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Application Note

Integrity Test Troubleshooting – Beyond Rewet and Retest

Integrity testing is a critical operation, especially for sterilizing grade filters used in biopharmaceutical processing. When performed correctly, an integrity test is a fast, definitive, non-destructive way to assure filter retention performance. Fortunately, there are few ways a non-integral filter will pass the integrity test, making it unlikely that a non-retentive filter would not be detected. Unfortunately, there are a lot of ways an integral filter can fail the integrity test, resulting in retests, lost time, lost productivity and potentially lost product.

Filter integrity tests are primarily based on capillary forces that hold liquid in the pores of wet membranes. The smaller the pores, the stronger the capillary forces. The bubble point test measures the force in gas pressure required to overcome the capillary forces, and therefore provides an assessment of pore size. The diffusion type tests measure gas flow across the wet membrane at a pressure below the bubble point. If the flow rate is above an established maximum, then the excess flow rate is attributed to a leak or defect in the filter. Test errors come from any phenomena impacting capillary forces, gas diffusion, or gas flow or pressure measurement accuracy.

It is a common assumption that false integrity failures are the result of incomplete membrane wetting. Incomplete wetting is certainly a common problem, but it is not the only potential problem. Simply rewetting and retesting may or may not produce a passing result and may not reveal the root cause of the problem. In this Application Note, we will consider all the potential sources of test error and apply a logical approach to resolution and retesting. The goals are to strengthen confidence in the result, provide justification for retests, and ultimately, to understand specific challenges and eliminate them to assure the integrity test can be performed correctly the first time.

General Integrity Test Result Categories

It is helpful to consider the general magnitude of test results. These can be categorized into four ranges:

Pass	Bubble point and/or diffusion are in specification and in typical range. For example, a filter with a minimum bubble point of 50 psi might have results in the range of 52 to 58 psi. Or a maximum diffusion rate of 13.3 mL/min and typical results range 8 to 12 mL/min. When passing results in the typical range are achieved, we have the highest confidence in filter integrity.
Gross Failure	For example, high gas flow at low pressure is observed with either bubble point or diffusion tests. Gross failure is typical of truly non-integral filters. If a filter is damaged due to high differential pressure, physical impact, or excessive heat, the resulting defect will be orders of magnitude larger than the pore size of the integral filter. The result is high gas flow at low pressure. When high gas flow at low pressure is observed, troubleshooting and retest procedures should be applied, but there would be low expectation that retests will show the filter to be truly integral.
Marginal Failure	A marginal failure is defined as a bubble point less than the specification, but more than approximately 70% of the specification, or a diffusional flow rate greater than the specification but less than approximately 150% of the specification. For example, bubble point specification is 50 psi and actual result is 48 psi, or the diffusion specification is 13.3 mL/min and actual result is 20 mL/min. Typically marginal results are not due to oversized pores or defects, but due to phenomena impacting capillary forces or gas diffusion (i.e. low surface tension, poor wetting) or test error. When marginal results are observed, troubleshooting and retest procedures should be applied and there would be a high expectation that retests will show the filter to be truly integral.
Invalid Test	An automatic integrity tester may stop the test prematurely or finish the test with an error message for a variety of reasons. An invalid test result is meant to indicate to the end user that the measurements it encountered are not what the tester was expecting. For instance, a tester may give an invalid test result if it measures a flow rate much lower than the specification, which can indicate a problem with the test execution, such as a closed valve that should be open. The error messages and what triggers them will vary between each manufacturer's integrity tester but are important tools for troubleshooting. People responsible for executing tests or reviewing results should be trained to recognize when a test is invalid, document the troubleshooting efforts, and initiate a retest.

Potential Causes of Integrity Test Failure

To think beyond 'poor wetting' as the root cause of all integrity test failures, it is valuable to consider all the potential causes of failure. Table 1 lists potential integrity test failure modes for the filter (column 1) or test method (column 2). These lists are not intended to be exhaustive. When developing a troubleshooting guide for a particular installation, users should consider process specific parameters of the application.

