Life Sciences

Water Intrusion Test

USTR 2047

Studies on the Theoretical Basis of the Water Intrusion Test (WIT)

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Summary

There is an increasing requirement to integrity test sterile air filters *in situ*. The water intrusion test (WIT) offers benefits over existing methods, but its scientific basis is not well understood.

This study investigates what is being measured and provides data on the factors influencing the test. The role of evaporative flow when testing intact filters is clearly demonstrated and the effect of water quality and temperature quantified.

This improved understanding of the scientific basis of the test allows the factors influencing it to be controlled and enables a reliable correlation with bacterial challenge to be established.

Introduction

Hydrophobic sterilising-grade membrane filters are widely used for the filtration of gases in the pharmaceutical industry. The hydrophobic properties are necessary to prevent wetting-out of the filters in the presence of water, which would result in high pressure drop. The hydrophobic properties also have a major influence on the practicalities of integrity testing.

Traditional integrity test methods for membrane filters, such as the Forward Flow test and the bubble point test, require that the filter membrane is fully wetted. For hydrophobic filters, this requires the use of an organic solvent, typically isopropyl alcohol (IPA). Use of these solvents imposes certain constraints which must be taken into consideration. In many cases, this has resulted in filters being tested 'off-line', which requires removing either the full assembly or the filter cartridge from the process line and performing the integrity test away from the production area where management of solvent handling is easier.

The major limitations of this approach are that the cartridge to housing seal may not be tested, there is potential for incorrect re-installation, and testing of the steam sterilised assembly prior to use requires aseptic manipulations. There is therefore an increasing need for an integrity test that can be performed *in situ* and does not require the use of organic solvents.

The water intrusion test potentially meets these requirements. An upstream water-based test is attractive as it is non-contaminating, eliminates the use of alcohol, uses a non-flammable test liquid and does not require downstream manipulations.

An intrusion test for the determination of pore-size distribution was first published in 1921 (1) and published for membrane filters in 1954 (2). These tests used mercury and are therefore not suitable for routine testing in production. The first attempt to perform a test with water as test fluid was reported in 1980 (3). This test was a gross failure test of flat-sheet filter membranes and was not correlated to bacterial removal.

The first attempt to correlate a water intrusion measurement to bacteria challenge for hydrophobic membrane filter cartridges was presented in 1990 (4). The test described was still based on the theory that pressurised water intrudes slowly into the matrix of an intact hydrophobic membrane and can be measured as a small volume change on the upstream side even at moderate pressures.

Damaged filters would show a much higher volume change due to the passage of water through the membrane and this would allow the test to discriminate between filters that passed or failed the bacterial challenge test. According to this theory, it would be expected that an intact filter would show no further intrusion of water into the membrane matrix and the volume change would approach zero after a certain test time. However, this conflicts with other observations that clearly demonstrate a small but continuous volume change over extended time periods. This has created a degree of uncertainty over the scientific basis of the water intrusion test which has deterred some potential users from adopting it.

Automated pressure-decay integrity test instruments such as 'Sartorius' 'Sartocheck' 3 and 'Millipore' 'Integritest' Exacta, incorporate options for water-based integrity testing of hydrophobic filters. In these instruments, pressure decay is measured over a fixed time period. This value is then automatically converted to a calculated averaged flow and reported as the test result.

The availability of advanced direct flow measurement instruments such as the 'Palltronic' 'TruFlow' and 'Flowstar', which continuously measure flow during the entire test sequence, has enabled further studies to be performed to investigate the theoretical basis of the test. This paper investigates the theoretical basis of the water intrusion test using direct flow measurement and the influence of factors such as water quality and temperature.

Material and Methods

1. Filter Assembly

The test system used for these studies is shown schematically in **Figure 1**. All tests were performed with a new, dry 'Pall' 'Emflon' PFR filter cartridge incorporating a hydrophobic PTFE membrane, 25cm in length (Pall no. AB1PFR7PVH4), in a **Pall** stainless steel housing (part no. SASM011) and supplied by Pall Europe Limited, Portsmouth, UK. In principle, this test system can be applied to any sterilising-grade hydrophobic membrane filter.

