive flow rate. The net volume can either be determined automatically or entered manually; however, it is imperative that the automatic volume measurement or manual volume entry be accurate, as any inaccuracy in the volume will manifest itself as an inaccuracy in the measured diffusion or water intrusion.

If automated volume measurement is activated, check if the value for the determined volume is 'typical' by comparing with earlier measurements.

If the net-volume was put in manually, check the input value and if there have been any changes to the filtration system since qualification of the net volume.

If the net-volume was put in manually, measure the net volume again by using a Sartocheck®. For net volumes above 14 Litres the external reference tank must be connected.

If the safety parameters are activated the measurement of the net volume can used for identification of faulty integrity test setups. This is available only for Sartocheck® 5 Plus and Sartocheck® 4 Plus Filter Testers.

2.7. Choice of the test gas

For Bubble Point testing, the test value is independent from the test gas used, as the test gas is used only to determine the pressure at which the largest pores are evacuated. The WIT is not dependent on the test gas used.
However, different test gases can have an influence on the Diffusion test value due to the solubility and diffusivity of those gasses in conjunction with the wetting solution. Example: If Nitrogen is used instead of compressed air for water-based test solutions, the test result will be reduced by a factor of 0.82.

To exclude the influence of a false measurement due to the test gas, check if the correct test gas was used for the Diffusion test.

Repeat the Diffusion test using the specified test gas. The standard test gas is compressed air.

2.8. Filter integrity test device

Filter integrity test instruments from manufacturers other than Sartorius can be used for all three integrity test methods, which can be applied to hydrophobic filters.

Sartocheck® 5 and Sartocheck® 5 Plus Filter Testers measure the integrity of filters by using pressure decay as the core measurement method that relies upon high-precision pressure transducers. Those pressure transducers must remain within a tight tolerance to yield accurate measurements. Out-of-tolerance excursions can result in inaccurate measurements, so annual calibration (and possible adjustment) of the pressure transducers using NIST-traceable reference pressure gauges is recommended.

To exclude the influence of an out of tolerance integrity tester, check the calibration. interval of the used test device.

Check if the pressure sensor works properly.

If available, use a different filter test device.

In the unlikely event of a pressure reading offset, during the annual calibration of the Sartocheck®, the Sartorius Service team can help you to calculate the impact on measured values.

3. Diffusion Test

3.1. Parameter setting

Hydrophobic filters can be Diffusion tested after wetting with IPA/water 60:40 (or 70:30) Vol. %. Other wetting agents are possible if validated.

Check if the filter was wetted completely.
Make sure that the filter housing or capsule was vented before flushing the filter.

Repeat filter flush prior to another filter test.

The temperature range during a Diffusion test has to be kept stable (± 2°C) at ambient temperature (20 – 25°C).

The Diffusion test requires as input parameters a stabilization time, test time and a maximum Diffusion test limit.
The stabilization time depends on the size of the test filter. For filters of the size 9 or smaller, a minimum stabilization time of 3 minutes is recommended, and for a single 5" to 30" filter, increase that time to 5 minutes. For testing of multi-round filter setups, please contact the Sartorius Tech Support team.

For the test time of single filters, 3 minutes are sufficient. Invoking the Auto Test Time feature can truncate that test time considerably if the measured diffusion value is below the maximum limit and the measured value is stable (not erratic or trending upward toward eventual failure). The IPA/water-based Diffusion test limits can be found in the respective filter validation guides.

If the pressure drop for a passing filter consistently is higher than 100 mbar, it is recommended to use a spool piece and increase the net volume of the filter integrity test.

4. Bubble-Point Test

4.1. Parameter settings

Hydrophobic filters can be Bubble Point tested after wetting with IPA/water 60:40 (or 70:30) Vol. %. Other wetting agents are possible, if validated.

Check if the filter was wetted completely.

Make sure that the filter housing or capsule was vented before flushing the filter.

Repeat filter flush prior to another filter test.

The temperature range during a Bubble Point test must be kept stable (± 2°C) at ambient temperature (20 – 25°C).

Sartocheck® 5 and Sartocheck® 5 Plus Filter Testers require for the Bubble Point test the Diffusion test limits and the input of a minimum Bubble Point. For the Sartocheck® 5 series, a maximum diffusion limit must be entered to ensure the optimal Bubble Point parameters are applied to the Bubble Point algorithm for the size filter being tested. This is synonymous with Test Class on the previous Sartocheck® models. Below is a table that relates max diffusion to test class for the different size filters.

