Millipore®
Preparation, Separation,
Filtration & Testing Products

Application Note

Multi-Tier Approach to Assuring Virus Retention and Integrity of Viresolve® Pro Devices

Introduction

Manufacturers of biopharmaceuticals are required to characterize the ability of key process steps to clear viruses. Typically, the entire manufacturing process is qualified to attain a cumulative virus reduction factor, which significantly contributes to the documentation of virus safety. In many of these manufacturing processes, a virus retentive filter is utilized to achieve a robust and effective virus clearance step.

The key to the implementation of virus filtration is the assurance of virus retention and device integrity. The Viresolve® Pro solution is based on a highly retentive parvovirus membrane, packaged in a range of devices, that is in-line with customer process development, validation and production stages.

A critical aspect of the Viresolve® Pro solution is to present users with an industry-leading level of virus clearance assurance by offering effective performance, as well as integrity testing methods. This document provides a summary of our multi-tier approach to assuring virus filter performance and integrity, which comprises complementary steps taken by us and the end user.

Our multi-tier approach to assuring virus filter performance and integrity.

- TIER 5. End-User Validation and Testing
- TIER 4. Device 100% Tests
- TIER 3. Device Release Tests
- TIER 2. Membrane Release Tests
- TIER 1. Process and Product Validation

TIER 1. Process and Product Validation

Membrane process validation must occur before the membrane is used in the manufacturing of filtration devices. Device process validation occurs before finished devices can be released to manufacturing and shipped to end users.

A validated process provides assurance that the manufacturing process consistently yields product that meets predefined quality attributes. As part of the process validation, each of the critical manufacturing parameters has been identified. Tight manufacturing windows have also been assigned to ensure continued control over the manufacturing process.

Viresolve® Pro devices are manufactured in Our state-of-the-art manufacturing facility, which adheres to cGMP, and whose Quality Management System is approved by an accredited registering body to the appropriate ISO® 9001 quality standards.

TIER 2. Membrane Release Tests

The Viresolve® Pro membrane process includes the following lot release tests using a predefined sampling plan:

1. Bacteriophage φX-174 retention test
2. Protein capacity test (using human IgG as a model protein)
3. Water permeability test
4. Visual inspection of membrane

2.1 Bacteriophage φX-174 Retention Test

The retention of 28 nm φX-174 marker virus under controlled conditions provides evidence that the membrane meets or exceeds the minimum virus clearance target. φX-174 bacteriophage is a single stranded RNA virus from cycstoviridae with a diameter of 28 nanometers. This test is performed on each membrane lot and roll based on a predetermined sampling plan. The test involves filtering φX-174-human-derived IgG (hIgG) solution through the membrane samples in a Micro device format. A filtrate grab sample is collected at 75% flow decay point from the initial buffer flux.

This test offers the following benefits:

- Representative of the product application
- Serves as a Quality Control test as well as a foundation for measuring and tracking membrane performance
- Consistent with the PDA Small Virus Filter Task Force recommendations (PDA Technical Report No. 41, Virus Filtration, PDA J Pharm Sci Technol Suppl Vol 59 No. S-2)

2.2 Protein Capacity Test

Consistent protein capacity performance for a given process is a critical expectation from virus end users. Given that capacity performance is primarily established during the membrane manufacturing process, Viresolve® Pro membrane lot release tests include a protein capacity test using hIgG as a surrogate protein marker. The hIgG molecule exhibits similar biochemical and physical properties to other mammalian-derived antibodies typical in customer applications.

In this test, a φX-174 and hIgG solution is filtered through the membrane samples. The capacity is measured to a 75% flow decay point (from initial buffer flux) and the results are normalized using a control membrane. The membrane roll average throughput values must fall within the 2-sided specification.

While hIgG capacity results are not predictive of the specific capacities obtained from a particular customer process stream, which are a function of the virus filter, process stream characteristics and processing conditions, the data in **Figure 1** demonstrates that general ranking of membrane obtained using our test method (hIgG) is consistent with that observed when tested with a customer's MAb. Six different membrane samples with a range in capacity were evaluated in triplicate with both hIgG and a customer's MAb.

2.3 Water Permeability Test

Water permeability, along with protein capacity and virus retention, is an important performance measurement and is tested and controlled to ensure consistent product performance in the final application, and to enable fast processing at the virus filtration unit operation. Samples of Viresolve® Pro phobic membrane are tested for water permeability as an in-process test prior to membrane hydrophilization. After hydrophilization, philic (final version) membrane is tested for water permeability as part of the membrane release testing using a predefined sampling plan. In the test, single layer membrane samples are tested at 25 psig and temperature normalized to 25°C. Phobic membrane is first prepared by wetting in alcohol and exchanging in water while philic membrane is wetted in water.