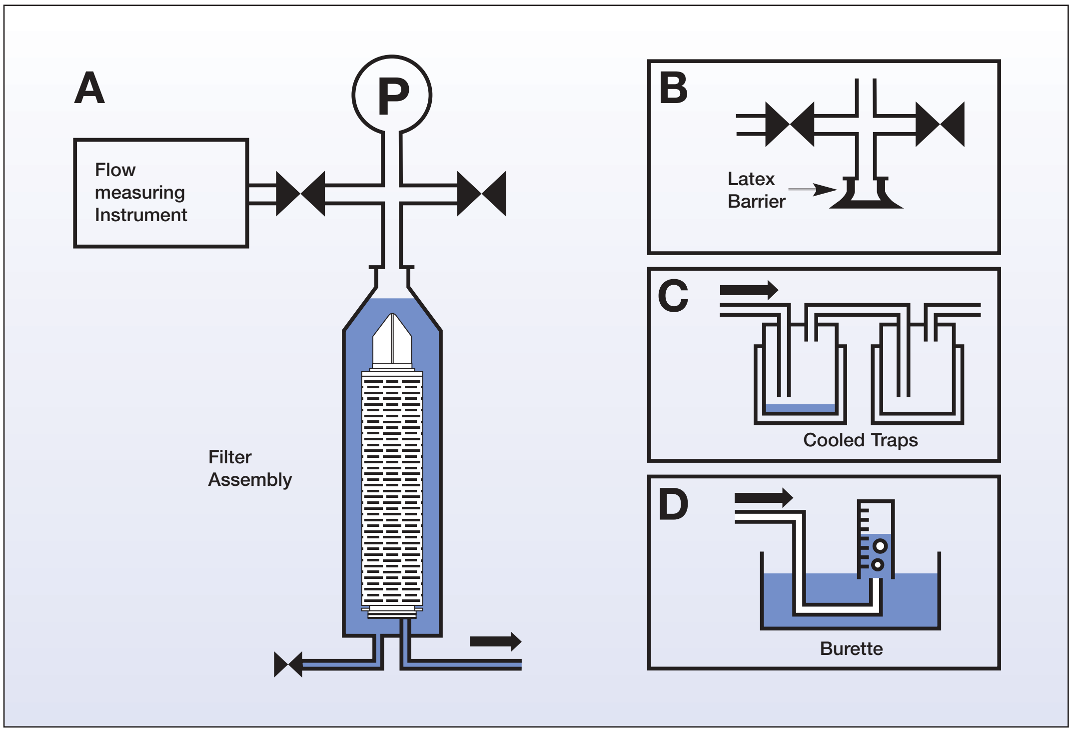

Figure 1: *System for performing the Water Intrusion Test*

Note: *A) Basic system*
B) Installation of a latex barrier between gas phase and water phase
C) Cooled traps installed downstream of the filter
D) Burette installed downstream of the filter

For specific tests the system was modified as given in **Figure 1B** and **1C**. **Figure 1B** shows the installation of a latex barrier. This barrier is installed by placing a latex balloon on top of the housing after filling with water and before pressurising the system. This barrier separates the water phase from the gas phase.

In **Figure 1C**, the two cooled traps (ice bath) shown are installed on the outlet of the filter housing. The first trap serves to collect the water passing through the filter, while the second trap excludes water vapour coming from the environment.

In **Figure 1D**, a burette is connected to the downstream side of the filter assembly and measures the volume of gas displaced by any transmembrane flow.

2. Flow Measuring instrument

The flow measuring instrument shown in **Figure 1** was a **Palltronic Flowstar** or a **Palltronic Truflow** (Pall Europe Limited, Portsmouth, UK). These devices pressurise the system on the upstream side and measure the flow into the system required to maintain this pressure.

3. Water

Unless stated otherwise, deionised water with a conductivity of 2-3 $\mu S/cm^{-1}$ was used.

For tests with water saturated with air, this water was prepared by bubbling air through the water for 30 minutes. Deaerated water, was produced by boiling the water then cooling it in a sealed container before use.

The conductivity of the water was measured using a conductivity probe type WTW LF90/KLE1 (WTW, Weilheim, Germany).

4. Temperature

The temperature of the water and the environment was 20 °C during the tests. For measurements at higher or lower temperatures, the filter assembly as given in **Figure 1A** was installed in a chamber with controlled temperature and the tests started when the temperature of the water and the environment were equal.

Results

Effect of pressure on water flow through a hydrophobic filter

In this study, the upstream flow was measured for a range of applied pressures.

The results are presented graphically in **Figure 2**.