Table 1

Filter Related Failure Modes	Test Method Failure Modes
Membrane damage	Wrong test selected
O-ring damage	Wrong test gas used
Device damage	Leaks
Surface tension suppression	Instrument/gauges out of calibration
Poor wetting	Temperature change
Air lock	Valves improperly open or closed
Wrong membrane	Untrained operator
	Wrong wetting fluid

Develop a Procedure for Troubleshooting Filter Integrity Tests

A well-constructed SOP is essential for efficient troubleshooting. The SOP should answer two fundamental questions:

- Is the filter integral or not? Answering this question accurately is critical, especially in post-use integrity testing situations where batch disposition depends on filter integrity.
- Why did it fail? Identifying a root cause of failure will allow that root cause to be addressed and corrective actions taken to minimize or eliminate future false failures. Knowing why a test failed is also valuable for justifying a retest. There is a common misconception that two retests are allowed and then the filter must be considered non-integral. Blindly performing two retests without consideration for root cause is inefficient and a potential compliance issue. On the other hand, any number of retests might be considered if the root cause of the previous test result can be clearly identified and documented.

Develop a Troubleshooting Flow Chart

A flow chart based on an understanding of integrity testing principles and applies clear logic is a central component to a good integrity test troubleshooting SOP. There are many good examples, including in our Wetting Guides (lit. no. P35515 and UG4224EN available at **SigmaAldrich.com**) and PDA Technical Report 26. Specific site or application conditions and constraints need to be considered for each end user. But here we provide a general troubleshooting flow chart and the logic used to create it.

For the initial test, it is always important to follow as closely as possible the filter manufacturers wetting and testing recommendations. For Durapore® or Millipore Express® hydrophilic membrane devices, the recommendations include:

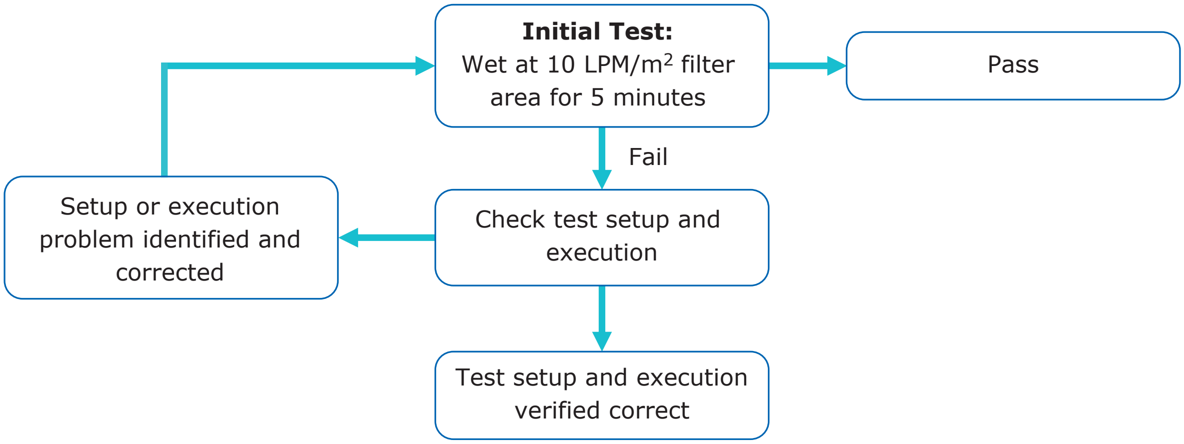
- Fill system slowly
- Vent completely
- A one minute static high pressure hold
- 5 minutes of flow at least 10 LPM/m^2 filter area

These initial wetting conditions have been proven to be robust and capable of resulting in "First Time/Every Time" integrity test success.

If the integrity test fails after the initial wetting procedure, often the typical reaction is simply re-wet and retest. If the reason for initial test failure is poor wetting, simply re-wetting may fix the problem and result in a passing result. But, as listed in Table 1 column 1, poor wetting is only one of many reasons for test failure. Ignoring the possibilities beyond poor wetting can result in repeated failures, frustration, lost time, or lost product.