Max. Diffusion (SC5/5+)	Test Class (SC4/4+)	Over-proportionality Criterion (A1)	Bulk Flow Criterion (A2)
< 9 mL/min	Small	5	50 mL/min
> 9 and < 50 mL/min	Standard	15	150 mL/min
> 50 mL/min	Special systems (large)	30	240 mL/min

In some cases, the Sartocheck® integrity tester is not able to identify an over-proportional increase of the flow in the course of a bubble point test. Reasons can be certain membrane structures as well as influence of some wetting solution or product residues. If the Bubble Point is clearly passed, the value for the Over-proportional Criterion (A1) should be chosen smaller.

5. Water Intrusion Test

5.1. General remarks

The Water Intrusion test is a very sensitive test method for hydrophobic filters using pure water. Because the amount of water intruding into a membrane is an order of magnitude lower than the air diffusion rate of a filter, the test is susceptible to interference. The influence of a subtle temperature shift in the gas volume during a WIT might be of the same magnitude as the intrusion rate itself. For that reason, the temperature during a Water Intrusion Test must be kept even more stable. It is proposed to keep the temperature at 20 – 25°C ± 1°C for all components involved in the integrity test.
The water quality also plays an important role during the WIT. The surface tension must be at least 70-72 mN/m for a successful test.
For an integrity test prior to use after sterilization (PUPSIT) alcohol-based testing is not applicable. WIT on the other hand can be used on an already sterile system, e.g. fermenter.
The hydrophobic membrane during WIT must be submersed under water for the whole test time. If the water level is lower the pressure will quickly drop.

✓ Check if the water level inside the filter housing is high enough.

Check the surface tension of the water used for testing (70-72 mN/m).

5.2. Parameter settings

Due to the sensitivity of the WIT, the compaction of the membrane pleats must be completed prior to volume determination and the subsequent test phase. Recommended test parameters:

Stabilization time 1:	3 minutes
Stabilization time 2:	10 minutes
Test time:	10 minutes (may be shorter if Auto Test Time is invoked)

A longer stabilization time can also help for temperature equilibration.

✓ Check the times for stabilization and testing. If necessary, choose longer times.

5.3. Water flow and gas flow-based test limits

For Sartorius Sartocheck® 4 Plus Filter Tester and older, it was possible to choose between two different reporting methods for water intrusion testing. The tests are identical in terms of how they were conducted but use different parameters for the calculation. One test uses the actual test pressure (WFT), whereas the other is based upon standard atmospheric pressure (WIT).
In order to eliminate the risk of mixing up critical test parameters during programming of the parameter settings for the Water Intrusion Test, Sartorius now allows only the use of Water Flow Rate in the Sartocheck® 5 and 5 Plus Filter Testers devices when performing a WIT.

✓ Check if the correct Water Intrusion filter test program and limits are used.

