Integrity testing

Application Support Publication

aerospace
climate control
electromechanical
filtration
fluid & gas handling
hydraulics
pneumatics
process control
sealing & shielding

Parker domnick hunter

ENGINEERING YOUR SUCCESS.

Parker domnick hunter Process Filtration

Setting the standard

Parker domnick hunter brings extensive experience through our Scientists, Engineers and sales representatives to the process of offering specific filtration systems to meet the needs of your production process. Support services are available covering a wide range of activities including scale-up advice from laboratory through pilot scales to production systems, validation support, design and manufacturing of custom housings and filtration products and on-site technical support.

Committed to quality

Quality is of paramount importance to Parker domnick hunter. As such we have been certified to ISO9001 since 1987, providing a quality management system that covers the entire organization including R & D, production, warehousing, materials management and customer support. In addition, our manufacturing facilities operate to the principles of cGMP.

This commitment is underlined by our registration to ISO 14001 and certification to current ISO9001.

Validation and product certification

To certify that Parker domnick hunter products meet the required regulatory and quality standards of the industries that we supply, all filters are supplied with a certificate of conformance. These certificates are linked to validation documents for both prefilter and sterilizing grade membrane filter products that define methodologies and data appropriate to each filter type. This information typically includes:

- Technical specifications.
- Biological safety testing including current USP <88> Class VI - 121 °C Plastics.
- Extractable testing including 21CFR211.72 and 210.3(b), 6 for fibre releasing filters.
- Purified water filtration quality including TOC, bacterial endotoxins, conductivity and particle release.
- Chemical compatibility information.
- Thermal stability.
- Correlation of an appropriate non-destructive integrity test to a defined bacterial challenge.
- Where appropriate this data is included in Parker domnick hunter's Drug Master File No. 7564 held at the US Food and Drug Administration repository.

Validation support services

Parker domnick hunter has extensive laboratory facilities and trained personnel capable of providing a range of validation services to support manufacturers in meeting their requirements for process validation relating to the use of filtration products.

Contents

Introduction

Microporous filter products are used by a number of industries to achieve required levels of purity in both gases and liquids. The verification of filter performance has been identified as an important process monitor in several industries, especially in those applications where product sterilization or microbial bioburden reduction is required. This has led to the development of a number of integrity test procedures, based around industry preference such as in the beverage sector or regulatory definition as in the pharmaceutical industry.

Integrity testing offers non-destructive methods for proving the capability of a filter product to meet its stated performance when it is installed in an application. This gives confidence to filter users that both before and after filtration processes the installed filter will achieve the required performance. In particular, integrity tests enable users to verify that no filter damage has occurred during storage, installation into the filter housing or following procedures such as chemical cleaning or in-situ steam sterilization prior to use or during the subsequent manufacturing process.

The integrity test principle

Establishing correlation to bacterial challenge data

During the development phase for new filter products, filter cartridges are validated for performance to industry defined standards.

A sterilizing grade filter is defined by the pharmaceutical industry as rated at 0.22 micron or less, capable of producing a sterile filtrate when challenged with 10^7 cfu (colony forming unit) of *Brevundimonas diminuta* ATCC® 19146 per cm^2 of effective filtration area (EFA) under process conditions.

This is the only specification that is currently documented in regulatory guidelines, however other filter ratings are assessed similarly using different challenge organisms, for example:

0.1 micron	*Ralstonia picketti*
0.45 micron	*Serratia marcescens*

As part of the validation process in the development of a membrane material and subsequently a filter product, a large number of samples are taken from the manufacturing process and subjected to bacterial challenge with the organisms identified above. The process for performing a microorganism challenge with *Brevundimonas diminuta* is defined in ASTM (American Society for Testing and Materials) standard F838-05. This standard describes the 'Standard Test Method for Determining Bacterial Retention of Membrane Filters Utilized for Liquid Filtration'. The ASTM F838-05 guideline provides guidelines on the testing methodology, the organism type (how it is grown, correct size, viability, etc.) and the challenge level.

Prior to bacterial challenge of each filter product, it is tested using an appropriate non-destructive integrity test. The data obtained is used to establish a correlation between the non-destructive test value and the sterility of filtrate produced.

The objective of this 'correlation' process is to identify the boundary value, given by the non-destructive test for a filter, below which filters consistently provide a sterile filtrate following bacterial challenge. Once this boundary value for the non-destructive integrity test has been identified, it is usual to apply an additional safety factor in defining the user integrity test limit. Parker domnick hunter applies a safety factor, such that the recommended value for the integrity test limit is 85% of the highest value at which a sterile filtrate was obtained during the bacterial challenge. The results of these tests are usually displayed in a chart similar to the one shown in figure 1.

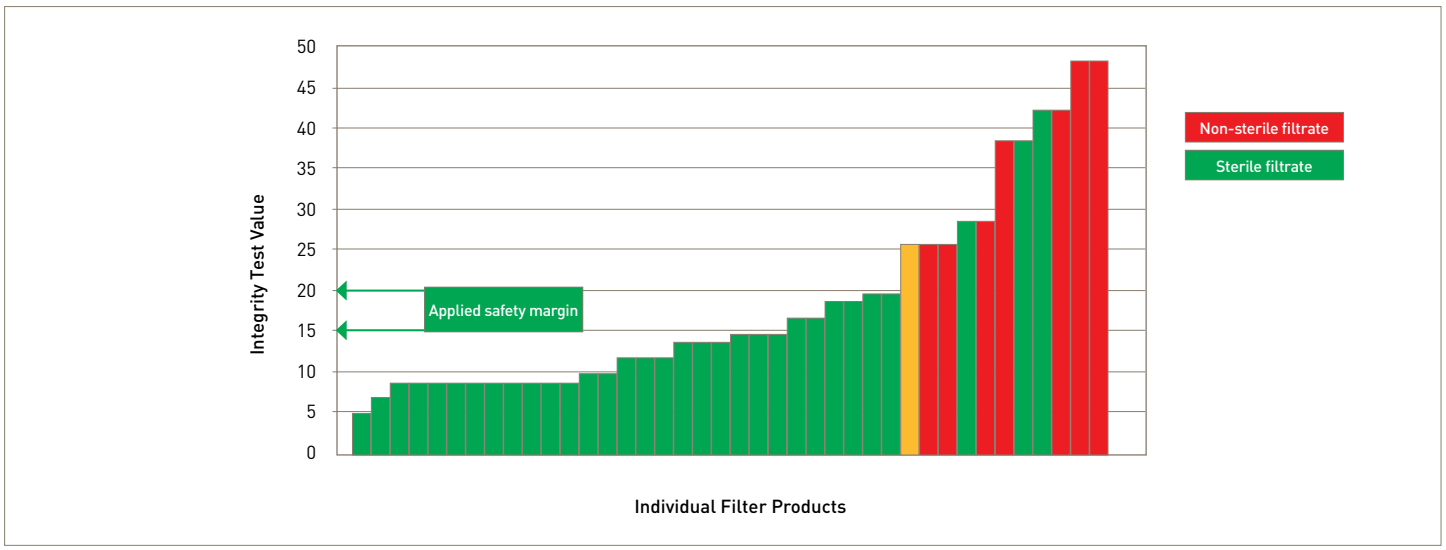


Figure 1 - Correlation of bacterial challenge to integrity test values

In this example, the boundary integrity test value was 20 mL/min, below which all filters test produced a sterile filtrate. Following the application of an additional safety margin, the user integrity test limit for this product would be set at 15 mL/min..

The correlation data and validation work for each specific filter type can be found in the validation guides.

More in depth information can be found in following PDA Technical Reports:

- *PDA Technical Report No. 26 Sterilizing Filtration of Liquids*
- *PDA Technical Report No. 40 Sterilizing Filtration of Gases*

Integrity test methods

There are three integrity test methods used to verify the performance of wettable filters (hydrophobic and hydrophilic membrane filters). There are also two methods solely for the use of hydrophobic filters. This is shown in table 1. The methods are detailed in Liquid integrity tests and Aerosol integrity test.

Integrity test	Bacterial challenge test	Use
Bubble point	Liquid	Hydrophobic and hydrophilic membrane filters
Diffusional flow	Liquid	Hydrophobic and hydrophilic membrane filters
Pressure decay	Liquid	Hydrophobic and hydrophilic membrane filters
Water intrusion	Liquid	Hydrophobic membrane filters
Aerosol penetration	Aerosol	Hydrophobic membrane and depth filters

Table 1

Liquid integrity tests

Physical principals

Surface tension and capillary action

Surface tension causes the liquid to behave like an elastic sheet, holding the liquid within the pores of the membrane (different liquids have different surface tensions).

If a material is hydrophobic this means that it does not possess an affinity to water, which leads to beading on the surface of the material. This is caused by the cohesive forces of the water being greater than the adhesive forces of the material. To fully wet out a hydrophobic filter a low surface tension liquid such as a solvent (IPA, ethanol) needs to be used.

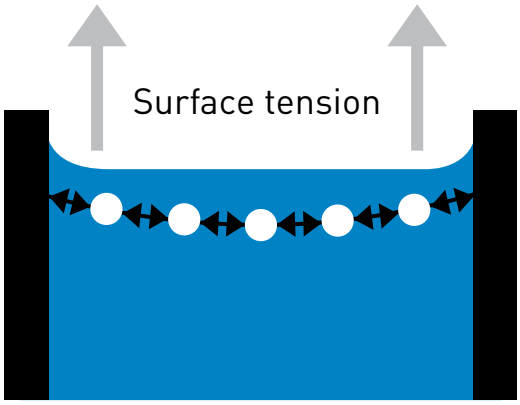


Figure 2 - Surface tension

Capillary action is a result of adhesion and surface tension. Adhesion of the wetting solution to the walls of the capillary tube will cause an upward force on the liquid at the edges i.e. it is the ability of a liquid to be drawn into a fine tube or pore.

The smaller the diameter of the tube the higher the liquid will be drawn up the tube or pore, as the surface tension is greater across the smaller liquid surface. As a result of surface tension the solution used to wet out the filter membrane will impact upon the test result.

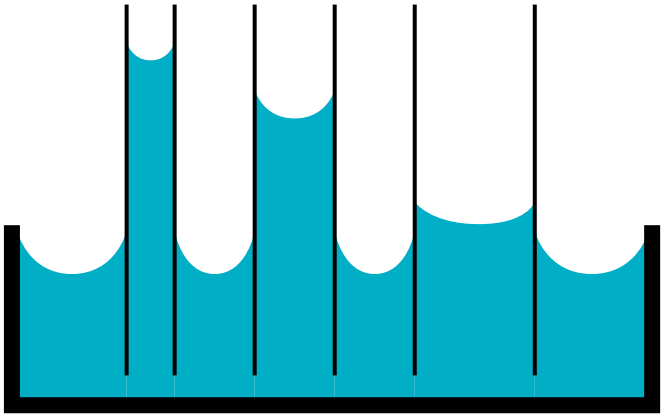


Figure 3 - Capillary action

Bubble point equation and pore size

Bubble point is based on the fact that liquid is held in the pores of the filter by surface tension and capillary forces. The minimum pressure required to force liquid out of the pores is a measure of the pore diameter. The pressure required to force liquid out of a liquid-filled capillary must be sufficient to overcome surface tension and is a direct measure of effective tube diameter.

This is described in the bubble point equation (derived from the Young–Laplace equation , i.e. a nonlinear partial differential equation that describes the capillary pressure difference sustained across the interface between two static fluids, such as water and air, due to the phenomenon of surface tension.)

$$P = \frac{4\kappa\gamma\cos\Theta}{d}$$

Where:

P = the differential pressure at which a given pore will open
κ = correction factor for the shape of the largest pores
γ = the surface tension of the wetting liquid
$\cos\Theta$ = the contact ('wetting') angle between the liquid and the membrane (the angle at which liquid / vapour inter phase meets a solid surface)
d = the diameter of the largest pores

If the wetting liquid and membrane surface chemistry are held constant the relationship between pore diameter and pressure required to free the wetting liquid is inversely proportional. This is the bubble point, and this therefore reflects the set of largest pores in the membrane.

$$d = \frac{\kappa_2}{P}$$

Where:

d = the diameter of the largest pores
κ_2 = correction factor for the shape of the largest pores
P = the differential pressure at which a given pore will open

From the bubble point equation we can see that, for example, if one pore is $^1/_4$ the diameter of another, you require four times the pressure to eliminate the wetting agent from that pore.