Prior to a retest, the failure modes in Table 1 column 2 can be addressed by examining the system and may be identified without retest. Others, such as temperature changes require training to understand the gas flow curve generated during the test and being able to recognize the impact of various faults on the curve trend. For example, a plot of flow rate versus time during the diffusion test is expected to approximate a horizontal line. If the slope of the line changes during the diffusion test, this may indicate a temperature change which should be addressed before retesting.

If the initial test fails, the next step in the troubleshooting flow chart is to check the test set-up and execution.



When the test is verified to be run correctly, the cause of failure may be due to one of the items in **Table 1 column 1.**

- Filter, seal, or device damage
- Surface tension suppression
- Poor wetting
- Air lock

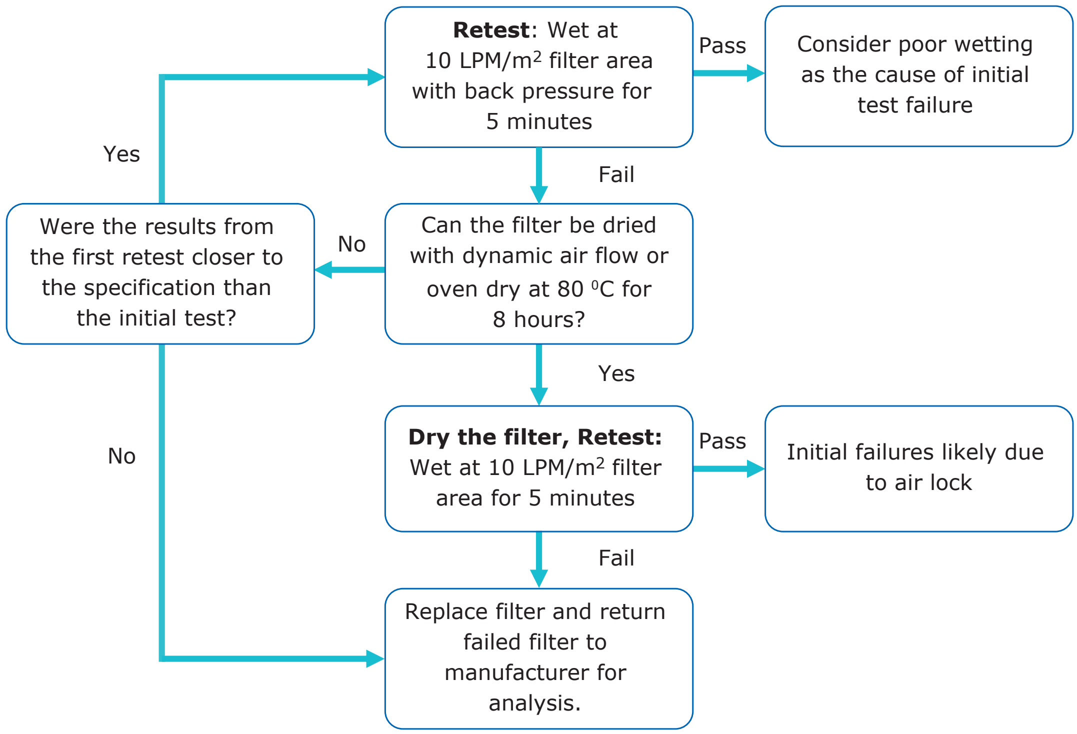
Enhanced wetting and retesting is now needed. There are several options for enhanced wetting. First, we must consider the options for enhanced wetting.