5.4. Test setup

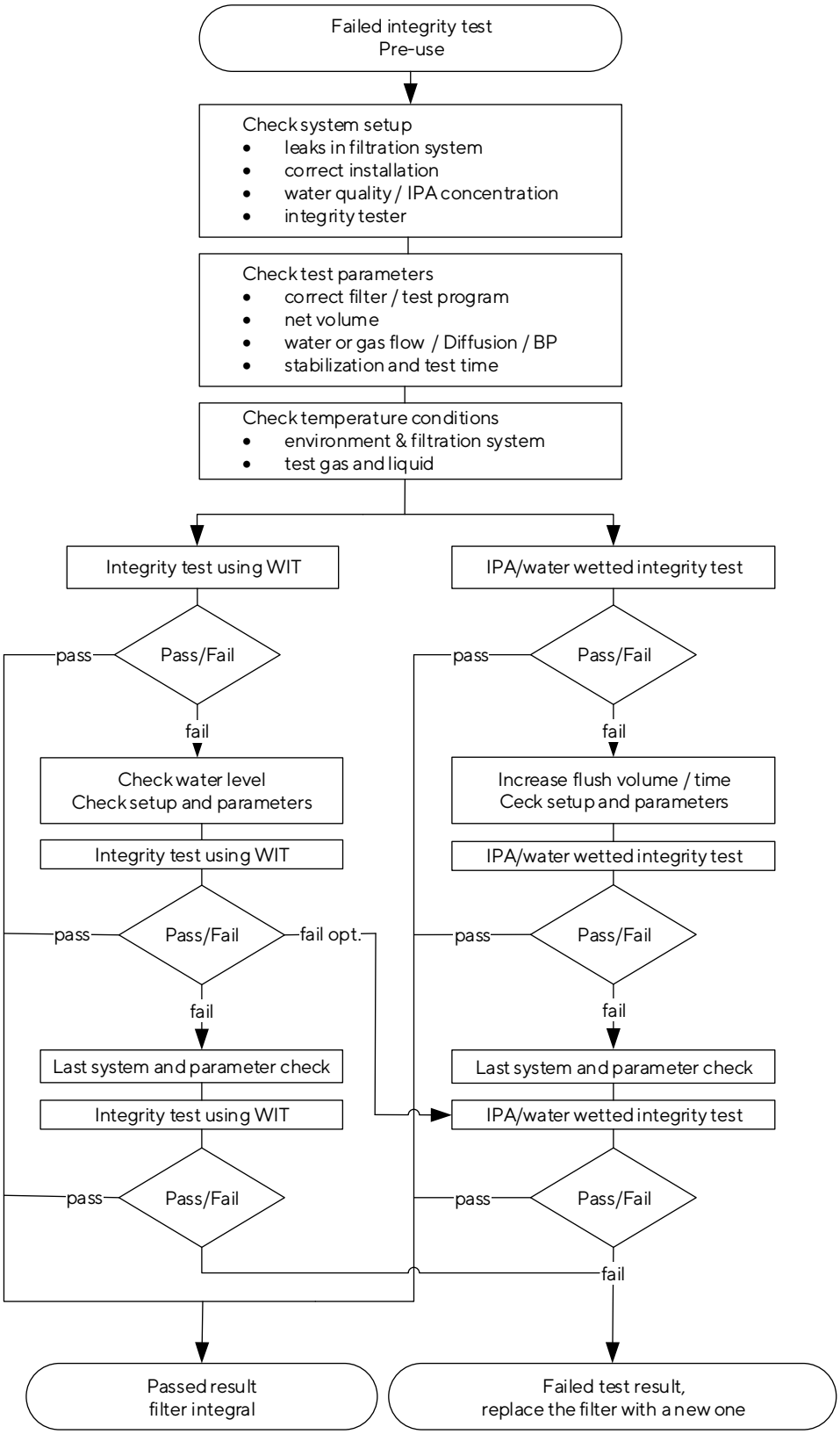
In many applications it is a regulatory requirement to the filters 'in situ' using WIT, in the production system. This verifies the absence of bypasses at the O-rings or valves and filter housing. For sensitivity reasons inherent to the WIT, the upstream volume of the filter is kept small. Valves should be located close to the filter housing, and dead spaces which could trap air and therefore disallow filling with water during the test are avoided.

For testing with IPA/water as well as for most filter capsules, the test is usually being performed offline. This should be done at a place with most stable environmental conditions (temperature).