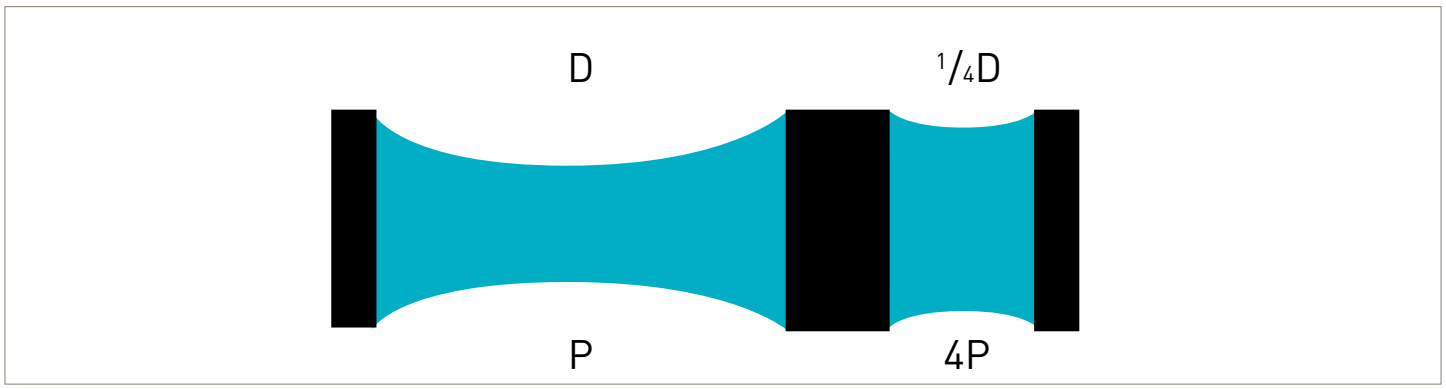


Figure 4 - Pore diameter in relation to bubble point pressure

Diffusion

When the filter membrane is fully wetted, all the pores within the membrane are filled by the wetting solution. This wetting solution is held in place by surface tension and associated capillary forces. These forces create a barrier between the upstream and downstream of the filter membrane.

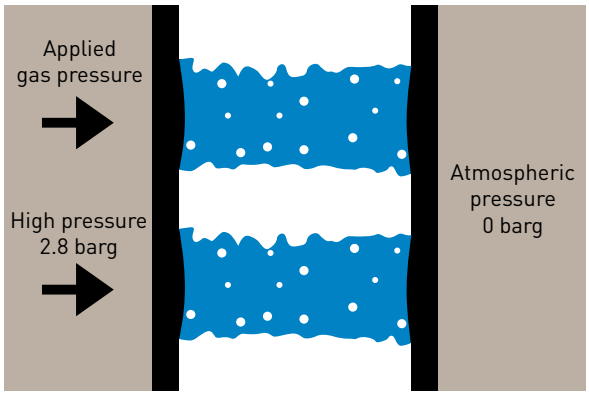


Figure 5 - Gas diffusion through a wetted membrane

When air or nitrogen pressure is applied to the upstream of a wetted filter the gas molecules on the high pressure upstream dissolve in the liquid layer, as per Henry's Law which states that a gas dissolves in a liquid in proportion to its partial pressure over the liquid. The dissolved gas will diffuse to the downstream side, which is at atmospheric pressure. As the gas passes across the pore depth the pressure drops and the gas comes out of solution under the lower pressure, according to the same law – this is the diffusive airflow.

The rate of gas diffusion through the liquid follows Fick's Law, re-modelled by Reti (1977), that states:

$$N = D\ H\ (p1 - p2)\ \rho / L$$

Where:

N	= permeation rate
D	= diffusivity of the gas in the liquid
H	= solubility of liquid in the membrane (= the depth of the membrane if the pores are filled with liquid)
(p1 - p2)	= transmembrane pressure
ρ	= total membrane porosity
L	= depth of the liquid

This results in the diffusion rate being directly related to the applied differential pressure and is therefore linear for a given value of the other variables.

Typical gas flow curve

The basic principle used in most methodologies is the measurement of a gas flow due to an applied pressure differential through a fully wetted filter media. This principle is usually applied only to membrane based filter products. This is because the pore structure and size distribution is normally sub-micron and the mass flow of gas measured across the membrane through the structure can be accurately measured with enough sensitivity to differentiate between a 'good' and 'bad' structure.

Consider a microporous membrane material wetted thoroughly by a suitable wetting fluid. All the pores are filled with the wetting liquid and held in the pores by surface tension and associated capillary forces.

If a differential pressure is now applied using an applied gas pressure to one side of the wetted membrane, two things can happen:

1. The gas can dissolve in the liquid in the pores -
   The amount of gas that dissolves is dependent on the solubility of the gas in the liquid and also the applied pressure. This phenomenon results in a high concentration of dissolved gas at the pressurised side of the membrane, and a low concentration at the low-pressure side. The gas molecules therefore diffuse across the pore structure to the low-pressure side due to this concentration gradient and come out of solution on the low-pressure side. The resulting transfer of gas is measured as a gas flow is therefore referred to as diffusional flow.

2. If pressure is increased high enough the wetting liquid can be forced from the larger pores -
   The wetting liquid is held in the pores by capillary forces. These forces are built up due to surface interactions between wetting liquids and the polymers that make up the membrane. If sufficient external force is applied, for example, by an applied gas pressure, then the capillary forces can be overcome and the pores can be totally emptied of wetting liquid. At this point the surface of the membrane is seen to bubble, as the escaping gas flows directly through a shallow pool of the liquid on the downstream side, hence the term bubble point. The larger the pore the lower the force required to vent it of liquid. Therefore, theoretically, the first bubble seen indicates the largest pore in the membrane structure.

The flow through a wetted membrane filter can therefore be summarized by three distinct zones.

At low applied pressure, the increase in flow rate is almost linear when plotted on log / log axes with applied differential pressure – this is the Diffusional Flow Zone.

As the pressure approaches the 'bubble point', the curve turns non-linear as greater numbers of pores are vented by the applied pressure – this is the Transitional Flow Zone.

Once all of the pores have been vented, the curve returns to virtually a linear form on log / log axes – this is the Mass Flow Zone.

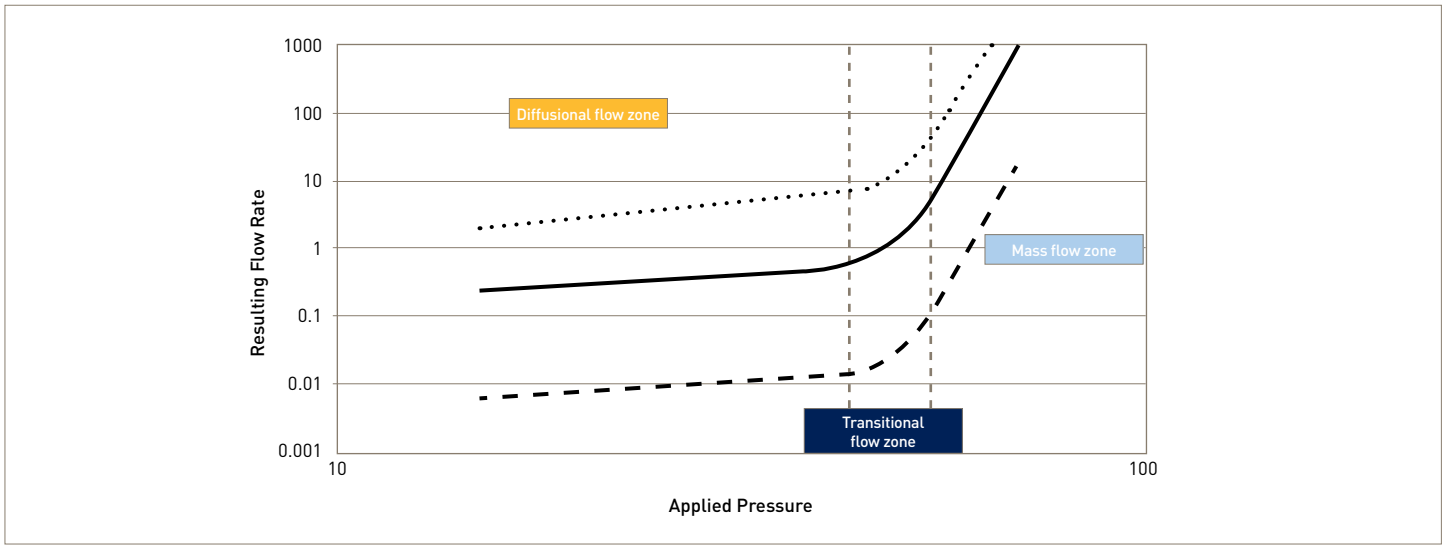


Figure 6 - Gas flow through a wetted membrane

Three simple curves plotted on log / log axes are illustrated in Figure 6 showing the flows for three different areas of membrane. It is evident from the curves that the distribution of the pore size in a membrane will determine how short the transitional zone will be. If the distribution is narrow, then all the pores will vent at approximately the same applied pressure and the 'knee' in the curve will be sharp. The wider the distribution, the broader the knee in the curve will be.

The above principles have lead to the development of two distinct integrity test methods for liquid membrane filters:

- determining the bubble point of a membrane *or*
- determining the diffusional flow of gas across a membrane.

Liquid integrity test methods

Bubble point

The bubble point is the minimum applied pressure required to vent the largest pores i.e. the pressure at which the liquid is ejected from the largest pores, thus allowing mass flow of gas.

This test is carried out by connecting a compressed air supply to the upstream side of the wetted filter, increasing the applied pressure and then monitoring either downstream visually in a manual test or upstream using an automated tester, such as the PORECHECK IV, which allows measurement of the gas flow rate and can therefore identify sudden changes in a much more accurate and repeatable way.

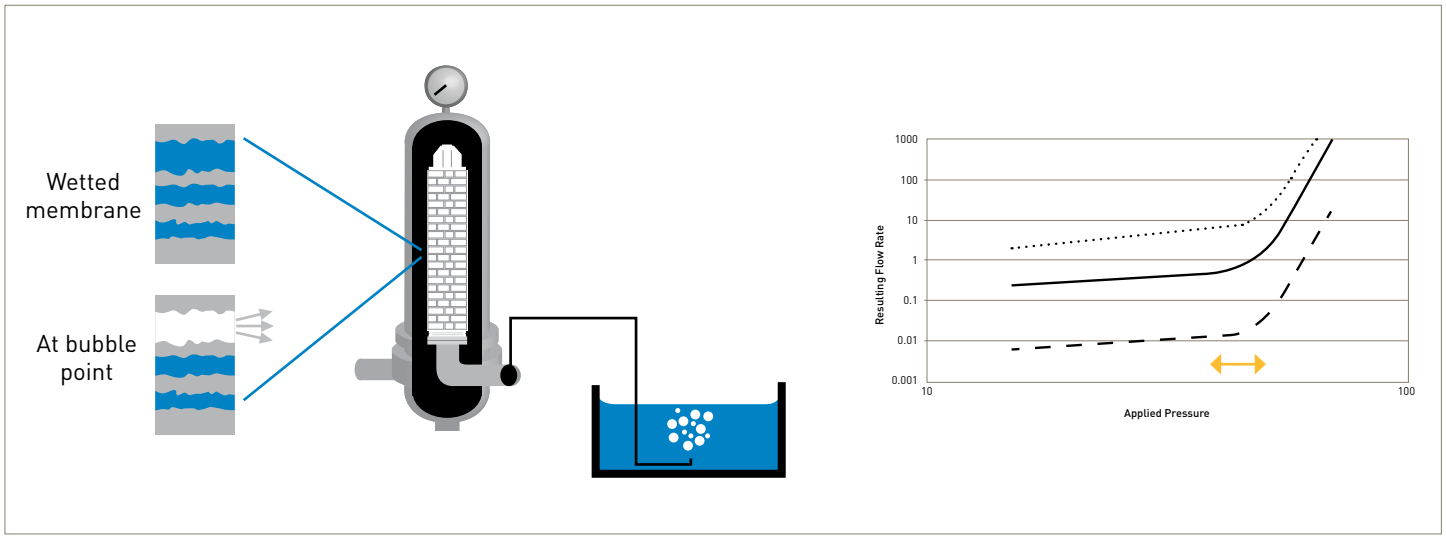


Figure 7 - Bubble point set up

Different Wetting Fluids

The surface tension of the wetting fluid will affect the bubble point directly. If a filter has been used with a specific liquid product, remnants of that product may be difficult to remove from the filter media, or that product may have a direct effect on the surface chemistry of the filter media. If any product fluid still remains in the filter, then once wetted with water this may effect the surface tension. Also, if there are changes in surface chemistry in the filter media due to contact with the fluid this may change the wetting angle between the liquid and the membrane and hence the bubble point. To overcome these difficulties and avoid the requirement for prolonged flushing protocols, it may be prudent to correlate bubble point values in water for a particular filter to bubble point values in the product fluid to be filtered. The Parenteral Drug Association (PDA) Technical Report No. 26 – Sterilizing Filtration of Liquids revised in 2008 gives a protocol to allow this correlation to be produced.