2.4 Visual Inspection of Membrane

The Viresolve® Pro membrane casting and hydrophilization processes include a frequent sampling of membrane to search for holes, scratches or other defects.

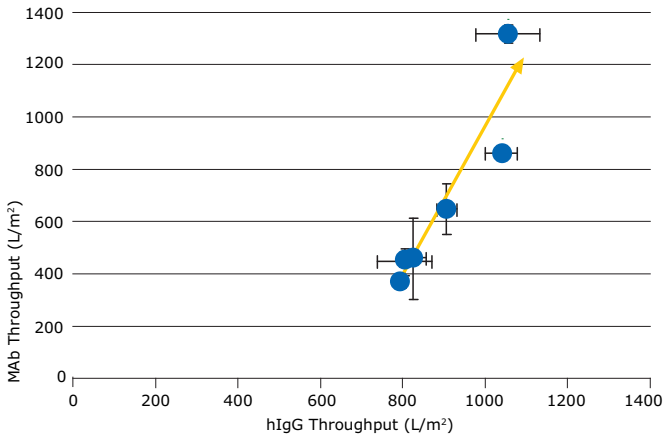


Figure 1.

TIER 3. Viresolve® Pro Device Release Tests

The lot release testing of Viresolve® Pro devices includes:

1. Bacteriophage φX-174 retention test
2. Housing burst test
3. In-Process manufacturing controls

3.1 Bacteriophage φX-174 Retention Test

Samples from each Viresolve® Pro device lot are tested for the ability to retain the φX-174 marker virus. This test confirms that the device manufacturing process is free of systematic issues that could compromise the inherent retention performance of the Viresolve® Pro membrane established during the membrane manufacturing process, and verified during the membrane release testing.

3.2. Housing Burst test

Samples from each Viresolve® Pro device lot are tested to determine the maximum pressure the device can withstand. During this test, the device is filled with water and the pressure inside of the device is increased at a fixed rate until failure occurs. The specification requires that the samples maintain integrity up to a pressure that corresponds to twice the rated pressure.

3.3 In-Process Manufacturing Controls

In addition to the device release tests, the following in-process manufacturing controls are used:

1. Luster sensors performed during membrane bonding operations confirms correct membrane orientation.
2. Visual inspection after the membrane bonding step (for Viresolve® Pro Modus and Magnus devices) confirms that appropriate membrane/plate bond has been achieved.
3. Verification that the correct thermal energy is being applied to all portions of the bonding surfaces.
4. Electronic servo-controlled motors are utilized to position the parts during heat, welding, and cooling portions of the bonding cycle.

TIER 4. Device 100% Tests

The Viresolve® Pro devices are subject to the following 100% device tests:

1. Pressure hold test
2. Water flux test
3. Air/water diffusion test
4. Binary Gas Test (BGT)

4.1 Pressure Hold Test

The pressure hold test assures that the interface between the interior and exterior of the device is leak-free to ensure that the device will not leak during usage. It does not challenge filter integrity. During this test, the device is brought up to no less than 1.5 times the rated pressure using compressed air and allowed to stabilize. The internal volume is then isolated and the internal pressure is carefully monitored for a specific period of time.

4.2 Water Flux Test

Water flux is a measure of the internal resistance to water flow. All Viresolve® Pro Modus and Magnus devices are wet challenged with water at a constant pressure, and the flow rate is measured and compared to the specification.

4.3 Air/Water Diffusion Test

All Viresolve® Pro Magnus and Modus devices are integrity tested for air/water diffusion using a test method that is similar to the air/water diffusion test recommended for pre- and/or post-use testing by the end user. The air/water diffusion test can provide up to 4.5–5 virus log reduction value (LRV) assurance for devices with membrane of the proper pore size distribution.

4.4 Binary Gas Test

Our Binary Gas Test (BGT) method is an innovative, highly sensitive device test that utilizes a two component gas mixture in which there is a large difference in permeability between the two gases across a wetted membrane. Concentration is measured on the downstream side. A deviation from the expected concentration is an indication of the presence of a defect that could negatively affect the virus retention capabilities of the device. The Binary Gas Test is performed on 100% of Viresolve® Pro Magnus and Modus devices as well as Viresolve® Pro Micro devices intended for virus validation studies. (Please see page 6 for more details on the Binary Gas Test.) The addition of BGT to our multi-tier approach has further strengthened the virus retention assurance delivered by the Viresolve® Pro solution.

TIER 5. End User Validation and Testing

Tiers 1–4 provide assurance that the Viresolve® Pro solution meets its performance specifications in terms of virus retention, device integrity, etc. The user is responsible for ensuring that the filters have not been damaged during shipping, and are installed, tested and utilized correctly.