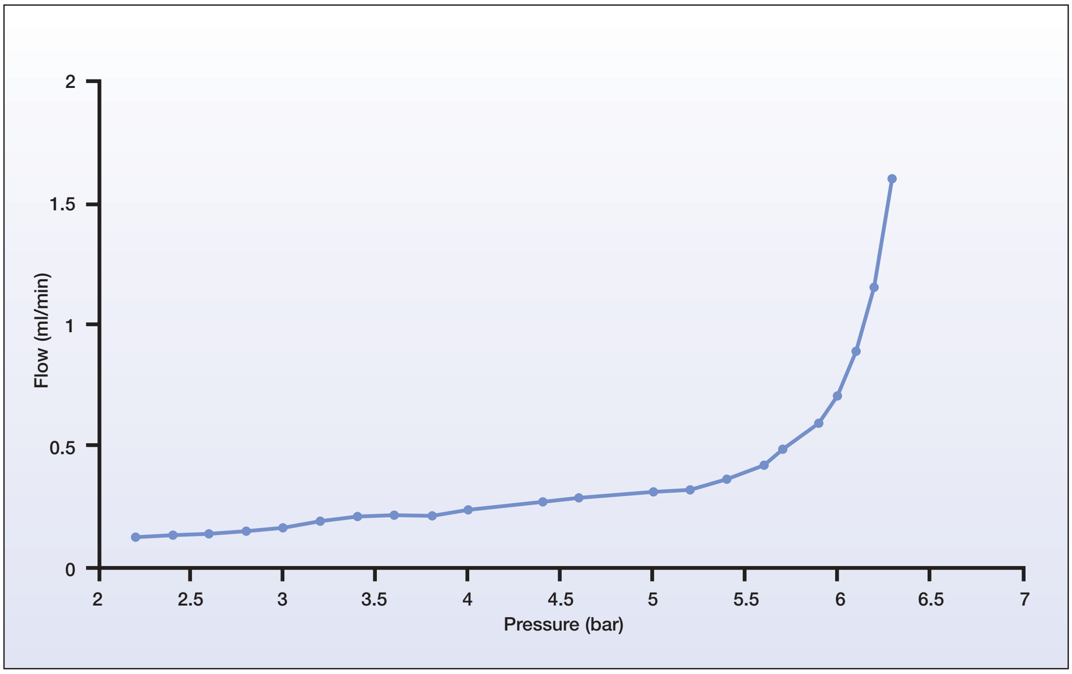

Figure 2: *Flow rate as a function of pressure*

At low pressures, a small but measurable flow was recorded. This stable flow increased with increasing pressure. In the region of 5.5 - 6.0 bar, a rapid increase in flow occurred. This non-linear increase is consistent with water breakthrough through the larger pores of the membrane. The pressure-dependent flow below this breakthrough zone must be associated with another mechanism.

As the water intrusion testing is performed at the lower end of this pressure range, typically 1.8 to 2.5 bar, it is important to understand the basis of the measured flow and the factors influencing it. Additional studies were therefore performed to investigate this further.

Measurement of Flow with Time

Measured flow on the upstream side of the filter could be explained by a number of effects. These can be more clearly understood when one considers the dynamics of the test sequence.

Pressurisation is achieved by the introduction of compressed air. It results in (1) compression of the pleated filter structure; (2) expulsion of air trapped in the membrane pleats; (3) cooling of the gas headspace following compressive heating (adiabatic effect); (4) water intrusion into the membrane structure; (5) dissolution of gas into the water, and (6) water flow through the membrane.

These effects were assessed by measuring the upstream flow as a function of time.

The results are given in **Figure 3**.