Issues regarding Bubble point testing

- Parker domnick hunter does not recommend the use of bubble point as an integrity test method for cartridges as the bubble point value has not been directly correlated to bacterial challenge testing. However the values are given for use as an indicator of product integrity.
- Less sensitive compared to Pressure Decay/Diffusional flow, especially for larger multi-round housings.
- Traditional / manual method requires a downstream connection which is often not acceptable. Test instruments avoid the use of a downstream connection.
- The test cannot be used for testing sterilizing grade filters rated at 0.1 µm and higher as the bubble point pressure can be significantly higher than the rated operating pressure of the membrane. The bubble pointing of the product could end in physical damage of the membrane or in the case of capsule product, the product housing and seals.
- Different filter areas : The size of a filter does not effect the intrinsic bubble point of the material used to construct it. Generally however, a larger area or filter material will give an indicated bubble point lower than expected compared to a smaller area in manual tests.
- Temperature changes impacts the surface tension of the wetting fluid, changing the bubble point. However an increase in temperature also raises the upstream pressure.
- Rapid changes in pressure : The procedure to find the bubble point of a particular membrane based filter requires the pressure to be raised. The rate at which the pressure is increased can have a dramatic effect on the measured bubble point. The more rapid the pressure increase the less time the system has to stabilise and due to lags in the diffusional flow equalisation rate and the sampling system if downstream monitoring is used, the more likely it is to measure a false high (good) bubble point. Standardized protocols and automated test equipment mitigate against this potential source of error.

- The higher the hydrophobicity of the membrane and other constructional materials for the filter, the greater the potential for variation in bubble point due to incomplete wetting. Not all filters of the same ratings will exhibit the same bubble point. This is due to the wetting angle differences with each polymer and liquid combination. Also remember that rating is usually measured against an ability to remove a specified organism from a fluid stream, not necessarily the effective pore size.
- Automated testing algorithm differences : Automated bubble point test equipment supplied by different manufacturers may determine the bubble point using different physical parameter measurement e.g. using measurement of pressure loss or measurement of direct mass flow. Instruments using the same principle may also potentially use different algorithms to calculate the bubble point e.g. the pressure at which the flowrate starts to go non- linear compared to the pressure at which extrapolations of the linear portions of the flow curve intersect. This can result in slight differences in measured bubble point comparing one manufacturer's machine to another and variation depending upon which method or machine was applied to producing the initial correlation to bacterial challenge data.

Diffusional flow / pressure decay

Diffusional flow based tests operate with applied pressure to measure gas flows in the diffusional zone.

There are three different measurement methods used for diffusional flow:

- Pressure decay / pressure hold measurement
- Diffusional flow measurement from pressure decay
- Diffusional flow measurement by mass flow

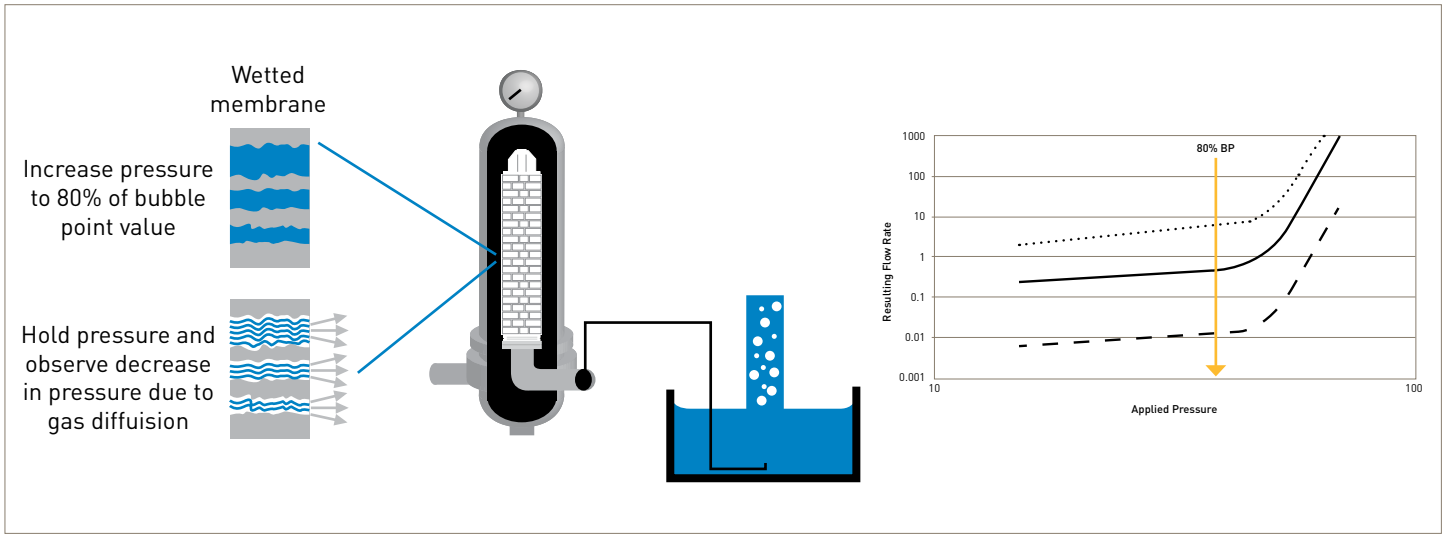


Figure 8 - Diffusional flow / pressure decay set up

Pressure decay / pressure hold

This, the most commonly adopted method, measures the gas pressure loss from a pressurized, sealed upstream volume due to diffusional flow across a wetted membrane filter . The gas pressure loss is equated to a gas flow rate through the filter membrane via the Ideal gas law. The upstream volume and maximum allowable diffusion rate need to be known to calculate the pressure decay limit.

$$\text{Ideal gas law:} \quad P \times V = n \times R \times T$$

Where:

P	= pressure
V	= volume
N	= amount of substance of gas
R	= universal gas constant
T	= temperature
P x V	= constant (when T = constant)

During the pressure decay test, gas initially contained in a closed system of known volume V1 at pressure P1 is left for a set time to diffuse out of the system through a wetted filter. The change in pressure P (P2) then relates directly to the mass of gas lost (V2) through diffusion, and if the time is known the measured pressure decay can be compared to the maximum allowable pressure decay.

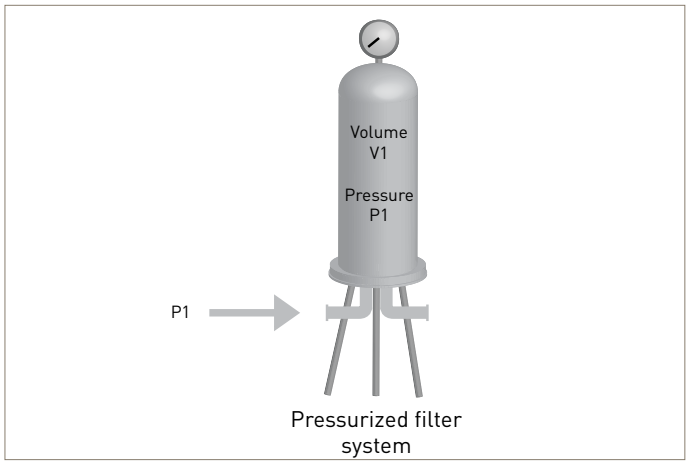


Figure 9 - Stabilization period

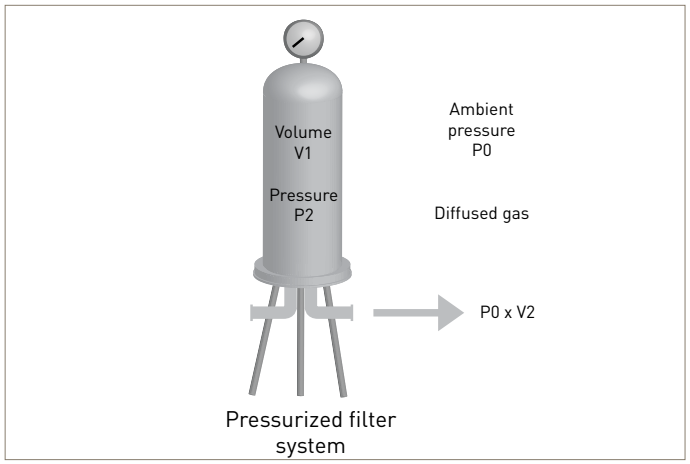


Figure 10 - Test period

$$P1xV1 = P2xV1 + P0xV2$$

Where:

P1 = upstream start pressure
P2 = upstream finish pressure
P0 = atmospheric pressure
V1 = upstream volume
V2 = diffused gas volume

$$(P1-P2) = \frac{V2\ xP0}{V1}$$

$$\text{and diffusion rate} = V2/\text{time}$$

$$\text{Pressure decay (dP)} \sim \frac{\text{Diffusion rate}}{\text{Upstream volume}}$$

Diffusional flow measurement from pressure decay

Indirect measurement of diffusional flow is achieved by including in the measurement system a method of measuring the upstream volume. This removes the uncertainty of the Pressure Decay method, which relies on an accurate volume being already known.

The Parker domnick hunter PORECHECK 4 incorporates a procedure for physically measuring the upstream volume. This is achieved by filling a vessel of known volume (located within the instrument) with compressed air or nitrogen gas and then venting it into the upstream void. The new pressure resulting in the upstream void added to the defined volume can allow the upstream volume to be calculated by the use of Boyle's law (pxV= k). A pressure decay test can then be run as normal, and the loss in pressure related directly back to a volumetric flow of gas, the diffusional flow.

Diffusional flow measurement by mass flow

Direct measurement using mass flow transducer technology can eliminate the requirement for knowing the upstream volume of the system being tested. Mass flow sensors can be used to measure the gas flow directly at a constant maintained pressure differential. This technique is currently only limited to a few particular test instruments and is not applied widely.

Advantages of diffusional flow and pressure decay

- Highly sensitive test : The diffusional flow test methods assess the effective porosity of a filter membrane. Any flaw in the membrane will be identified due to an increase in diffusional flow. Any change in structure not only associated with the 'largest' pore as measured by the bubble point method will also be identified.
- Diffusional flow data from Parker domnick hunter filters are correlated to a bacterial challenge and have a high margin of safety.
- Tests the complete filter system - cartridge and housing.
- Upstream test method - no downstream test point required.
- Measurements of high area filters: increases in the area of a filter membrane in a product can give problems with the identification of the bubble point, as the impact of the high levels of diffusional flow blur the transition point. Most manufacturers will recommend the use of diffusional flow for larger filter systems.

Issues of diffusional flow and pressure decay

- The pressure decay result is dependent on the housing upstream volume plus any associated pipe work to be known accurately. Using the wrong upstream volume can lead to false results being recorded. Parker domnick hunter supplies details of Parker domnick hunter housing volumes with Parker domnick hunter filter products. In cases where alternative suppliers have supplied the housings, this data may not be readily available.
- Any temperature fluctuations affect the result (more than bubble point testing). A temperature increase will mask the pressure drop by an increase in the upstream pressure due to the temperature, this can create a false positive integrity test result. An upstream pressure change caused by temperature changes can be calculated as follows:

$$dP = Ptest\ (T1-T0)\ /\ T0\ \text{(based on Gay-Lussac's law : P/T =k)}$$

Where :

dP = upstream pressure change (mbar)
Ptest = diffusion test pressure (mbar)
T0 = absolute temperature (K) at time 0
T1 = absolute temperature (K) at time t

A temperature change in the system from 20 °C to 21 °C at a test pressure of 2.8 barg results in a pressure change of 9.6 mbar, a significant quantity!

Water intrusion testing

The water intrusion volume is the volume of water that penetrates (intrudes) into the structure of a hydrophobic membrane at a given applied pressure (typically held for 10 minutes).

The water intrusion test (WIT) or water flow test (WFT) has been designed to eradicate the need for solvent wetting with a 'contaminant' such as IPA (isopropyl alcohol) or ethanol to integrity test hydrophobic PTFE membrane filters. Other liquid based tests for hydrophobic PTFE membrane filters require the membrane to be wet out with a solvent prior to testing.

The use of IPA, especially if attempting to test multi-round systems can also pose a health and safety issue due to flammability.

The water intrusion test method measures the dry membrane's resistance to wetting with DI /WFI grade water during an applied gas pressure.

Definitions

- Water flow is a measure of the actual water volume change at test pressure, which is ~ 3.5 times lower than water intrusion and is expressed in µL/t or mL/t (where t = time).
- The water intrusion value is referred to as the increase in compressed gas volume, as a result of water flow, normalized to atmospheric pressure and expressed in NmL/t (where t = time).

The test methodology for water flow and water intrusion is identical, it is only the expression of the result and the limits that differ.

Practical principle

During a water intrusion test the upstream volume of the filter housing is flooded with DI / WFI grade water and pressurized to a test pressure of 2.5 barg.

The volume increase of the upstream compressed volume caused by the decrease in water volume upstream of the housing is measured by the integrity test instrument either by:

- Pressure drop (following isolation of the pressure source)
- Gas flow required to maintain the test pressure

The test result is expressed as either the WI value or WF value. The PORECHECK 4 unit measures the pressure drop over the test time (10 minutes) and calculates the actual water flow rate at 2.5 barg (3.5 bara).

