BIOTECH

Application Note

USD 3033a

Best practices for successful filter integrity testing using the water intrusion test (WIT) method

Contents

1 Purpose

The water intrusion test (WIT) is widely used in the pharmaceutical industry to test the integrity of vent filtration for holding tanks and bioreactors, autoclave and lyophilizer vent filters. The purpose of this document is to present the collected best methods/practices to give filter end users the best possible chance of performing a successful WIT. It presents the factors that influence the WIT, the most common reasons for "false failure" results associated with the WIT, and how to overcome them.

Following these methods/practices will provide the end user with the most advanced way to test critical gas and air vent filters to ensure the highest process safety and to demonstrate compliance with regulatory requirements according to FDA[1] and European Guidelines[2], which state that "the integrity of critical sterile gas and air vent filters (that are directly linked to the sterility of the product) should be verified by testing after use, with the filter remaining in the filter assembly or housing."

2 Introduction

Hydrophobic filter cartridges which utilize sterilizing grade polyvinylidene fluoride (PVDF) or polytetrafluoroethylene (PTFE) membranes are used in the pharmaceutical industry to sterilize compressed air or gas for product contact, as well as acting as sterile tank vents. The hydrophobic nature of the membrane is useful in preventing the membrane from wetting out under conditions of high humidity and aerosolized water over long service periods. However, the hydrophobic membrane also makes integrity testing *in situ* (as recommended in EU-Annex 1 "Manufacture of Sterile Medicinal Products" (2022), 8.88) difficult as the membrane must be fully wetted prior to testing by the widely accepted integrity test methods of bubble point and forward flow. Typically, low surface tension organic solvents (e.g. iso-propyl alcohol) mixed with water, which are often considered to be contaminative fluids, are used in order to fully wet the membranes. Once the filter is wet it can be tested successfully, but then has to be fully dried before it can be returned to service.

The WIT, widely accepted in the pharmaceutical and biotech industry, overcomes the need to wet the filter membrane out as it relies on the hydrophobic nature of the membrane in order to perform the test successfully.

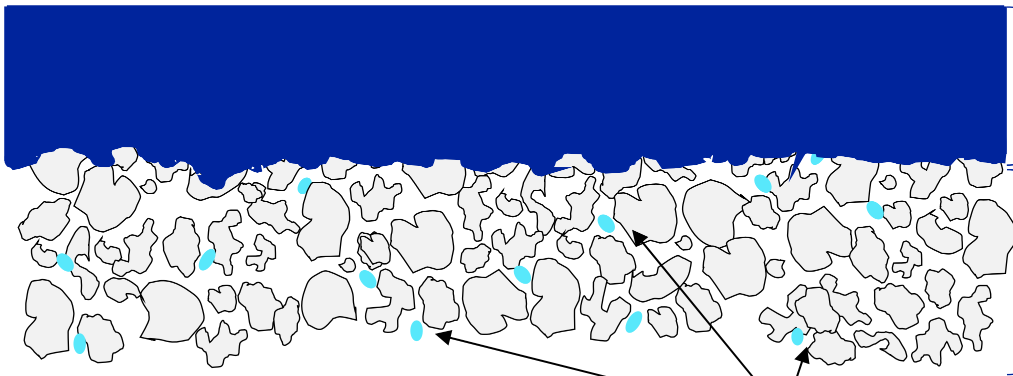
This offers the end user several benefits:

- Since the membrane does not wet out during the WIT, the filter can be returned to service after draining and a minimal drying period reducing filter downtimes
- The WIT can be performed on filters in a sterilized system, as there is no manipulation of the downstream (sterile) side of the filter, eliminating potential downstream contamination issues
- Since only water for injection (WFI) or deionized (DI) water is in contact with the membrane during the test, there is no contamination of the membrane or downstream process with solvents designed to wet the membrane out for integrity test purposes.