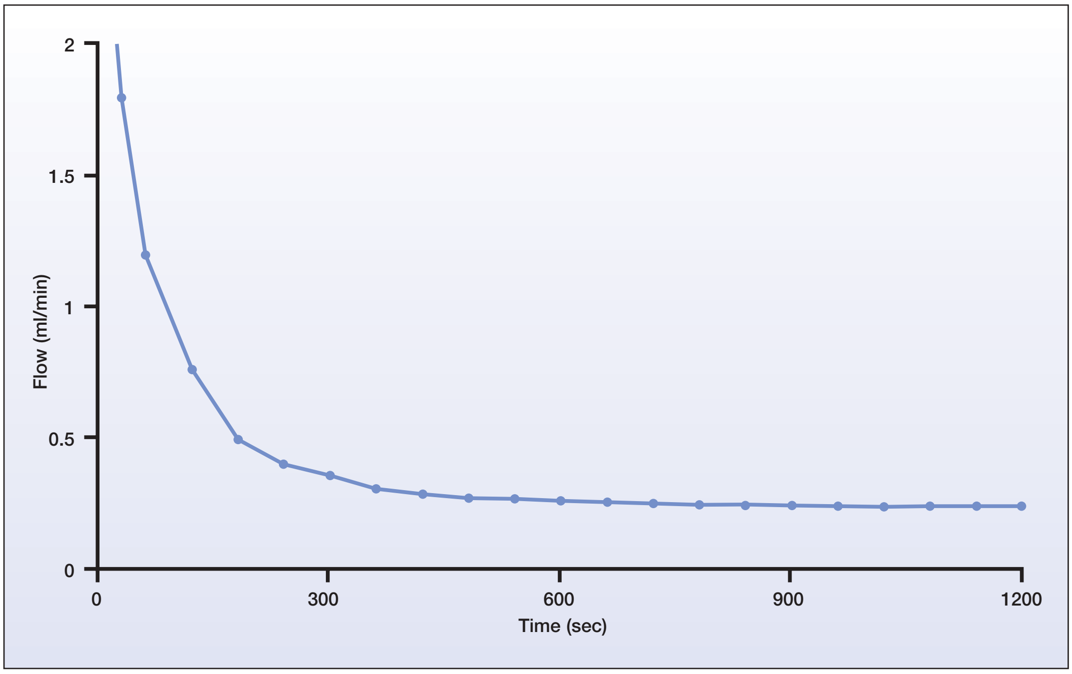

Figure 3: *Flow rate as a function of time*

During the early stage of the test, relatively high flows can be seen and all of the factors (1) to (6) noted above could contribute at this stage. However, the flow stabilises after approximately 20 minutes and then remains constant. This result and other extended time studies show that intact filters, after an initial stabilisation period, have a constant, measurable flow rate. This measurement cannot be explained by transient effects due to factors (1) to (4) listed above, as they would be expected to decrease to zero with time.

Further studies were therefore performed to assess whether this flow is associated with the transfer of gas into the water, or passage of water through the membrane.

Comparison of the flow measured upstream and downstream of the filter

The water intrusion test is performed on the upstream side of the filter, and one of the basic issues concerns whether the stable flow measured on the upstream side of the filter results directly from flow across the filter. To test this, a burette was installed on the downstream side to measure the displaced volume on the downstream side of the filter and to compare it to the result of the flow-measuring device on the upstream side.

The results, summarised in **Table 1**, show that the flow measured on the upstream side is directly related to a volume transfer across the filter membrane.

Table 1: Flow measured on the upstream and on the downstream side of the filter

Flow upstream (ml/min)	Flow downstream (ml/min)	Filter Serial No.
0.12	0.11	IA2417074
0.11	0.11	IA2417074
0.14	0.12	IA4294014
0.12	0.12	IA4294014
0.13	0.11	IA2417074
0.12	0.10	IA2417074
0.15	0.16	IA4294014
0.13	0.13	IA4294014

Influence of Impermeable Barrier at Gas/Liquid Interface and of the aeration of water on the Flow rate

It has been suggested that a mechanism for the flow across the filter membrane could be pressurised gas dissolving into the test liquid, diffusing into and through the liquid and passing through the membrane to the downstream side of the filter. This gas transfer could account for the measured flow.

In order to evaluate the contribution of gas transfer into the liquid phase on the measured flow, an impermeable latex barrier was placed between the test liquid and the gas headspace. Flow measurements with the barrier in place were compared to the results using the same filter without the barrier. The results of this study are summarised in **Table 2**.

Table 2: Effect of a barrier on water intrusion flow rates

Flow without barrier (ml/min)	Flow with barrier (ml/min)	Filter Serial No.
0.13	0.15	IA4294014
0.13	0.14	IA6279246
0.13	0.14	IA4294014

The results show that the barrier had no effect on the measured flow rate. If the background flow of an intact filter was due primarily to the dissolution and transfer of gas to the downstream side, a major reduction in flow would have been expected with the barrier in place.

Further studies were performed to evaluate the possible transfer of gas through the liquid phase by comparing the effects of aerated and de-aerated water. If background flow was associated with dissolution of gas, then significant differences in water intrusion flow rates would be expected between the two test liquids.

The water intrusion results are presented in **Table 3**, and show that the level of aeration has no significant effect on the flow after stabilisation.