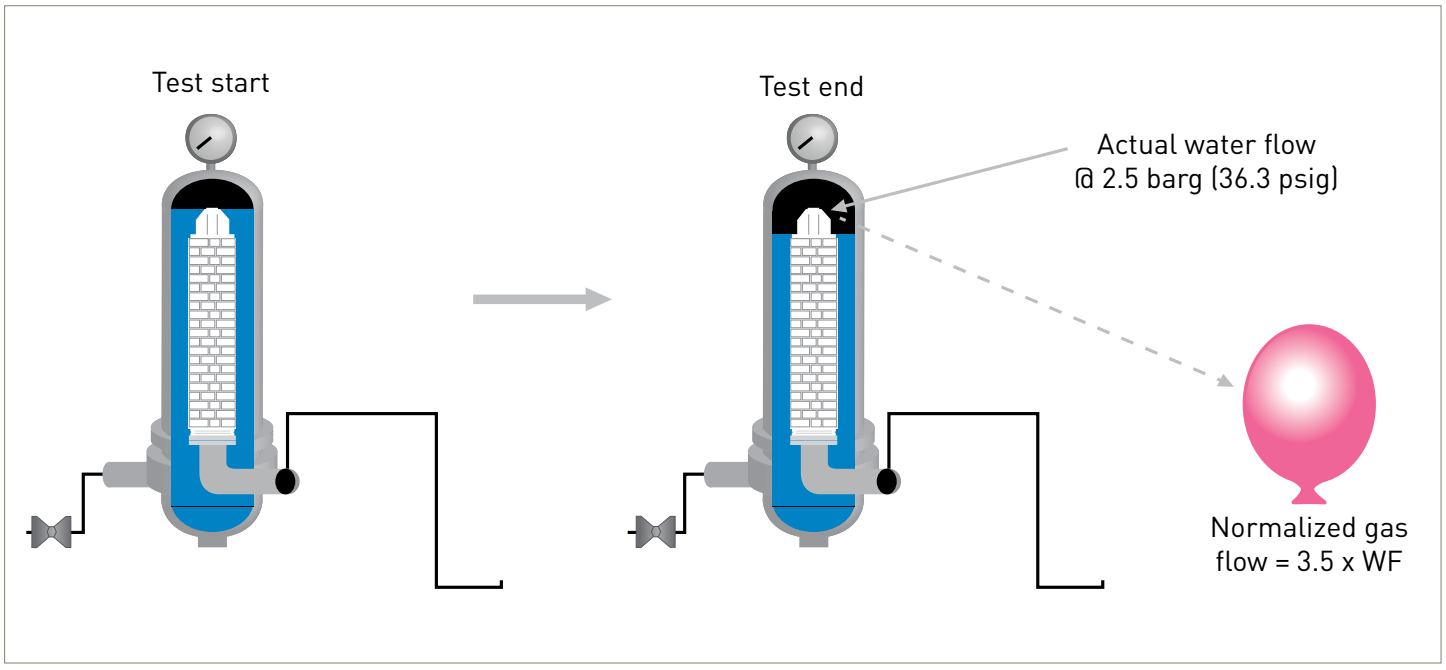


Figure 11 - Illustration of pressure drop i.e. change in volume

Theoretical principles

The decrease in water volume can be divided into two phases. The first is during the stabilisation phase, the second during the test phase.

a) Stabilization phase

The largest pressure drop occurs during initial pressurization and stabilization due to:

- pleat compression
- cartridge deformation
- expulsion of air within the membrane support layers

The PORECHECK 4 stabilizes the upstream side of the filter at 2.5 barg by adding pressurized air during the stabilization phase (10 minutes).

b) Testing phase

After stabilization of the system, the water volume continues to decrease and this is caused by a combination of two separate mechanisms.

Evaporation:

Evaporation at the high pressure downstream interface, as shown below, results in a reduced upstream water volume. This is the primary cause of water loss / flow leading to a pressure drop during the test phase.

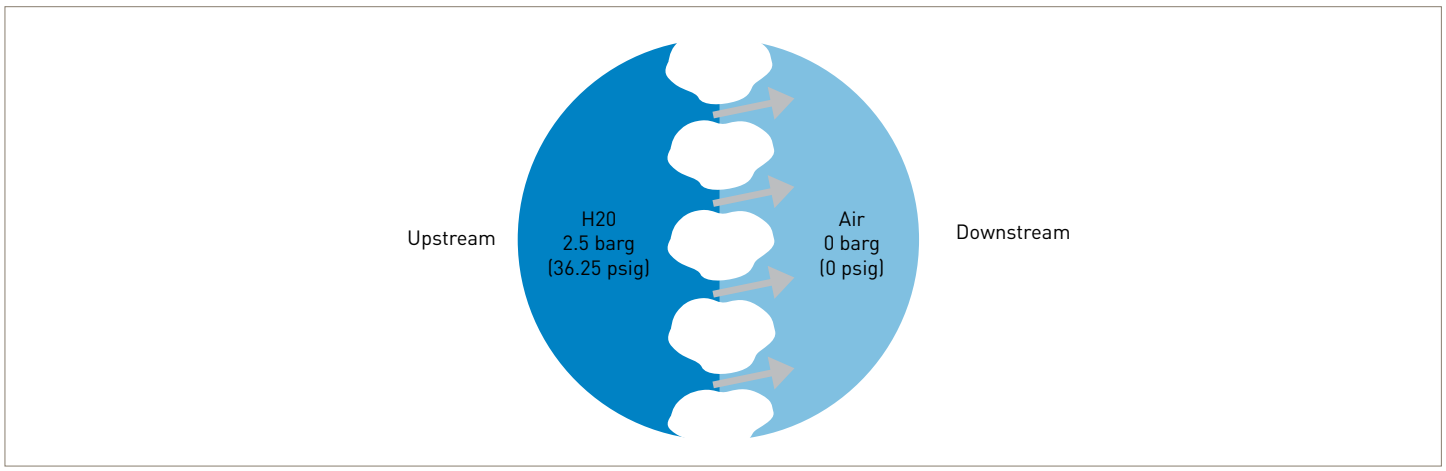


Figure 12 - Evaporation

Intrusion into pore structure:

Water gradually intrudes into the membrane over the period of the test, with the presence of larger pores in the filter being detected by an increased flow due to bulk water flow through these pores. Tests conducted at Parker domnick hunter show that no continuous intrusion occurs in the majority of the membrane structure but confirms that continuous intrusion into a minority of the larger pores can take place resulting in a small amount of water on the downstream side of the membrane. If continuous intrusion did occur through all the pores it has been calculated that the membrane structure would completely 'wet out' in approximately three hours. This does not happen as demonstrated by the fact that air can pass through the cartridge immediately after a WI test.

PORECHECK 4 - Test set-up

The PORECHECK 4 instrument calculates the water flow based on measuring the pressure loss and applying Boyle's law to calculate the actual volumetric change of the hardware volume.

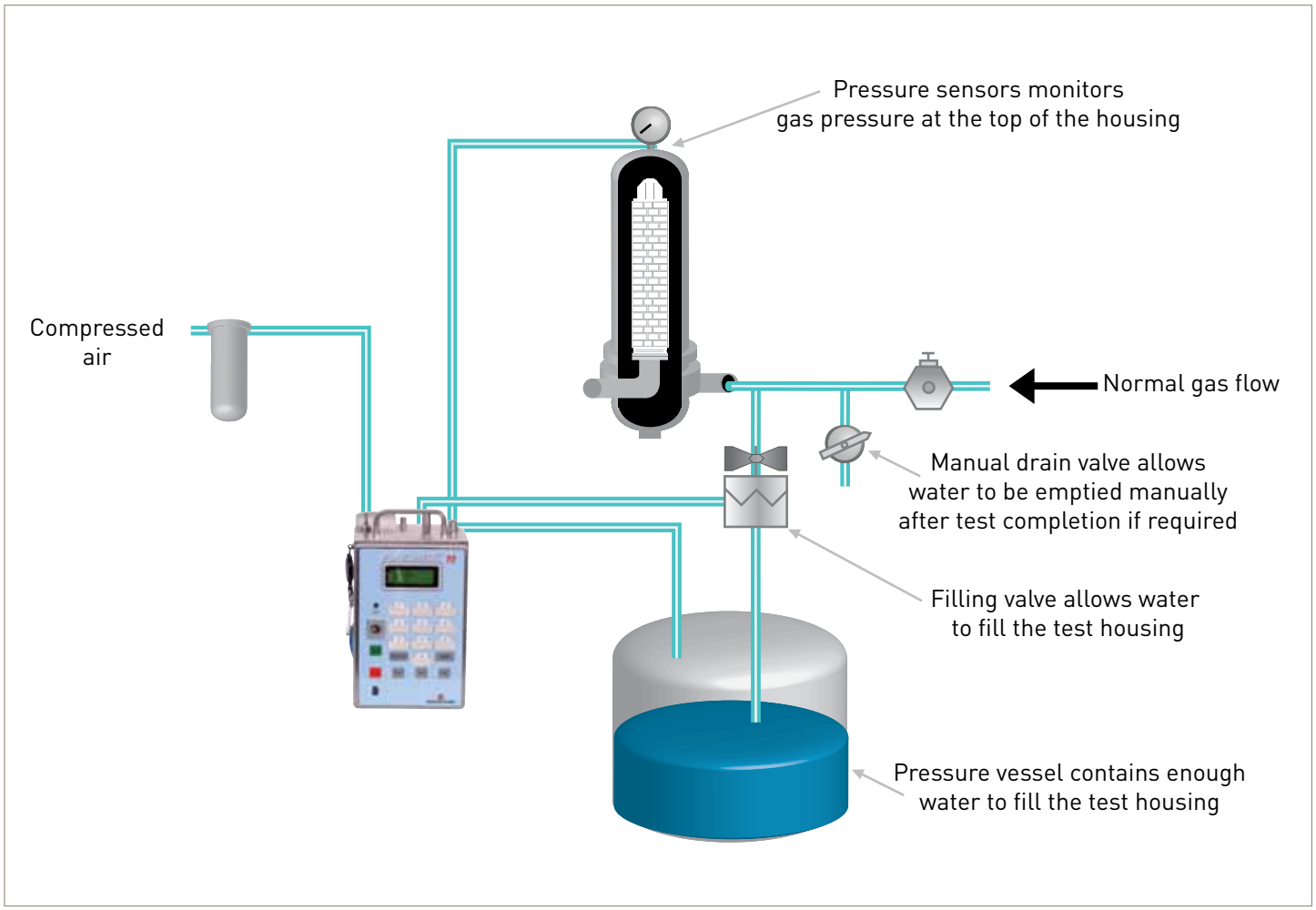
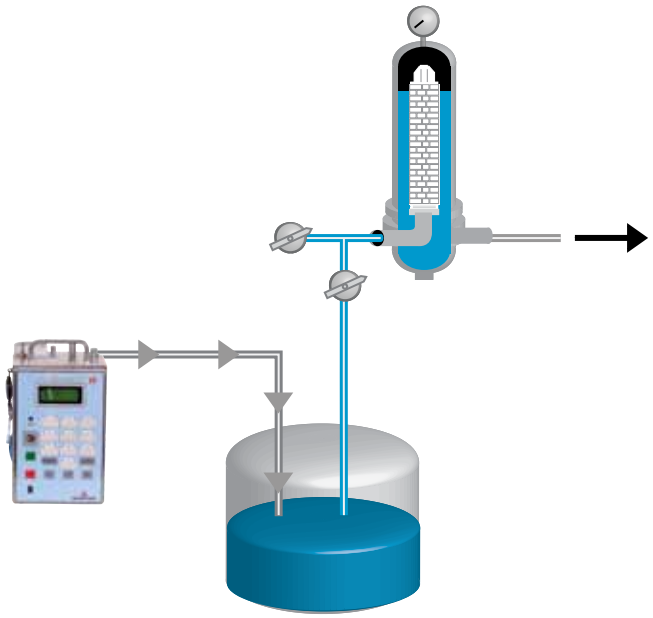
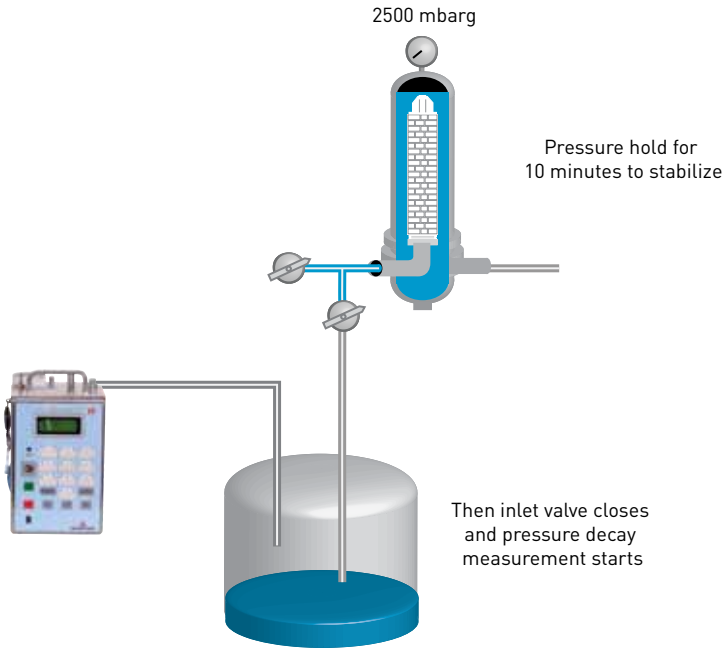