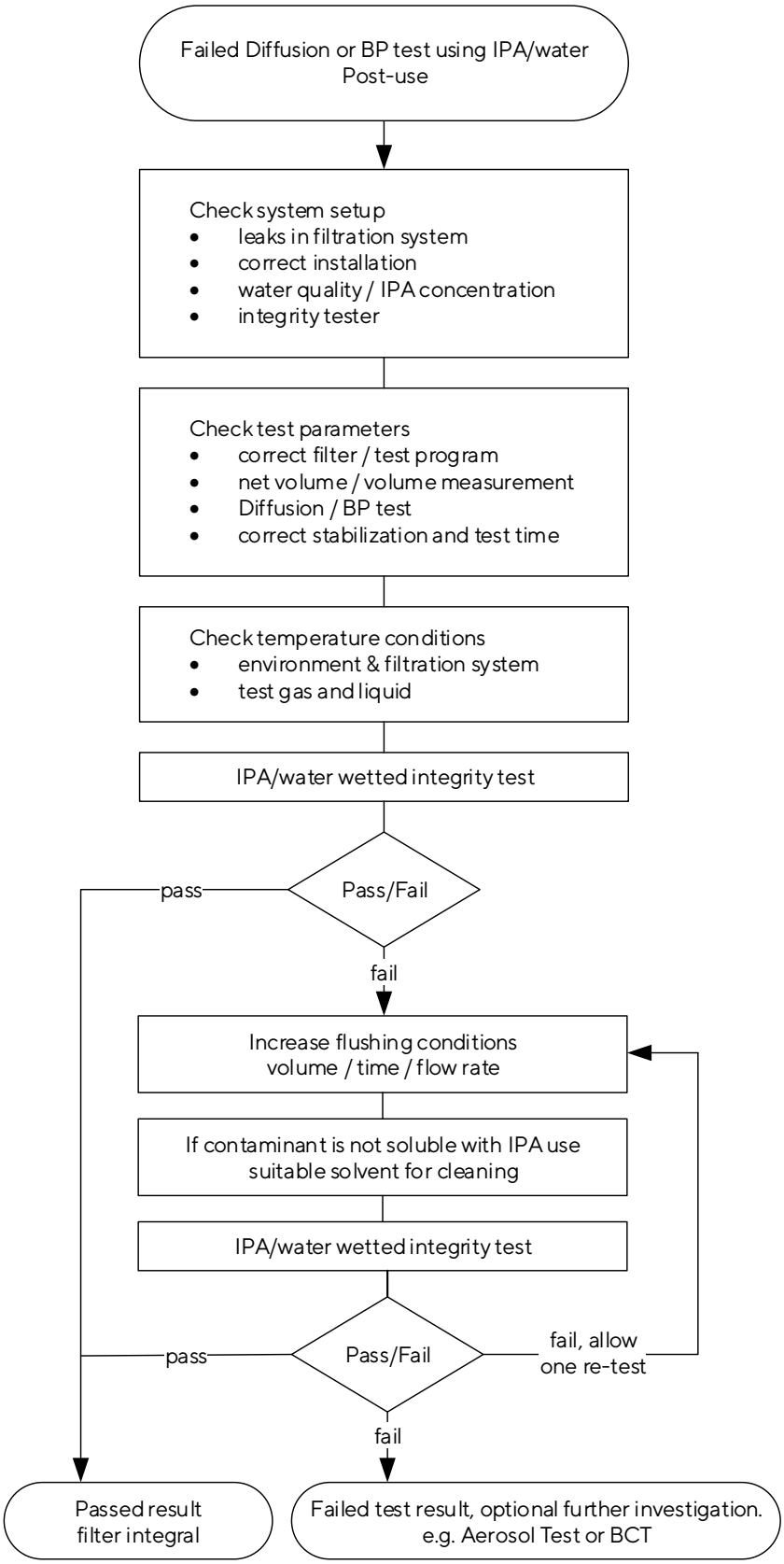
6. Flowcharts for integrity testing

In the guidelines no general recommendation for the number of re-test for failed filter elements is given. The flowcharts follow the procedures proposed in the PDA Technical Report No. 26. In this document filters can be re-tested three times in total.
Based on process and product specific risk assessments, also different regulations can be in place. The allowed number of re-test should be stated in the respective SOP.

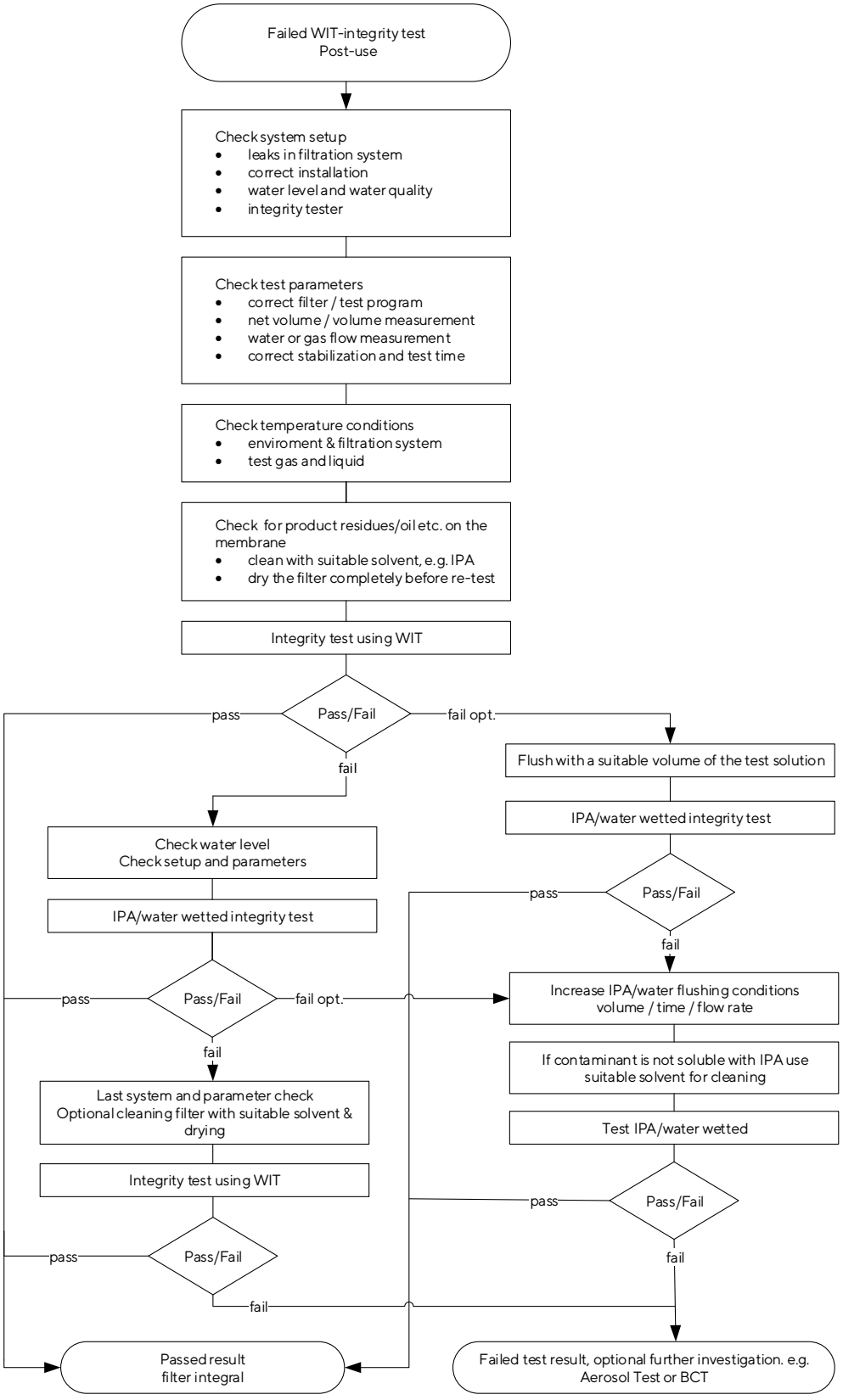
6.1. Flowchart for IT of a new filter pre-use