Table 3: Effect of water aeration on the measured flow

Water Quality	Measured Flow (ml/min)
Aerated	0.16 (Test 1) 0.16 (Test 2)
Deaerated	0.16 (Test 1) 0.17 (Test 2)

These data are consistent with the results obtained using the impermeable barrier and suggest that any dissolution of gas into the water phase does not contribute significantly to the measured flow of an intact filter.

It was therefore concluded that the measured flow was due primarily to passage of water in vapour or liquid phase through the membrane.

Detection of Water on the Downstream Side

The upstream flow rates measured on intact filters after stabilisation are typically in the order of 0.2 to 0.3 ml/min for a 25cm length filter. Furthermore, over the time span of the test, free water is not normally observed on the downstream side.

In order to establish the presence of water downstream it was necessary to ensure that both free water and water vapour could be collected in a controlled way. This was achieved using the equipment shown in **Figure 1C**.

A water intrusion test was performed on an intact filter. Because of the low flow it was necessary to extend the test time to 300 minutes to collect a significant amount of water on the downstream side.

Initial tests using potable water (that is, not de-ionised) showed that the water collected downstream had lower conductivity than that upstream. This suggested a possible mechanism involving vapour-phase flow through the membrane. To test this theory further, a 0.2% sodium chloride solution with high conductivity was used as the test liquid and the experiments repeated. The results are shown in **Table 4**.

Table 4: Conductivity of water upstream and downstream of the filter

Conductivity (µS/cm^{-1})	
Upstream	**Downstream**
3900	100

The water collected downstream was of much lower conductivity than the sodium chloride solution, indicating the transfer of water molecules through the membrane but not sodium chloride ions. These results support the theory that the stabilised flow for an intact filter is primarily due to evaporation of water within the membrane, and vapour-phase flow through the membrane to the downstream side. This process results in the loss of water volume from the upstream side which can be measured under constant pressure conditions as flow on the upstream side.

The conductivity of the water collected downstream was higher than would be expected for pure condensate. This can be explained by the contribution of ionic contaminants in the downstream system.

Influence of Ionic Content of Water on Measured Flow

Previous investigations (5) showed a relationship between the water quality and the measured flow. Further studies were therefore performed to understand the possible reasons for this relationship. A series of tests was performed on the same filter using liquids with a wide range of conductivity, achieved by adding Sodium Chloride to the water. The results are shown in **Table 5**. The surface tension of the test liquids was also measured to ensure that this was not a contributory factor.

Table 5: Influence of the conductivity of the water on the measured flow

Conductivity ($\mu S/cm^{-1}$)	Surface Tension (dyn/cm)	Flow (ml/min)
0.2	71.9	0.18
2.3	70.6	0.15
727	70.5	0.12
3900	68.5	0.07
15700	67.1	<0.04

The results confirm that the ionic content of the water has a significant influence on the measured flow. Increasing ionic strength and conductivity reduces the flow rate. This would be expected if the measured flow of an intact filter is primarily due to evaporation, as higher ionic strength would lead to lower evaporation. This would not be expected if flow of liquid water was the primary mechanism.

This study also shows the importance of controlling the quality of water used for routine testing. De-ionised water in the form of Purified Water or WFI is the preferred choice as it is readily available and appropriate for pharmaceutical use.

Effect of Water Temperature on Measured Flow

The influence of water temperature on the water intrusion test is unclear from earlier publications. Studies reported in 1990 (4) show a decrease in flow with increasing temperature while other studies (6) indicate an increase in flow with increasing temperature. Tests were performed at different temperatures but under conditions where the environmental temperature and water temperature were equal. In this way, any effects of environmental temperature were eliminated.