Figure 13 - WIT set-up



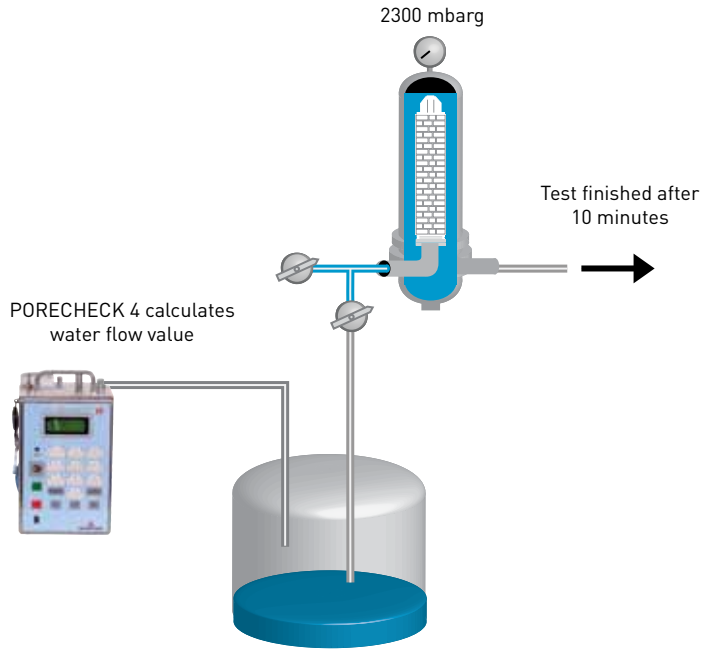
The PORECHECK 4 unit fills the housing with water up to a pressure of 2500 mbarg (3500 mbara). The hardware volume is measured during commissioning with the PORECHECK 4 instrument.

Figure 14 - WIT start-up



Stabilization phase: 10 min
P_1 = 2500 mbarg (3500 mbara)
V_1 = 200 mL (hardware volume)

Figure 15 - WIT stabilization phase



Test phase: 10 minutes
Pressure drop is measured and recorded.
Example: Pressure at the end of the test
P_2 = 2300 mbarg (3300 mbara)

Figure 16 - WIT test phase

Boyle's law:

$$P_1V_1 = P_2V_2$$

$$V_2 = 212.12 \text{ mL}$$

Actual water volume change = 212.12 – 200 = 12.12 ml over 10 mins

Water volume change = 1.212 ml/min = 1212 µL/min

Note: The water intrusion test result can be expressed in two ways:

Water flow (µL/t or mL/t)	Water intrusion (NmL/t)
Actual water volume change at test pressure	The increase in compressed gas volume, as a result of water flow, normalized to atmospheric pressure
Example: 1.212 ml/min = 1212 µl/min	Example: 1.212 ml/min *3.5 = 4.2 Nml/min

WIT advantages

- The water intrusion test method is correlated to the liquid bacterial challenge which guarantees filter performance.
- No downstream connection is required making testing easier and more practical.
- There are no potential contaminants being introduced to the process as it uses high purity water and can therefore be integrity tested in-situ.
- Post testing drying cycle is shorter than equivalent drying cycle post solvent wetting.

WIT issues

There are a number of issues associated with the water intrusion integrity test method: -

- The water intrusion test method is more time consuming than the pressure decay / diffusional flow test methods.
- WIT is suitable only in clean operating environments. The test is affected by contamination on the membrane surface, so should only be implemented on sterile gas cartridges which are adequately protected by both prefilters and steam filters.

Key considerations when water intrusion testing

A number of critical areas can be identified as worthy of consideration when conducting a water intrusion test;

Water condition	• Temperature • Quality
Cartridge condition	• Contamination • Dryness • Temperature
Filling the upstream volume with water with pressure	
Performing the test	• Fluctuation in ambient temperature • Housing integrity
Post test filter use	

Water condition

Temperature - As the water loss on the upstream side of the filter is due primarily to evaporation of water through the membrane it is essential that the temperature of the water is equal to that of the ambient air. If the temperature of the water is greater than that of the air the evaporation losses will be greater and hence a larger WI value will be recorded. Therefore the water used for test purposes should be stored in the test area for a minimum of 24 hours for the temperature to equilibrate.

The actual temperature of the water will also influence the measured water intrusion value. Correlation of water intrusion test values to a liquid bacterial challenge have been conducted in line with industry standards at a test liquid temperature of 19 - 21 °C (66 - 70 °F). If this temperature range cannot be achieved in practice, an appropriate correction factor should be used. See the graph detailed below. The test should ideally not be performed at temperatures below 15 °C (59 °F) or exceeding 25 °C (77 °F).

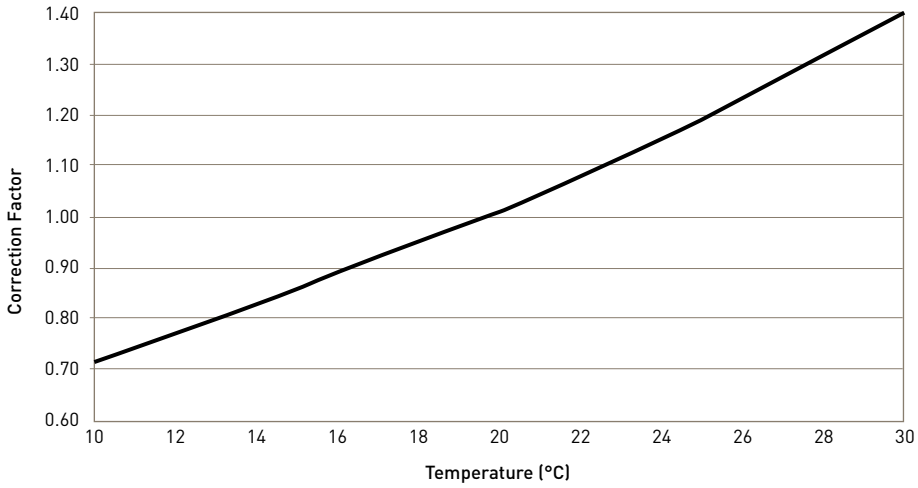


Figure 17 - Correction factors for WI at various temperatures

Quality - The quality of water is very important if consistent results are to be achieved. It is recommended that DI or WFI quality water is used. Using untreated tap water can result in spurious values being recorded due to possible contaminants.

Cartridge condition

Contamination - Cartridge contamination may influence results if these contaminants affect the hydrophobicity of the membrane. Possible contaminants include:

- Chemical additives used in steam boilers. This becomes evident when too much additive is used due to boiler shut down or low steam usage. It would normally also be associated with a discolouration of the cartridge; typically a green or red tinge.
- Rust from corroded pipework.
- Contaminants from fermenter broth on off-gas filters. Fermentation broth often includes antifoams and these can be deposited onto the membrane throughout the fermentation in the form of aerosols. The antifoams often reduce the hydrophobicity of the membrane again leading to incorrect water intrusion values.
- Other contaminants such as from cleaning procedure.

Dryness - The cartridge needs to be fully dried to ensure the WI value is correct. Any residual water within the pore structure of the membrane will result in a higher WI value being recorded. The companies that have adopted the WI test have often incorporated a drying cycle (typically 60 °C for 1 hour) into their testing procedure to ensure consistent results. If a drying cycle is used it is essential to validate this to guarantee consistent results.

Temperature - As a result of a drying cycle the cartridge may be at an elevated temperature compared to ambient. It is essential that the cartridge is allowed to cool down to ambient conditions before the water is introduced into the housing. Experience has shown that as the cold water contacts the warm membrane a thin film of water is heated which can result in localized water penetration resulting in an abnormally high result. This can be most important when testing a vent filter on a WFI tank that utilizes a heater on the housing to prevent condensation during use.

Filling the upstream volume with water with pressure

When filling under pressure it is important that the cartridge is not pressurized above the test pressure. The housing should be filled through a drain port allowing the air to expel out through the vent. The fill rate should be low to reduce the risk of over pressurization.

Performing the integrity test

The integrity test sequence is typically 10 minutes stabilization and 10 minutes test.
The main factors that can affect the test at this stage are :

- Fluctuation in ambient temperature
- Problems with the integrity of the test system i.e. not leak tight.

Fluctuation in ambient temperature - As the values being measured are extremely small compared to a standard diffusional integrity test with an alcohol / water mix (typically $^{1}/_{20}$) the effects of temperature difference have to be considered. This includes not only ambient temperature fluctuations but also differences between the water and air temperature in the compressible volume. As discussed previously the test water should be at the same temperature as the test environment. If this is not ensured false failures / passes will be recorded.

Similarly it is important to control the ambient air temperature. However, whilst this may be readily achievable within a laboratory environment, in production, appropriate controls may not be possible. For example, using the WI test to integrity test filters on a bulk fermentation facility (multi-round housings) is not practical. For these reasons, implementation of the water intrusion test within a production environment requires careful risk assessment (contact Parker domnick hunter for further advice).

Housing integrity - As the water intrusion values measured are small, all leaks on the upstream / non sterile side of the housing have to be eliminated. This can involve the replacement of all isolation / vent valves within older facilities which can result in significant additional expense not visualized when the test introduction was agreed.

When testing small filtration systems, the maximum allowable WI value will be extremely low (e.g. 3.5 ml / 10 min). This means that any small leak in the system can result in an incorrect failure being recorded. Whilst this is obviously a fail safe result, i.e. an integral cartridge will register as a failure, it leads to frustration for the operator conducting the test and complicates manufacturing operations.

Post test filter use

After the test has been performed the water has to be drained from the upstream side of the filter. To facilitate draining, pressure should first be vented from the housing either manually or by the integrity test machine. Once the pressure has vented the water can be drained from the housing. The vent valve should remain open to aid draining and to prevent a partial vacuum forming.

It is recommended that the drain valve is left open for approximately 30 minutes to ensure the maximum amount of liquid is removed from the filtration support layers.

If air is processed immediately following the WI test residual water can be forced onto the surface of the membrane leading to high initial differential pressures. This is not generally problematic in pressurized systems but if the cartridge is being used as a vent the consequences can be a collapsed tank. A number of manufacturers cartridges have been tested and all would exhibit the same potential problem. It is therefore advised that a housing bandheater is used for at least 30 minutes to minimize the risk of high differential pressures being generated.

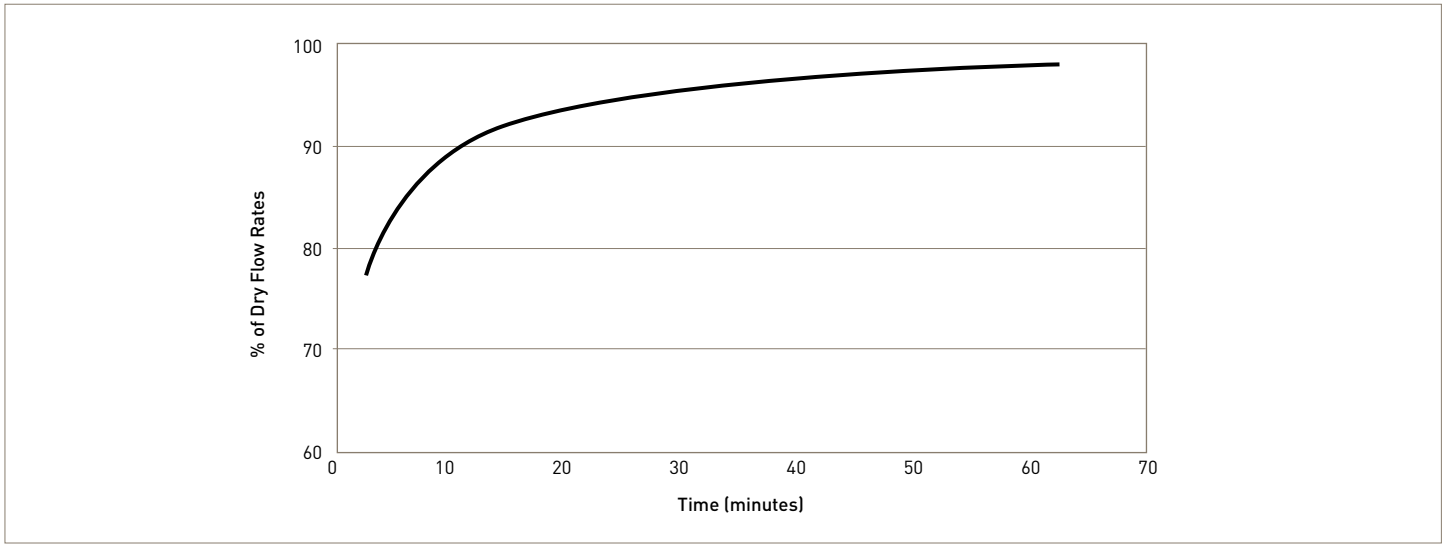


Figure 18 - Typical air flow recovery rate for a PTFE sterile gas cartridge

Aerosol integrity test

The aerosol challenge integrity test method has been used historically within the pharmaceutical industry for detecting failures in systems containing HEPA and ULPA grade filters. It consists of challenging a filter's ability to retain an aerosol particle of a particular size. An aerosol integrity test can be used to test gas filters where the filtered gas is not in direct contact with the exposed sterile product or surfaces as recommended in PDA's 'Sterilizing Filtration of Air' - technical report No. 40.