Air/Water Diffusion Test

We recommend an air/water diffusion test as an optional integrity test, due to the following benefits:

- Ease of use: water wetted test, simple procedure, non destructive test
- Results in approximately 40 minutes
- Accurate: downstream flow meter or our Integritest® 4 Automated Integrity Tester
- Sensitive to the type of defects that could be introduced during shipping, handling, installation and use
- Similar to the test performed by us which can facilitate device failure investigations

An end user's integrity test may be performed prior to, or after use utilizing either a downstream air flow rate measurement with a flow meter, or an integrity test instrument. We recommend the Integritest® 4 instrument which is designed to accurately measure air/water diffusion values for Viresolve® Pro devices. Please consult the User Guide for specific instructions for device installation, testing and use.

The 50 psig (3.45 Bar) air/water diffusion test was selected because of its suitability for detection of the type of defects that could be introduced during shipping, handling or processing of Viresolve® Pro Modus and Magnus devices.

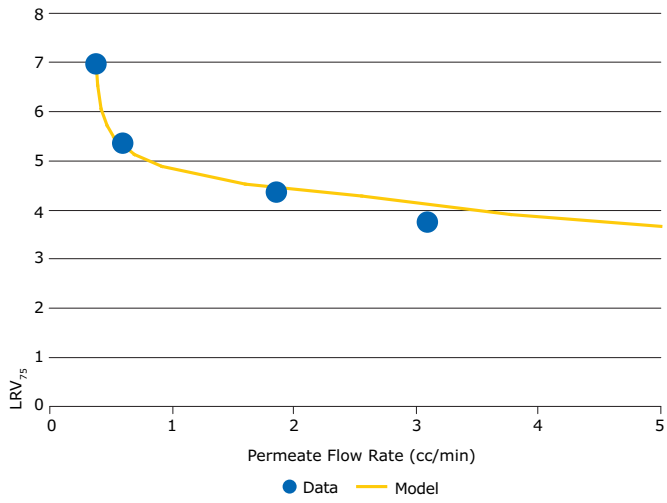


Figure 2.

Figure 2 shows an example of the air diffusion rates and virus retention performance of Viresolve® Pro devices that were fabricated with controlled defects. At the steep portion of curve in the 5–6 LRV range, small defects will result in significant effects on LRV but will have minor effects on air diffusion rate. These small defects, if present, will be detected by using the 100% Binary Gas Test and therefore, would not be released for shipping to end users. Presence of larger defects that are more likely to occur during shipping, handling and use will result in elevated air diffusion flow rates as illustrated at the right side of the curve knee. Devices with defects in this range can be detected by the end user using the recommended air/water diffusion test.

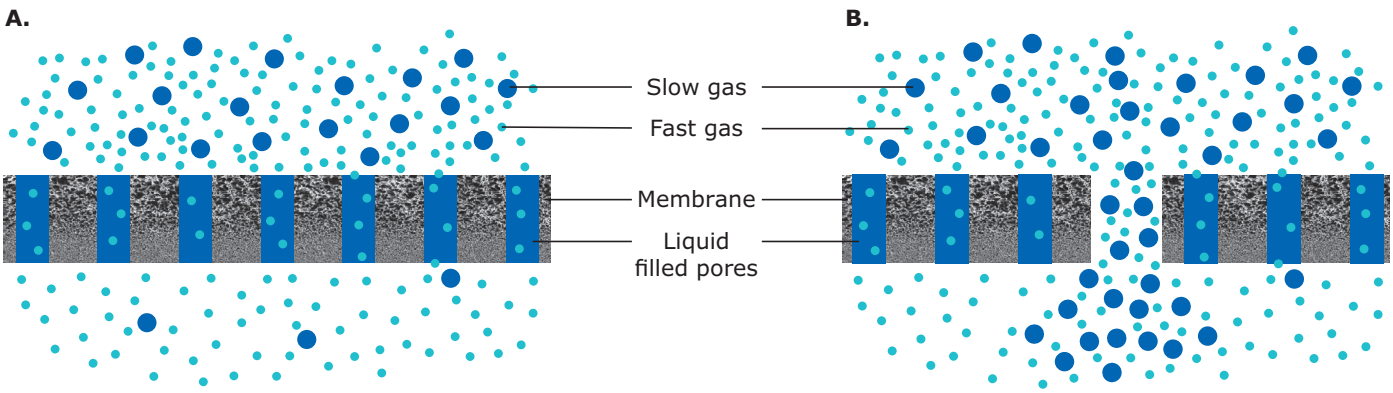
Conclusion

From membrane manufacturing to filter use, our multi-tier approach for ensuring virus retention and integrity of Viresolve® Pro devices creates a continuum of filter integrity testing. This approach places responsibility on the filter manufacturer to document the production of: (1) membrane that consistently provides robust virus clearance and (2) integral filter devices that house this membrane. The end user is then responsible for auditing the filter manufacturer to verify compliance to effective quality systems. As a result, users can be assured that filters will be produced and tested in a controlled manner. During day-to-day operations, the end user's primary responsibility is to ensure that filter integrity has been maintained from receipt to use.