6.2. Flowchart for alcohol wetted IT of a filter post-use



6.3. Flowchart for WIT of a filter post-use



7. Avoiding false passed ITs

False passed test results in any case must be avoided. The following root causes are potentially possible:

a. A pressure drop caused by a truly non-integral filter element potentially can be masked by a temperature increase that expands the test gas during the test phase.

Don't perform integrity tests if the system or the test solution is lower than the environmental temperature (e.g. bring a filter from a cooled room to ambient temperature).

- let the system equilibrate before starting the integrity test
- choose a longer stabilization time
- integrity tests below 20°C must be validated

b. The volume of downstream side of the filter must be large enough (e.g. large volume storage tank) in order to avoid any increase of pressure in the course of the integrity test. A valve downstream of a tested filter has to be open during the entire test; otherwise, pressure could begin to build, reducing the differential pressure across the membrane, ultimately reducing the flow rate.
Attention! In case of a PUPSIT, it must be ensured that the sterile filtrate outlet remains closed to the environment.

Make sure that during the integrity test no pressure increase downstream of the filter happens.

- use the safety parameters of the Sartocheck® 5 Plus. A low diffusion rate and/or a wrong volume measurement can point to a closed valve or too small downstream volume

c. If a wrong test program is used, e.g. for a larger filter, the test can be prone to a false passed test result.

d. Wrong parameter setting can also lead to a false pass test result.

Make sure that the test parameters are correct.

- use barcode scanner for selection of the correct test program

e. Different test gases will have an influence on the Diffusion test value due to solubility and diffusivity. Always ensure the pass/fail diffusion limits are correct for the test gas being used.

To exclude the influence of a false measurement due to the test gas.

- check if the correct test gas was used

More information about avoiding false passed integrity tests please refer to the "QRM Handbook - Conducting FMEA Using the Sartocheck® 5 Plus Filter Tester". For correct setup of the integrity test, the Sartorius Tech Support team can be asked for support.

8. Abbreviations

BCT	Bacteria Challenge Test
BP	Bubble Point (integrity test)
IPA	Isopropyl alcohol (2-Propanol)
IT	Integrity Test
mAb	monoclonal Antibody
Max.	Maximum
PDA	Parenteral Drug Association (PDA.org)
PUPSIT	Pre-Use Post-Sterilization Integrity Test
SC	Sartocheck® Filter Tester
SIP	Steaming In Place
SOP	Standard Operating Procedure
Vol.	Volume
WFT	Water Flow Test
WIT	Water Intrusion Test

9. Literature

PDA Technical Report No. 26, (TR 26) Revised 2008, Sterilizing Filtration of Liquids

PDA Technical Report No. 40, (TR 40) Sterilizing Filtration of Gases

QRM Handbook - Conducting FMEA Using the Sartocheck® 5 Plus Filter Tester, updated version can be downloaded at www.sartorius.com

Validation Guide Sartofluor® GA & Sartofluor® LG; Material No.: 1000054589

Validation Guide Sartopore® Air Midicaps®, Maxicaps® and Capsules; Publication No.: SPK5819-e200206

Validation Guide Sartopore® Air Midisart®; Publication No.: SPK5813-e180702