Physical principles

Gas filter efficiency principles - Gas filter efficiency is dependent on three different capture mechanisms. These are:

- Direct interception
- Inertial impaction
- Diffusion

Direct interception - Exclusion is due to the particle / microorganism size being greater in size than the average pore diameter or inter-fibre distance of the filtration medium, see figure 19. Typically, particles in the size range of 1.0 micron and above are removed by this mechanism.

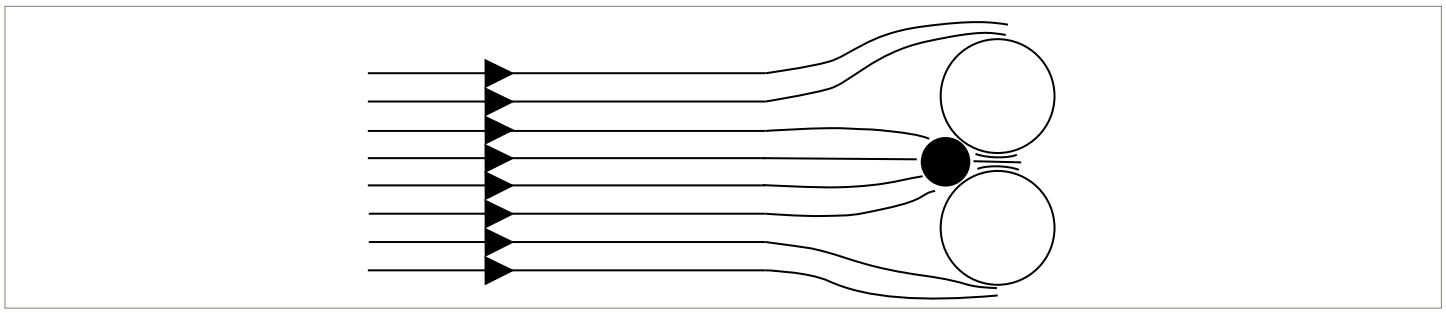


Figure 19 - Direct interception

Inertial impaction - Particles of a certain size are carried around the fibres / pore walls of the filter matrix in the gas stream line. However, particles of a certain mass will exit the streamline due to their inertia. They then impact on individual fibres and remain captured due to electrostatic forces (e.g. Van de Waals), see figure 20. Retention by this mechanism is generally accepted to be in the range of 0.3 – 1.0 micron.

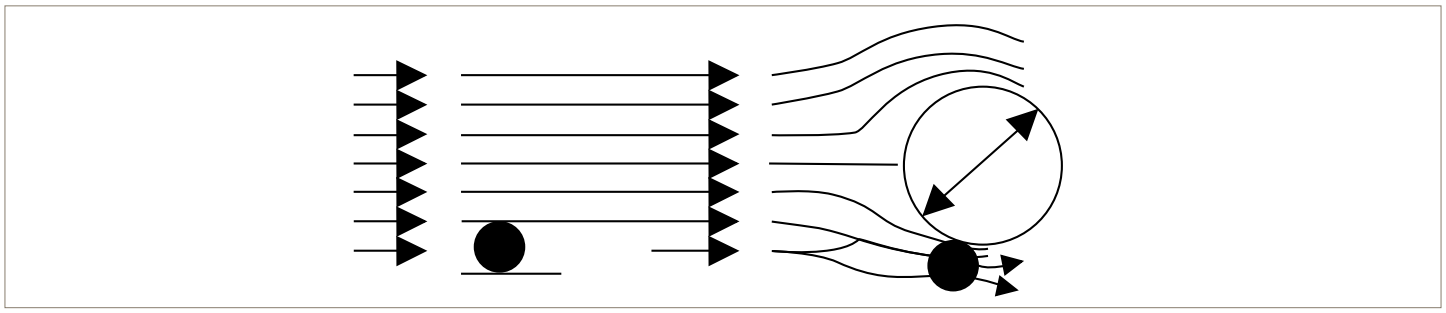


Figure 20 - Inertial impaction

Diffusion - When particles are small enough they are acted upon by the molecules of the gas being filtered. This means that even though the particles may be as small as 0.02 microns they have an effective diameter much larger. Also, due to their size, they travel at a lower speed than that of the carrier gas, see figure 21. These two phenomena mean that a very small particle can be removed from a gas stream using filtration media with an average pore size much larger than that of the particle being removed.

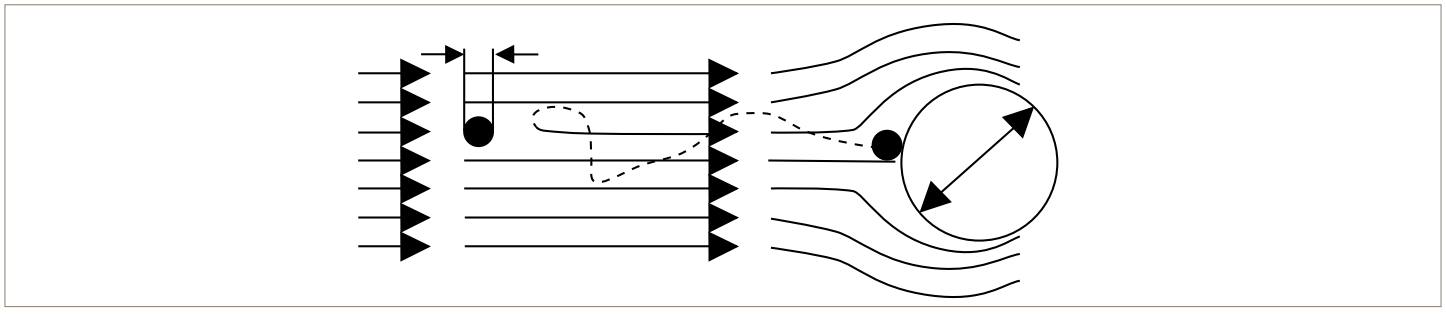


Figure 21 - Diffusion

Most penetrating particle size (MPPS) - The overall efficiency of a gas filter is the result of a combination of all these three mechanisms, and each mechanism has a particle size for which it is most efficient. This results in a particle size that is most difficult to remove because its size is between the ideal size for removal by a combination of diffusion and inertial impaction. This particle size is referred to as the MPPS. For a sterile gas filter this is recognized during normal process conditions as being in the order of 0.2 – 0.3 microns. As the gas velocities are substantially increased through the membrane the MPPS can reduce to around 0.07 micron but this is at velocities outside those experienced in a correctly designed system.

Aerosol challenge integrity test method - VALAIRDATA II

The VALAIRDATA II integrity test uses an aerosol challenge method based on generation of a defined aerosol from Shell Ondina EL oil. The oil meets the UK Mineral Hydrocarbons in Food Regulations 1966 (SI No 1073) and FDA Federal Code Regulation 178-3620(a) for food quality white oils.

The aerosol particles are generated from a highly refined mineral oil before challenging the filter. If any penetration of the aerosol occurs it will be detected by a laser particle counter downstream of the filter and a subsequent fail result is generated.

Aerosol penetration - This is the percentage of aerosol that penetrates to the downstream side of a hydrophobic test filter, during a gas borne aerosol challenge, using a high concentration of sub-micron aerosol.

Set up - When conducting an aerosol challenge integrity test a connection to both the upstream (dirty side) and downstream (sterile side) is required.

The basis of the VALAIRDATA II test is to challenge the sterile filter under test with a high concentration of aerosol in the size range of 0.2 to 0.3 microns (the most penetrating particle size). The presence or absence of aerosol on the sterile side of the filter is determined using a laser particle counter and the % penetration correlated to an aerosol bacterial challenge methodology. Unlike liquid based integrity test methods the VALAIRDATA II tests filters in the gas phase and is therefore more representative of the true efficiency of the gas filter. The other major benefit of this method is that the test times are a fraction of those required for a liquid based test.

The test has three key phases that are all automatically controlled by the VALAIRDATA II; aerosol generation, sensing and data collection. A typical test set up is shown below:

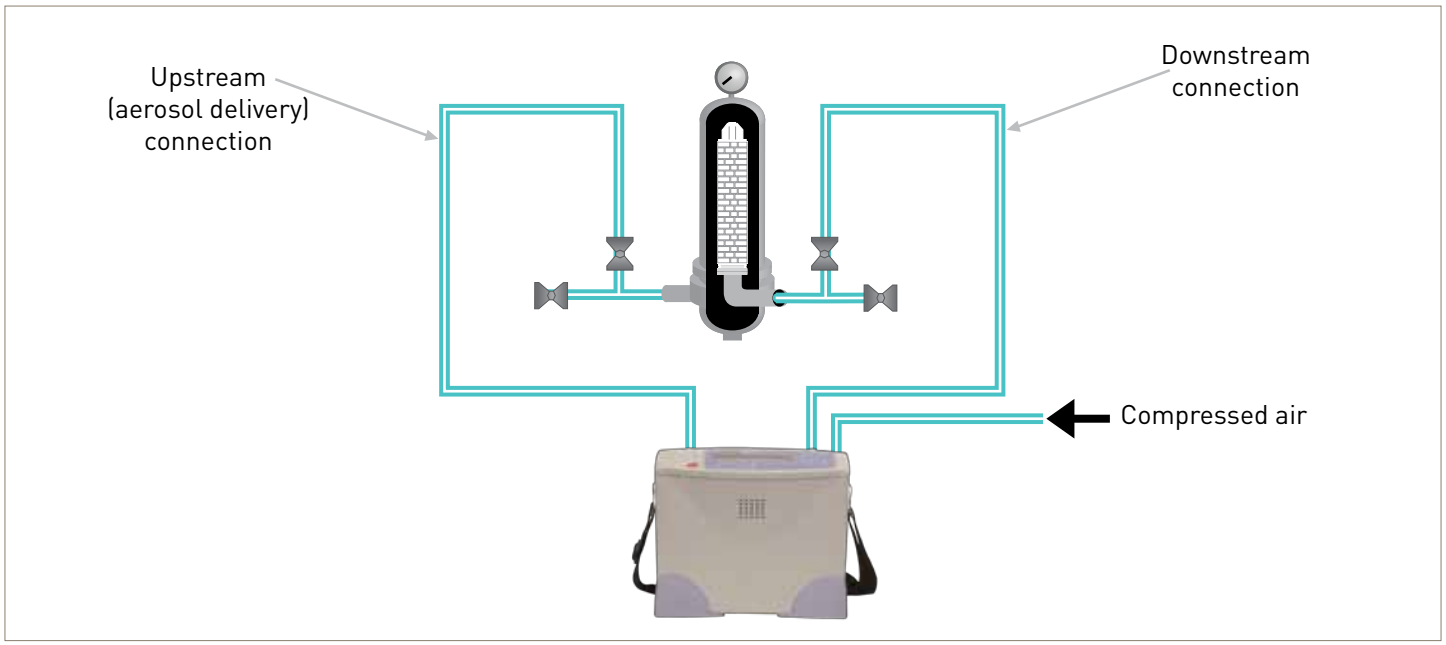


Figure 22 - Typical VALAIRDATA II set up

Aerosol generation phase - Aerosol containing a narrow distribution of particles within the most penetrating particle range is generated via a proprietary aerosol generator utilizing a submerged Laskin nozzle. Sterile compressed air is pumped through the nozzle at 14.5 l/min that generates a polydispersed aerosol. Aerosol of the desired distribution preferentially passes through a separation layer in the aerosol generator and the larger removed particles return to the challenge fluid sump. Air pressure, viscosity of the challenge fluid and fluid level govern the specification of the distribution. Internal pressure and level sensors are therefore incorporated to ensure the conditions for aerosol generation is retained during operation. A cross section of the aerosol generator is shown in figure 23.