3 Theoretical basis of the water intrusion test

For performing WIT, high quality water such as WFI or deionized water with a conductivity of 1–3 µS/cm is used to fill the upstream side of the filter cartridge or capsule installation before pressurizing the assembly with compressed air or nitrogen (see Figure 1). The test pressure for the WIT is chosen such that the water intrudes into the membrane surface but cannot wet the membrane. Water then evaporates from the surface of the liquid at the membrane interface and moves under the pressure gradient to the downstream side of the membrane. The rate at which water evaporates can be measured and correlated to a bacterial challenge to provide a non-destructive test that can be used to test the integrity of the filter *in situ*.

Figure 1
Water intrusion



Because of the nature of the test there are multiple factors which can influence the test which need to be carefully considered and controlled, in order to ensure a successful test outcome and minimize the number of false test failures[4].

4 Eight factors that can influence the WIT

Studies have shown that the basis of the WIT is the evaporative loss of water through the filter membrane[3]. A number of factors which have been shown to influence this evaporation and/or which cause an additional passage of water through the membrane and/or can have an influence on the measurement itself are discussed here.

4.1 Factor 1: Membrane contamination during installation

The WIT relies on the fact that the filter membrane is highly hydrophobic. Any contaminating species which alters the critical wetting surface tension of the membrane or the surface tension of the water used for the test could compromise the WIT, as it can allow water to penetrate the affected area at a greater rate than the pass/fail limit of the test. In order to avoid transfer of contaminating material like natural oils from the operator's hand onto the surface of the filter membrane, gloves should be worn during the handling and installation of the filter cartridge into the housing.

If a sanitizing solution with a low surface tension is used to wipe down the outside of the bag (in which the filter cartridge is supplied) then it should be allowed to dry before opening to ensure that no low surface tension liquids are in contact with the filter surface.

If the filter cartridge o-rings are lubricated to allow for easier installation, only WFI or deionized water should be used to prevent wetting of the membrane surface. Alcohols and other low vapor pressure fluids should never be used to lubricate o-rings.

4.2 Factor 2: Water quality

The conductivity of the water used to conduct a WIT has been shown to have an effect on the water intrusion result[1] obtained during a test, as evaporation of water is greater at lower conductivities. DI or preferably WFI quality water with a conductivity of 1–3 µS/cm are the preferred choices for WIT.

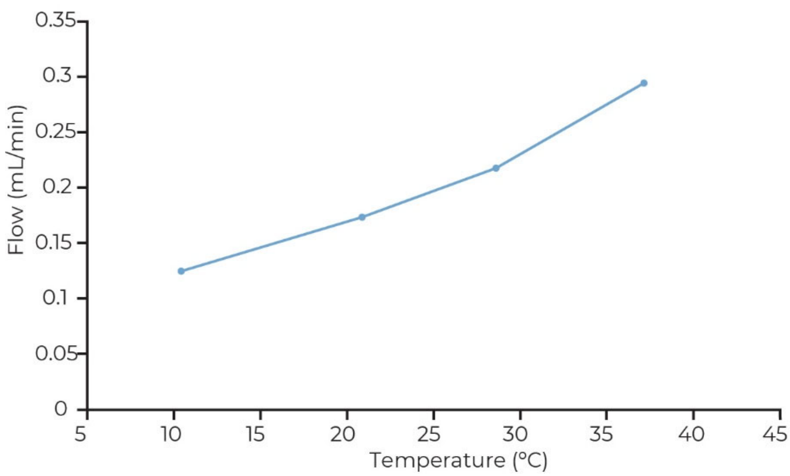
The use of purified water also ensures that it does not contain any material which could contaminate the filter, altering the hydrophobicity of the membrane and causing a higher water flow. Any water used for testing should be discarded after each test and fresh water decanted for all subsequent tests to prevent contaminant carryover as well as bacterial growth in the water used. In addition, if a filter housing is used for the test, it is essential that the housing is clean, since residual chemicals, debris or process fluid may also alter the hydrophobicity of the membrane and/or the water quality.

4.3 Factor 3: Water temperature

Since the WIT relies on evaporative flow of water across the membrane to be tested any increase in temperature will result in a higher evaporation rate leading to a higher water flow measured during the WIT. Water used for WIT should remain at a stable temperature of between 18 and 22 °C and must not change by more than ± 1 °C during the test. Similarly, temperatures below 18 °C will result in lower evaporative losses and could lead to falsely passing results.