Table 2

Wetting Option	Ease of Implementation	Failure Modes Addressed	Failure Modes Not Addressed	Pre- or Post-Use?
Longer Time / More Volume	Easy	Poor wetting, non-adsorbed surface-active residuals	Damage, air lock, adsorbed surface-active residuals	Both
Warm Water Flush	Easy if facilities are available	Poor wetting, non-adsorbed water- soluble surface-active residuals	Damage, air lock, adsorbed surface-active residuals	Both
Higher Flow Rate	Easy depending on system capabilities	Poor wetting, non-adsorbed surface-active residuals	Damage, air lock, adsorbed surface-active residuals	Both
Higher System Pressure	Easy	Poor Wetting, non-adsorbed surface-active residuals, air lock	Damage, adsorbed surface-active residuals	Both

The best option in Table 2 is high pressure wetting. High pressure wetting means restricting downstream flow to create higher system pressure. Typically, a system pressure of 40 psi is targeted but the enhanced wetting can be performed at whatever system capabilities are available and compatible with the pressure limit specifications of the filter device. Ideally, the flow rate of 10 LPM/m^2 filter area is maintained. With this technique, air trapped in the membrane may be forced into solution and flushed out, fully wetting, and solving the problem of poor membrane wetting. If the cause of failure is adsorbed surface-active compounds, pressure wetting may be ineffective at flushing these compounds from the membrane surface.

If the filter continues to fail after multiple wetting attempts, the problem may be air lock. Air lock is a phenomenon where the upstream and downstream surfaces of the membrane are wet simultaneously, trapping a pocket of air within the thickness of the membrane. Air lock can be very difficult to remove with flushing. The best option for eliminating air lock is complete drying by dynamic air flow for 2 hours or static drying using a cross flow oven at 80°C for 8 hours. Filter should then pass after standard water wetting.



In the SOP it may be wise to allow a provision for a retest at any step when a clear assignable cause for test inaccuracy is identified.

When all tests fail, it is recommended to return the filter to the vendor for confirmation and defect analysis. Defect analysis should be able to determine if loss of integrity is the result of physical impact, excessive pressure and/or temperature, a filter manufacturing defect, or another cause.

Considerations for Post-use Integrity Tests

The most effective for demonstrating filter integrity is alcohol wetting. Because an alcohol solution such as 70/30 IPA/water has a low surface tension it will wet the membrane very thoroughly, overcoming problems of poor water wetting. In addition, surface-active compounds that lower the surface tension of water are unlikely to impact the surface tension of alcohol solutions. Alcohol wetting and testing should determine whether a membrane is integral but will not answer the question “why did the first test fail?”.

Table 3

Wetting Option	Ease of Implementation	Failure Modes Addressed	Failure Modes Not Addressed	Pre- or Post-Use?
Alcohol	Complex when alcohol contamination in the process is a risk	Poor wetting, non-adsorbed surface-active residuals, adsorbed surface-active residuals, air lock	Damage	Post-use

Filters are generally tested with alcohol during a post-use test. If used for a pre-use test, all alcohol must be removed prior to starting the filtration process. Also, some filters are not compatible with alcohol, so any exposure of such filters to alcohol should be limited to post-use integrity testing only.