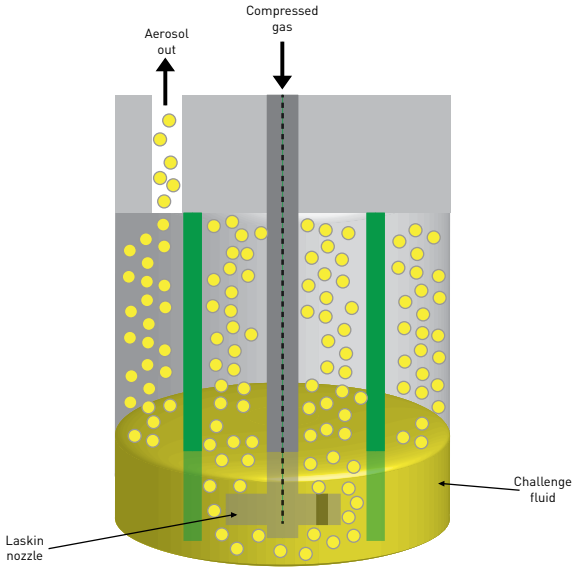


Figure 23 - Cross section of the aerosol generator

Sensing phase - High concentrations (approximately 1 x 10^{11} per minute) of the aerosol generated as described above then challenge the upstream surface of the test filter. Any aerosol that passes through the filter is then detected using a sensitive laser particle counter. Within the particle counting system, light from the laser is scattered by any penetrating aerosol particles onto the parabolic mirrors that focus the light onto a photodiode. The output from this diode is proportional to the size and number of particles in the air stream. Software in the VALAIRDATA II calculates the number of particles and compares this to the number typically generated in the challenge stream. The result is then displayed as a "PASS" if the filter under test is integral and has retained the aerosol challenge. If the filter has allowed penetration of aerosol, the system displays 'FAIL' and the percentage penetration. PASS / FAIL limits are determined by the correlation to an aerosol bacterial challenge.

A cross section of the laser particle counter is shown in figure 24:

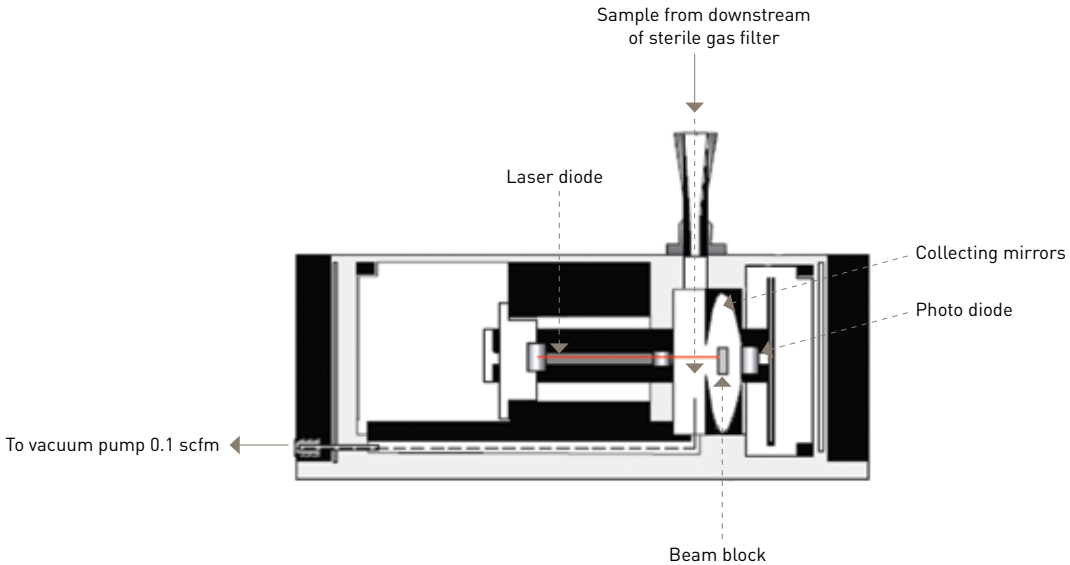


Figure 24 - Cross section of the laser particle counter

Maintaining sterility - A filter within a sterile system can be aerosol integrity tested using the VALAIRDATA II without compromizing the sterility on the downstream side of the filter.

In some instances users may want to integrity test air filters using the VALAIRDATA II aerosol integrity test following steam sterilization. As it is necessary to connect the VALAIRDATA II into the sterile side of the filtration system it is important to ensure that this connection does not compromize the sterility of this downstream section. To overcome this potential problem, sterility is maintained with the VALAIRDATA II by ensuring there is always a positive pressure within the filtration system during a test. Under these circumstances where a positive pressure is maintained, ingress into the downstream section is impossible. Studies carried out were conducted to show that the sterility of the downstream sections is maintained even if high levels of contamination exist around the downstream VALAIRDATA II connection.

This has been demonstrated in three separate tests where air contaminated with approx 10^7 cfu/litre of *Brevundimonas diminuta* was passed across the downstream integrity test point at 10 l/min, prior to, and during an integrity test.

The maintenance of sterility is guaranteed due to the VALAIRDATA II always providing a positive pressure to the system under test.

Advantages - Parker domnick hunter is the only filter manufacturer in the world with an aerosol challenge test instrument.

- The VALAIRDATA II test method is correlated to a bacterial aerosol challenge and therefore guaranteeing filter performance.
- The VALAIRDATA II test method is correlated to *MS-2 Coliphage* (virus) aerosol challenge.
- The test is quick and easy to use.
- The VALAIRDATA II unit tests the filter as an air and gas filter as it would be used within the application.
- The VALAIRDATA II can test both membrane and depth filters.
- The level of carry over of hydrocarbon following VALAIRDATA II integrity testing, in the absence of post test steam sterilization and at ambient or elevated operating temperature is negligible.

Issues

- The test is only correlated to a live aerosol bacterial challenge and not a liquid bacterial challenge. This limits some of the applications for which the instrument can be used.
- A downstream connection is required for testing, which means breaking into the sterile clean side, however it is proven that this does not impact on the downstream sterility.

Integrity test selection

Selection of integrity test

Parker domnick hunter recommends that the diffusional flow test be applied to most products in capsule and cartridge format.

Diffusional flow testing is simple to perform using automated test instruments such as the PORECHECK IV Integrity Tester, and avoids some difficulties in maintaining repeatability which is associated with the bubble point method.

In cases where the filter media area is small, i.e. discs, there is no real alternative to bubble point testing as the diffusional flowrates are so small that the sensitivity of the automated instruments do not allow their use.

When solvent wetting of a hydrophobic filter is not seen as a possibility (for example - vent filters on a WFI tank) the water intrusion method should be chosen.

For sterilizing gas filters that are used to filter gas that is not in direct contact with exposed sterile product on surfaces, the VALAIRDATA II aerosol challenge method can be used.

When to integrity test

Integrity testing serves to identify the pore size rating in accordance with the filter manufacturer's standards. Where the claimed purpose of the filter is to sterilize, pre- and post filtration integrity tests should be performed.

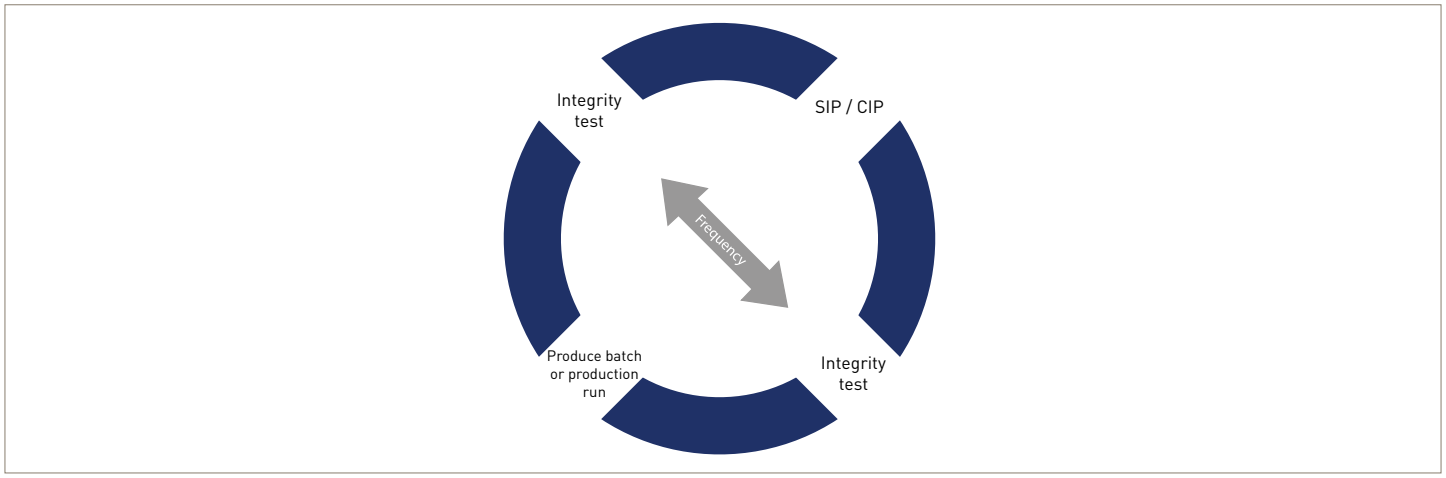


Figure 25 - When to test

Integrity testing requirements

A summary for the requirements for each test is shown in the table below:

Test method	Requirements for testing
Bubble point	Clean dry gas: Air quality = condensate and oil free (150 8573.1 class 1.4.2)
	Wetted filter*
	Sealed upstream volume, filters correctly installed
	Correct test parameters
Pressure decay / diffusional flow	Clean dry gas: Air quality = condensate and oil free (150 8573.1 class 1.4.2)
	Wetted filter*
	Sealed upstream volume, filters correctly installed
	Correct test parameters
	Known upstream volume
	Control of environment - temperature
WIT	Clean dry gas: Air quality = condensate and oil free (150 8573.1 class 1.4.2)
	Dry, clean hydrophobic filter
	Sealed upstream volume, filters correctly installed
	Correct test parameters
	Known hardware volume
	Control of environment - temperature
Aerosol Challenge	Clean dry gas: Air quality = condensate and oil free (150 8573.1 class 1.4.2)
	Dry hydrophobic filter
	Clean air lines
	Sealed upstream volume, filter correctly installed
	Downstream connection for measurement

**Note - To ensure the filter is full wetted, the Parker domnick hunter guideline for flushing is:*

- Flush for 10 minutes at approximately 10 L/min per 10¨ (250 mm) module.
- Dip wet for 10 minutes. The cartridge should be placed (closed) bomfin end first and allowed to sink under its own weight.

Failure analysis / troubleshooting

If a sterilizing filter fails an integrity test, it could be damaged, but there may be other causes for the failure that include incorrect assembly (incomplete sealing) and incomplete wetting.

To distinguish between filter damage and possible test problems or artifacts, the following verification steps can be taken:

Liquid integrity testing	Aerosol challenge integrity testing
The appropriate integrity test has been selected	Correct test parameters have been used
Correct test parameters have been used	The filter is dry
Correct wetting fluid and wetting procedure have been used	There are no leaks in the test system
There are no leaks in the test system	Equipment has been properly calibrated
Filter assembly temperature has remained stable and within specification during testing	Test set-up has been properly assembled and fuctions properly
Equipment has been properly calibrated	Correct filter has been installed
Test set-up has been properly assembled and functions properly	Correct number of filters have been installed
Correct filter has been installed	
Correct number of filters have been installed	

Liquid integrity test failures are generally caused by insufficient wetting of the filter. Incomplete wetting may be due to either inadequate flushing to wet out all the pores, adsorption of hydrophobic contaminants, or other formulation components that can change the surface wetting characteristics of the filter membrane. A change in wetting characteristics may affect integrity test performance. Also pores could dry out, if the time between wetting procedure and test procedure is too long, especially when using alcohol based wetting agents.

Diffusional flow

The following process flow diagram can be used when testing filters with diffusion flow / pressure decay.