Figure 2

Effect of temperature on the WIT result of a 254 mm (10 in.) cartridge under otherwise equal conditions



In order to achieve a stable temperature during the WIT, the water temperature used for WIT should be adjusted to the temperature of the room. If the water supply does not allow such a temperature control and adjustment, the water should be decanted into a container (carboy or similar) and left in the room in which the WIT is to be conducted so that the water temperature can equilibrate to the temperature of the room before testing commences.

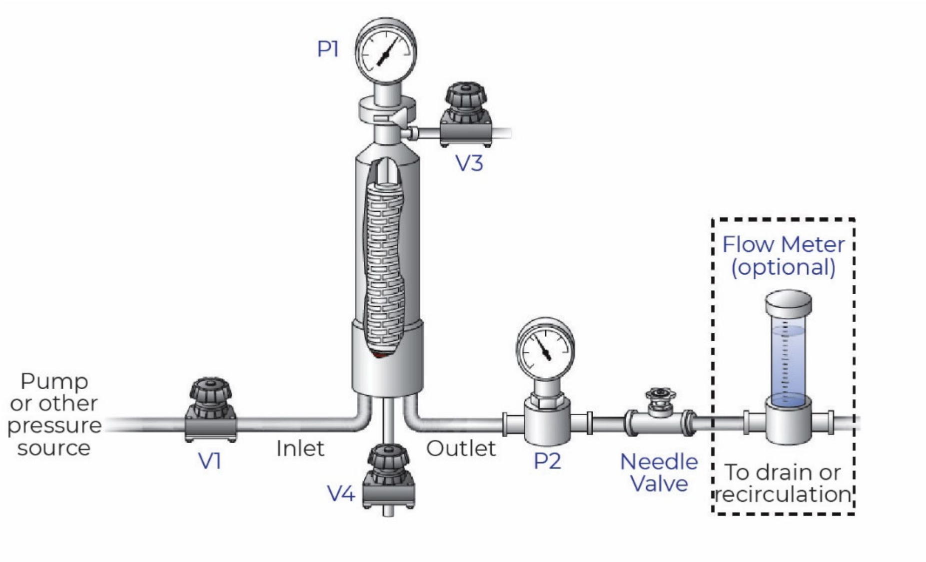
In addition, this will allow the water to degas after dispensing to remove as much dissolved air as possible before WIT. Small bubbles present in the water can prolong the required test time for a successful WIT (see Factor 8: Stabilization).

4.4 Factor 4: Filling of the filter capsule or housing

After installing the filter, the installation (housing or capsule) must be completely filled with water. This should be done by filling the housing slowly using the drain connection (V4 in Figure 3) at the base of the housing, up to the vent valve (V3 in Figure 3) on the top of the housing. This will prevent any dissolved gas from forming bubbles during turbulent flow, which may cause elongated stabilization times. The installation should be filled at a flow rate of 2–5 cm (1–2 in.) of vertical height per minute to avoid introduction of gas into the installation.

A pump or a pressurized vessel may be used to deliver water to the filter assembly provided that the flow rate is kept sufficiently low.

Figure 3
Apparatus set-up



The filter cartridge must be completely covered with water and be in an upright position as shown in Figure 3 to prevent air bypass through any membrane which is uncovered, leading to falsely high WIT values.

4.5 Factor 5: Environmental temperature

During the WIT, the room temperature cannot change by more than ± 1 °C. This is due to the possibility of expansion (if the room temperature increases) or contraction (if the room temperature decreases) of the compressed gas within the tubing connecting the filter installation to the integrity test equipment, and/or the headspace above the water level inside the filter capsule or housing. Any expansion or contraction will be measured by the integrity test equipment and can lead to falsely high or low WIT results.

The filter installation and electronic integrity test equipment must be placed away from any sources of heat (steam pipes etc.) or ventilating ducts to prevent the possibility of temperature changes during testing as noted above.