Results of these studies are summarised in **Figure 4.**

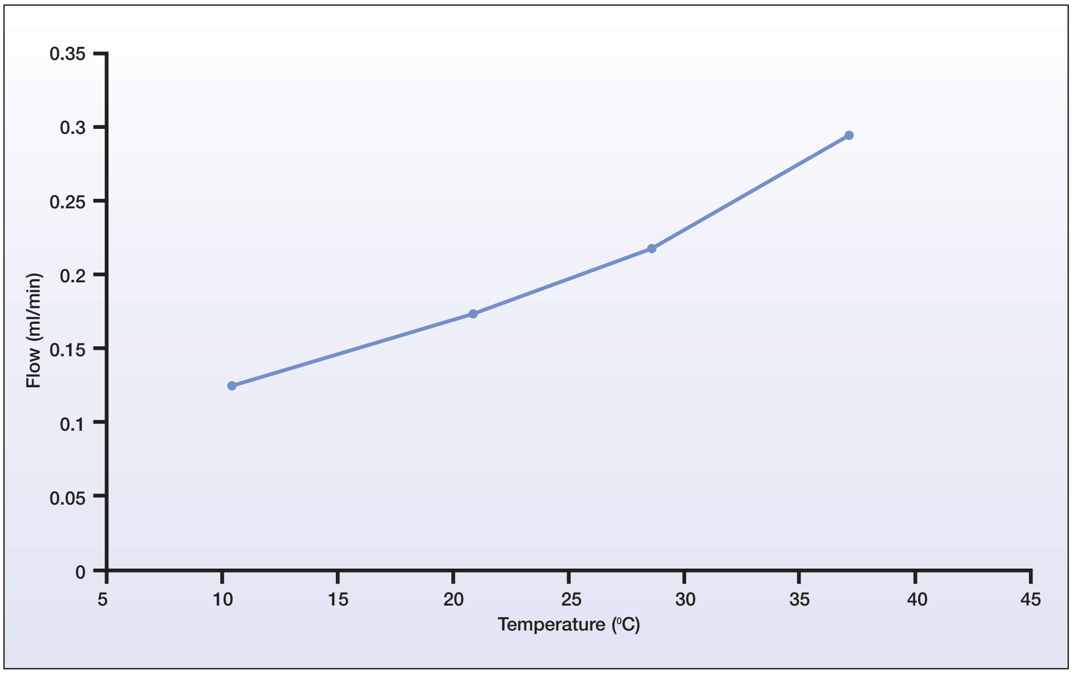

Figure 4: *Flow rate as a function of temperature*

The data show an increase in flow with temperature. This provides further evidence in support of an evaporative mechanism for intact filters, as the rate of evaporation would increase with temperature.

The results also show that water temperature must be considered when establishing test parameters for the water intrusion test.

Discussion

Two explanations have previously been proposed for the observed flow on the upstream side of an intact filter during a water intrusion test: water intrusion into the membrane structure (4), and pleat compression (7).

Water intrusion into the membrane structure may occur to some extent during the initial pressurisation stage but would be expected to be complete in a short time. The measured flow would then become zero. In practice, this does not occur and therefore intrusion into the membrane structure cannot explain the continuous and stable flow that is measured over extended time periods.

Pleat compression occurs during the initial stabilisation phase of the test due to the high differential pressure at which the test is performed. The compression is detected as a flow upstream. However, this process must eventually stop when full compression has been reached. Compression effects cannot therefore account for the continuous and constant flow observed after stabilisation.

The data presented in this paper instead support the theory that the continuous measurable flow on the upstream side of an intact filter is primarily caused by evaporation of water across the membrane. When the hydrophobic filter is covered with water and the water is pressurised, evaporation will occur within the membrane. The resulting vapour will pass through the membrane to the downstream side of the filter. This results in water loss from the upstream side which can be measured by sensitive flow-measurement instruments.

This theory is supported by the following results. In the first instance, the conductivity of the water collected downstream of the filter is significantly lower than the conductivity of the water on the upstream side. This would not be the case if the flow was primarily due to liquid flow through larger pores of the membrane.

Secondly, the ionic content of the test fluid has a strong impact on the flow. Flow is reduced with increasing ionic concentrations. As evaporation is known to be reduced with increasing ionic concentration, this supports the theory that the primary flow mechanism is based on evaporation.

Third, the water temperature has a direct influence on flow. Flow increases with increasing temperature. Since rate of evaporation also increases with increasing temperature, this is consistent with an evaporative mechanism.

The results presented in this study explain why intact filters show a small but measurable flow when tested by the water intrusion test. Filters with oversized pores will show a higher flow due to direct flow of liquid water through the membrane.

This improved understanding of the scientific basis of the test allows the factors influencing the test to be controlled and enables a reliable correlation to be established against a destructive bacterial challenge test (8,9).

Practical guidelines on how to perform the test in a production environment are also available (9).

Conclusions

The uncertainties surrounding the basis of the water intrusion test have been removed by this study. The role of evaporative flow in intact filters has been clearly demonstrated and other factors influencing the test have been identified.

The latest developments in flow measurement instrumentation can accurately measure the low flow rates associated with water intrusion testing. With suitably validated test devices used under controlled conditions, the water intrusion test can be used as a reliable integrity test for hydrophobic filters.

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PELEH/C.S/02.2002