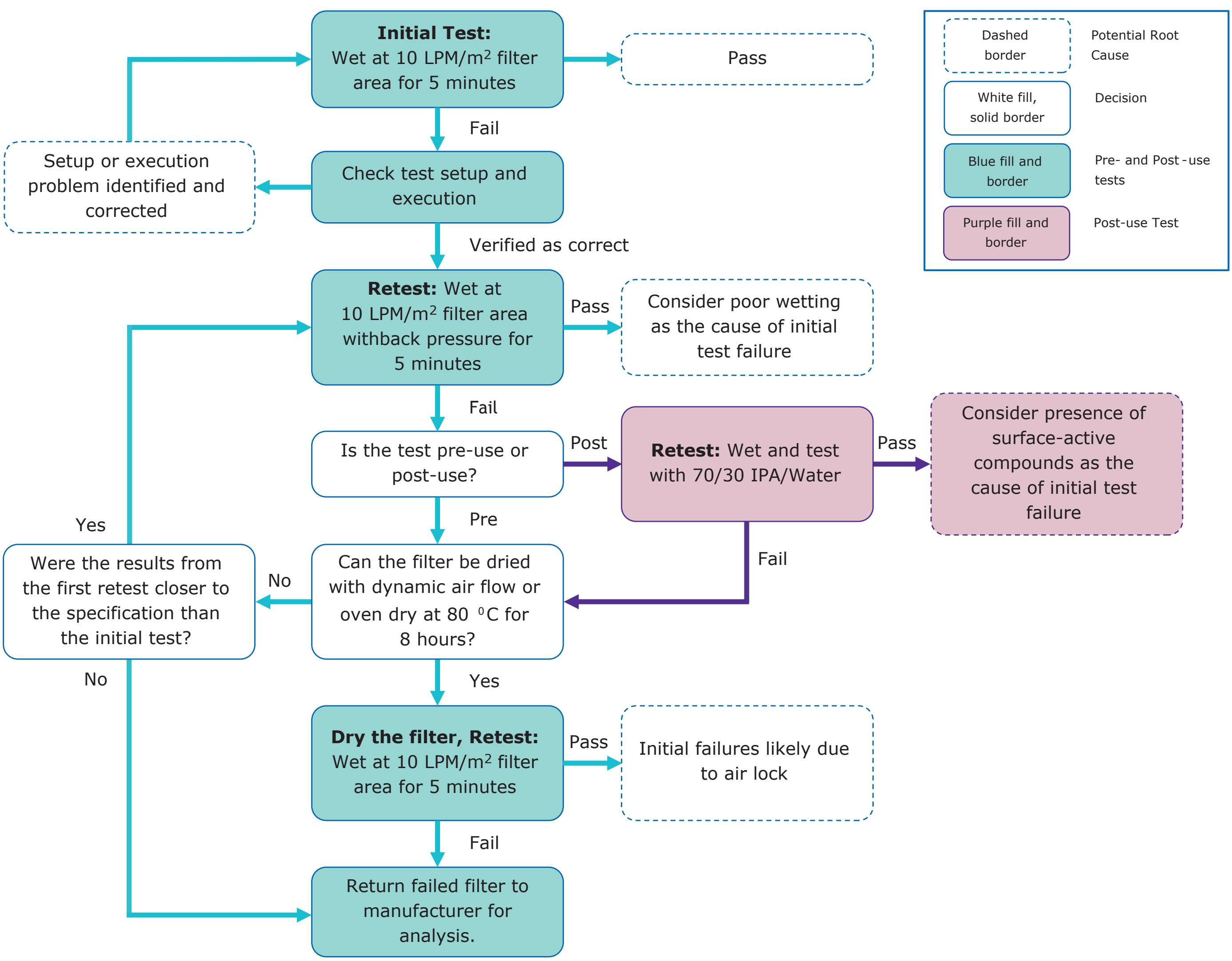
If retests are commonly performed in post-use testing, developing a more rigorous flushing procedure should be investigated to reduce time, resources, and deviations. This may include defining a minimum flush volumes, flowrates, and system pressures that result in at least 3 stable consecutive test values and are comparable with pre-use test values.

For certain processes, the amount of time or volume of water required to get passing post-use test results is impractical. In these cases, determining a product-specific test specification may be worthwhile. This means that the post-use test can be conducted immediately following the filtration process, eliminating the water flush step. Further information on this topic can be found in AN1505EN00: Establishing Product Specific Bubble Point Values for Sterilizing-Grade Filters (available at **SigmaAldrich.com**).

Summary

The complete troubleshooting flowchart is shown in the figure below. As considerations for pre-use and post-use testing differ, separate branches are shown for each scenario. The procedure may be considered as is or could be modified to incorporate process/product/site specific failure causes or testing constraints.

It is not always possible to have a definitive failure mode. For example, re-wetting with high pressure water may remove surface-active compounds simply because it is additional flushing. But a procedure based on logic and typical known integrity test failure modes, should provide an efficient process for resolving failures and direction for identifying and applying corrective actions.



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