4.6 Factor 6: Compressed gas quality

Clean dry (instrument quality) air or nitrogen should be used as the pressure source for all WIT. This will prevent the possibility of contaminant material being deposited onto the filter either during normal use or integrity testing.

4.7 Factor 7: Compressed gas temperature

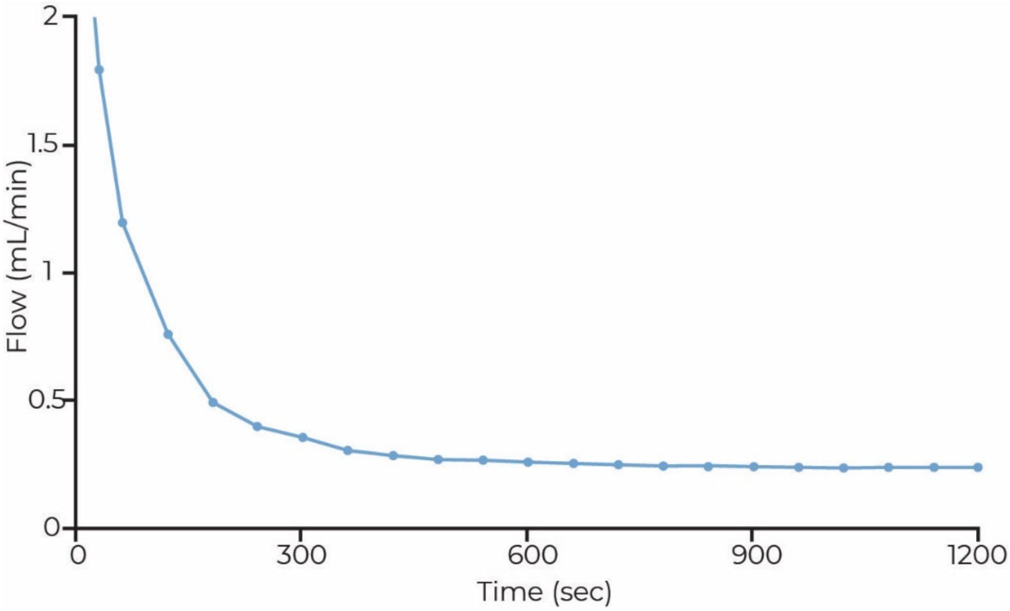
The gas used for WIT should be at ambient temperature so that it is neither heating up nor cooling down during the WIT. This will prevent contraction or expansion of the gas within the tubing or headspace of the filter installation.

4.8 Factor 8: Stabilization

Figure 4 shows the normal graph of evaporative flow plotted against time. As can be seen the flow rate drops quickly, then plateaus after about 600 seconds of test time have elapsed. This significant decrease in flow rate is due to the pleated filter structure being compressed, water being forced into the pleat structure, dissolution of gas into the water, expulsion of gas from the pleats, cooling of the gas in the headspace after compression and finally evaporation of water through the membrane. As the first five factors stabilize and are omitted with increasing measuring time only the evaporative loss of water remains resulting in the very low flow rate observed.

Figure 4

Water flow rate during a WIT plotted against time



All of the parameters mentioned have been shown to be time dependent (see Figure 4) such that the initial flow rate drops quickly (within 5 minutes) as the pleats compress, and the other factors exert less and less of an effect, until a plateau is reached which is due to evaporative loss of water through the membrane.

As a result, modern electronic filter integrity test equipment such as the Palltronic® Flowstar V and AquaWIT Filter Integrity Test Instruments include a pre-stabilization function which allows the end user to program a suitable time to allow all of the factors listed to stabilize so that they no longer contribute to the measured flow rate. Under normal circumstances, a pre-stabilization time of 10 minutes is used. However certain filter installations (e.g. systems with larger volumes and/or long connections between filter housing and test instrument) can require longer stabilization times.

The Palltronic AquaWIT IV Filter Integrity Test System is strongly recommended for routine WIT. It provides a fully automated WIT, including fill, test, drain and drying period. Further information on Palltronic filter integrity test equipment can be found on our website.