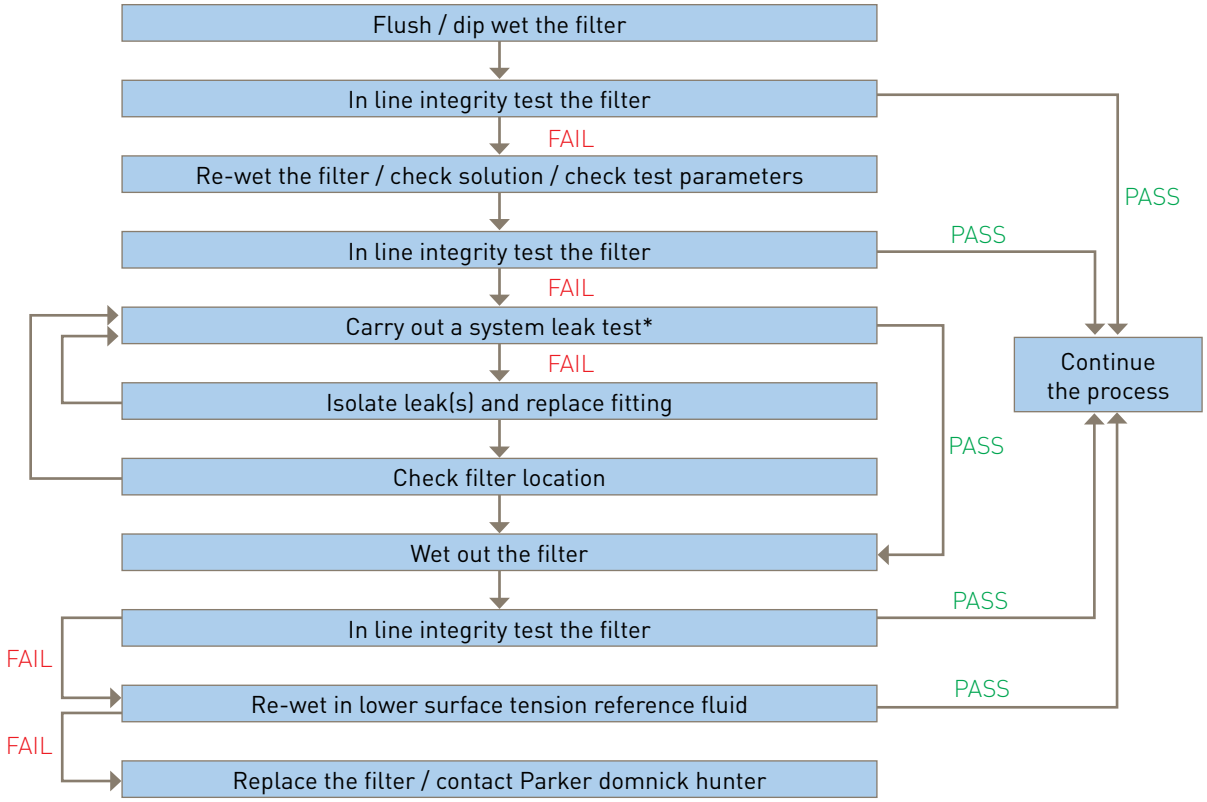


Figure 26 - Pressure decay / diffusional flow integrity testing process flow diagram

**Note: A system leak test is where the downstream of the housing is sealed off (or the filter is replaced with a blank), and the pressure drop is tested using a test pressure of 4 bar.*

Water intrusion test

The following process flow diagram can be used when testing filters with WIT:

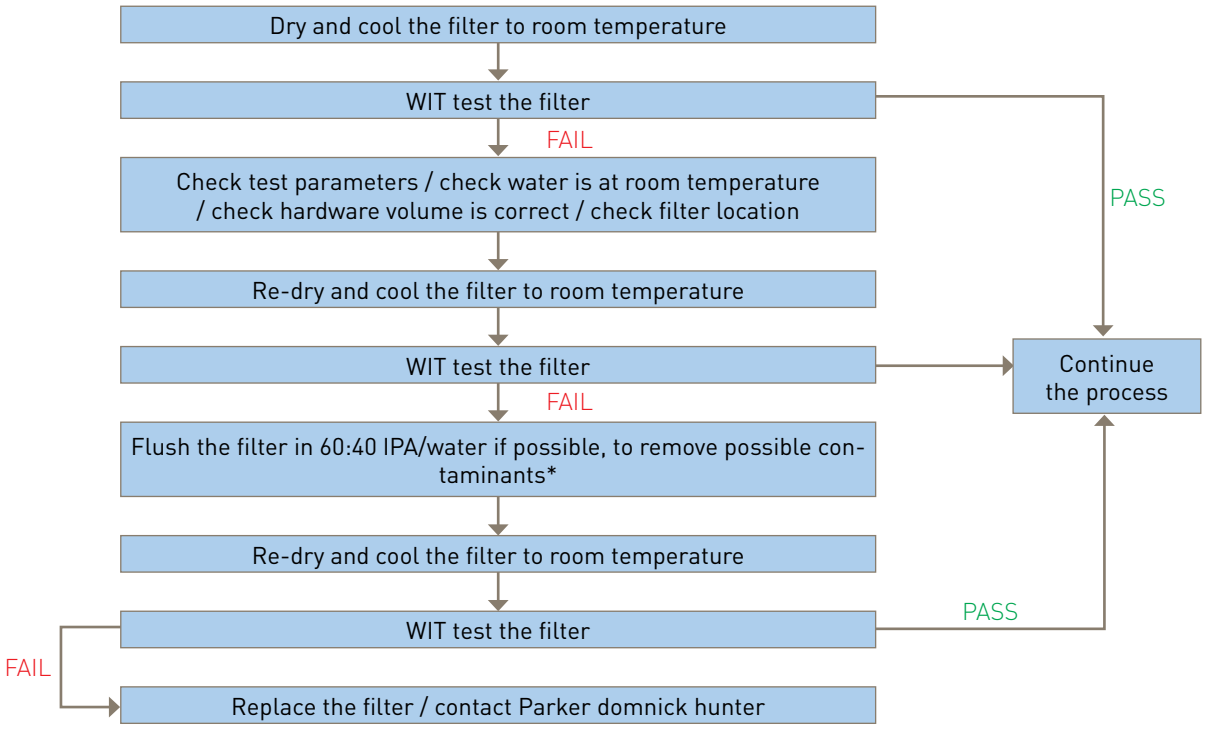


Figure 27 - WIT integrity testing process flow diagram

**Note: Diffusional flow testing can also be performed at this point, if applicable.*

Aerosol challenge

The following process flow diagram below can be followed when testing filters with the aerosol challenge:

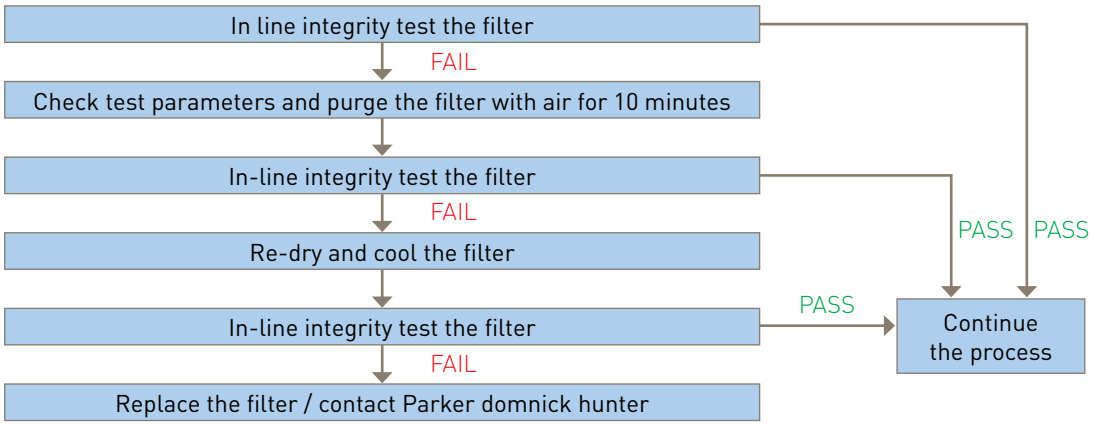


Figure 28 - Aerosol challenge integrity testing process flow diagram

Technical Support Group activities

Parker domnick hunter have a trained team of Scientists and Engineers available to answer questions regarding the technical capabilities of our products, to assist in the selection and design of appropriate filtration systems and to provide user training programs. The following services can be delivered both on-site and in-house:

- Filterability testing to optimize filter system design
- Advice on the development of integrity testing, steam sterilization and clean-in-place procedures
- Development of validation protocols
- Troubleshooting
- Facility audits to ensure continued optimization of filter use
- Operator training including filtration theory, filter system design and management, validation, etc.

For more information on any of the above support services please contact your local Parker domnick hunter representative.

email: tsg@parker.com
website: www.parker.com/processfiltration

Parker Worldwide

Europe, Middle East, Africa

AE – United Arab Emirates, Dubai
Tel: +971 4 8127100
parker.me@parker.com

AT – Austria, Wiener Neustadt
Tel: +43 (0)2622 23501-0
parker.austria@parker.com

AT – Eastern Europe, Wiener Neustadt
Tel: +43 (0)2622 23501 900
parker.easteurope@parker.com

AZ – Azerbaijan, Baku
Tel: +994 50 2233 458
parker.azerbaijan@parker.com

BE/LU – Belgium, Nivelles
Tel: +32 (0)67 280 900
parker.belgium@parker.com

BY – Belarus, Minsk
Tel: +375 17 209 9399
parker.belarus@parker.com

CH – Switzerland, Etoy
Tel: +41 (0)21 821 87 00
parker.switzerland@parker.com

CZ – Czech Republic, Klecany
Tel: +420 284 083 111
parker.czechrepublic@parker.com

DE – Germany, Kaarst
Tel: +49 (0)2131 4016 0
parker.germany@parker.com

DK – Denmark, Ballerup
Tel: +45 43 56 04 00
parker.denmark@parker.com

ES – Spain, Madrid
Tel: +34 902 330 001
parker.spain@parker.com

FI – Finland, Vantaa
Tel: +358 (0)20 753 2500
parker.finland@parker.com

FR – France, Contamine s/Arve
Tel: +33 (0)4 50 25 80 25
parker.france@parker.com

GR – Greece, Athens
Tel: +30 210 933 6450
parker.greece@parker.com

HU – Hungary, Budapest
Tel: +36 1 220 4155
parker.hungary@parker.com

IE – Ireland, Dublin
Tel: +353 (0)1 466 6370
parker.ireland@parker.com

IT – Italy, Corsico (MI)
Tel: +39 02 45 19 21
parker.italy@parker.com

KZ – Kazakhstan, Almaty
Tel: +7 7272 505 800
parker.easteurope@parker.com

NL – The Netherlands, Oldenzaal
Tel: +31 (0)541 585 000
parker.nl@parker.com

NO – Norway, Asker
Tel: +47 66 75 34 00
parker.norway@parker.com

PL – Poland, Warsaw
Tel: +48 (0)22 573 24 00
parker.poland@parker.com

PT – Portugal, Leca da Palmeira
Tel: +351 22 999 7360
parker.portugal@parker.com

RO – Romania, Bucharest
Tel: +40 21 252 1382
parker.romania@parker.com

RU – Russia, Moscow
Tel: +7 495 645-2156
parker.russia@parker.com

SE – Sweden, Spånga
Tel: +46 (0)8 59 79 50 00
parker.sweden@parker.com

SK – Slovakia, Banská Bystrica
Tel: +421 484 162 252
parker.slovakia@parker.com

SL – Slovenia, Novo Mesto
Tel: +386 7 337 6650
parker.slovenia@parker.com

TR – Turkey, Istanbul
Tel: +90 216 4997081
parker.turkey@parker.com

UA – Ukraine, Kiev
Tel +380 44 494 2731
parker.ukraine@parker.com

UK – United Kingdom, Warwick
Tel: +44 (0)1926 317 878
parker.uk@parker.com

ZA – South Africa, Kempton Park
Tel: +27 (0)11 961 0700
parker.southafrica@parker.com

North America

CA – Canada, Milton, Ontario
Tel: +1 905 693 3000

US – USA, Cleveland
Tel: +1 216 896 3000

Asia Pacific

AU – Australia, Castle Hill
Tel: +61 (0)2-9634 7777

CN – China, Shanghai
Tel: +86 21 2899 5000

HK – Hong Kong
Tel: +852 2428 8008

IN – India, Mumbai
Tel: +91 22 6513 7081-85

JP – Japan, Tokyo
Tel: +81 (0)3 6408 3901

KR – South Korea, Seoul
Tel: +82 2 559 0400

MY – Malaysia, Shah Alam
Tel: +60 3 7849 0800

NZ – New Zealand, Mt Wellington
Tel: +64 9 574 1744

SG – Singapore
Tel: +65 6887 6300

TH – Thailand, Bangkok
Tel: +662 717 8140

TW – Taiwan, Taipei
Tel: +886 2 2298 8987

South America

AR – Argentina, Buenos Aires
Tel: +54 3327 44 4129

BR – Brazil, Sao Jose dos Campos
Tel: +55 12 4009 3500

CL – Chile, Santiago
Tel: +56 2 623 1216

MX – Mexico, Apodaca
Tel: +52 81 8156 6000

VE – Venezuela, Caracas
Tel: +58 212 238 5422

Ed. 2010-09-29

AS_03_09/11 Rev. 1A

Parker domnick hunter

Parker Hannifin Manufacturing Ltd
domnick hunter
Process Filtration - Europe
Durham Road
Birtley, Co. Durham
DH3 2SF, England
phone +44 (0)191 4105121
fax +44 (0)191 4105312
email: dhprocess@parker.com
www.parker.com/processfiltration

Parker Hannifin Corporation
domnick hunter
Process Filtration - North America
2340 Eastman Avenue
Oxnard, California, USA 93030
toll free: +1 877 784 2234
phone: +1 805 604 3400
fax: +1 805 604 3401
email: dhpsales.na@parker.com
www.parker.com/processfiltration