The use of older technology, such as pressure hold devices is not recommended for WIT, due to poor accuracy inherent with indirect measurement. Some devices will require a conversion factor for testing Pall filters on these devices.

5 Summary

Table 1

Installation/handling

Best practice	Why?	How to achieve it?
Use of gloves	To minimize the chance of contamination of the filter membrane by the operator	Wear gloves while handling filter cartridges
Use only de-ionized or better water for lubrication of the o-rings prior to installation of the filter cartridge, where applicable	To avoid overspray of any fluid that may allow the hydrophobic membrane to become wetted, and to avoid damage to the adaptor	Use only high quality water (see 'Water' below)
Ensure the filter is completely covered with water before the WIT	To prevent air bypass through uncovered membrane surface	Where necessary, add extra upstream volume, such as a spool piece

If a sanitizing solution is used to wipe down the filter bag prior to opening, it must be completely dried before opening	To avoid any low surface tension fluid getting on the filter membrane	Allow for sufficient time in the standard operating procedures for the packaging to air-dry, before opening the primary packaging
Filter should be in the correct position for filling and testing	Air can get trapped in the pleats, which can lead to false failure	Ensure that the filter is held upright for filling and testing

Table 2

Water

Best practice	Why?	How to achieve it?
Should be at room temperature (18-22°C)	Increased temperatures affect water evaporation rates and temperature lower than 18°C may lead to a false integrity test pass. Dispensing water from a valve or tap may aerate the water, and lead to false failure	Fill a pre-cleaned or sterilized carboy (or similar vessel) with suitable quality water, and place it in the room to be used for testing overnight to equilibrate to the temperature of the environment
Should be de-ionized or better quality	Conductivity has an effect on water flow across the membrane	Use de-ionized water with a conductivity of 1–3 $\mu S/cm^{-1}$ or better
Water should be filled from the bottom of the housing up to the vent	To prevent air in solution in the water from forming gas bubbles during turbulent flow	Fill slowly from the base of the housing at a fill rate of 2–5 cm (1–2 in.) of vertical height per minute

Table 3

Air

Best practice	Why?	How to achieve it?
Should be clean dry air (CDA) or nitrogen gas	To prevent contamination of the filter	Adequate prefiltration/pretreatment of air or gas used in the WIT
Should be at ambient temperature	If the incoming air is either hot or cold, then contraction or expansion of gas volume during the course of the test can cause problems	See 'Integrity Test Instrument' below

Table 4

Palltronic Flowstar IV and AquaWIT IV integrity test instruments

Best practice	Why?	How to achieve it?
Should be placed away from heat sources and heating, ventilation, and air conditioning (HVAC) registers	Changes in the temperature of compressed air within the space above the filter in the housing or the tubing used to connect the integrity tester to the filter can cause a change of the gas pressure which is registered as a change in flow rate by the integrity test equipment	Position the integrity test equipment in an area away from HVAC registers, and away from sources of heat or cold
An additional pre-stabilization time can be set to 10 minutes at the normal water intrusion test pressure	To allow sufficient time for the transient effects of pleat compression, water penetration into the pleat structure, expulsion of gas from the pleats, dissolution of gas into the water and cooling of the gas in the headspace to dissipate	Set the pre-stabilization time to 10 minutes and the pre-stabilization pressure to 2.5 barg (36 psig)

6 References

1. FDA Guidance Document on High Purity Water Systems for Pharmaceutical Use, 1996

2. EU Guidelines to Good Manufacturing Practice, Annex 1

3. Jaenchen *et al*, Studies on the Theoretical Basis of the Water Intrusion Test (WIT), Pall publication USTR 2047

4. Identifying False Failures Using the Water Intrusion Test, Pall publication application note USD 3593

PALL CORPORATION

Corporate Headquarters
Port Washington, NY, USA
+1-800-717-7255 toll free (USA)
+1-516-484-5400 phone

European Headquarters
Fribourg, Switzerland
+41 (0)26 350 53 00 phone

Asia-Pacific Headquarters
Singapore
+65 6389 6500 phone

Visit us on the Web at www.pall.com/biotech
Contact us at www.pall.com/contact

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