Details of the Binary Gas Test

Principle

Binary Gas Testing (BGT) uses a two component gas mixture for diffusion across a wetted membrane. The two components diffuse through the liquid layer at different rates, thus the permeate stream is enriched with the more rapidly diffusing gas, and depleted for the more slowly diffusing gas. If a defect is present in the membrane, the bulk flow of gas through the defect will be of the same composition as the inlet high pressure side, thereby contaminating the permeate stream and resulting in an increase in concentration of the slower-permeating gas. A deviation from the expected concentration is therefore an indication of a defect. Our BGT method uses a gas pair in which there is a large difference in permeability between the two gases. **Figure A** depicts an integral wetted membrane, wherein the gas transport across the membrane is controlled by diffusion through the liquid layer. In this case, the concentration of the slow gas in the permeate stream is greatly diminished compared to the high pressure side of the membrane. **Figure B** shows that a defect or open pore allows the upstream gas to flow into the downstream side by convection, effecting an increase in permeate concentration of the slow gas. The permeate concentration can be precisely measured so that even an exceptionally small defect in the membrane can be detected.



Superior Applicability of the Binary Gas Test

While the air-liquid or nitrogen-liquid test may be impractical for small devices (e.g., 25 mm or 47 mm discs) due to the very low flow rates that must be measured, there is no such limitation for the BGT. As a result, BGT is applied by us in testing the full range of Viresolve® Pro devices—from Magnus 2.2 device (approximate EFA of 1.5 m^2) to Micro devices that are part of the Viresolve® Pro Validation Kit (approximate EFA of 3.1 cm^2).

Superior Sensitivity of the Binary Gas Test

The superior sensitivity of the BGT compared to the air/water diffusion test is shown in the data summarized in **Table 1**. Three devices, constructed from the same membrane lot, were tested with air/water diffusion, Binary Gas Tested and submitted for throughput as well as virus retention testing. While the BGT could discriminate among devices with small differences in retention, the air/water diffusion test could not.

In an additional set of experiments, Viresolve® Pro membranes (two layers assembled in a 142 mm filter holder; effective filtration area of 127 cm^2) contained single defects of a controlled size. These defects, in the range of 2–10 µm diameter, were introduced intentionally into both membrane layers by laser drilling.

The membrane devices were tested with air/water diffusion, BGT and then challenged with a solution of the bacteriophage φX-174 at 1 x 10^7 pfu/mL mixed with hIgG at 0.1 mg/mL in 50 mM acetate buffer. The virus log reduction value was measured at 75% flux decay (LRV_{75}) from the initial buffer flux.

Filter	Air/Water Flux[1] (cm^3 min-m^2)	Binary Gas Test Value[2]	75% Fouled LRV[3]
Device no. 1	12	72	5.9
Device no. 2	12	284	5.6
Device no. 3	11	760	5.0

1. At 50 psig.

2. ppmv of C_2F_6 in permeate stream using a 10/90% C_2F_6/CO_2 inlet at 50 psig.

3. Log reduction value of φX-174.

Table 1.

Figure 3 compares the air/water diffusion test to the BGT over a range of defect sizes. For the smallest defect size (2 µm), the diffusive air flow was elevated compared to the integral membrane, but was within the typical diffusive flow range of integral devices of this size. In contrast, the binary gas composition for the membrane with this same defect was over an order of magnitude higher than the integral composition; a very clear indication of a defect. Defect sizes larger than 2 µm were detectable by the air diffusion test, and even more easily by the BGT.

The impact of these defects on virus retention is shown in **Figure 4**. It can be seen that LRV_{75} shortfall due to a single defect can be closely predicted by the permeate gas composition and the binary gas model.

The BGT was demonstrated to give superior defect detection sensitivity in virus filters compared to the air/water diffusion test. While the air diffusion test provided an LRV assurance of ~ 4.5–5 for the virus filters studied, the Binary Gas Test provided an LRV assurance greater than 6. The BGT specifications are established to provide assurance that membranes with the proper virus retention properties have less than 0.5 LRV loss due to defects. Presence of larger defects that are more likely to occur during shipping, handling and use will result in elevated air diffusion flow rates as illustrated at the right side of the curve knee. Devices with defects in this range can be detected by the end user using the recommended air/water diffusion test.

The virus retention assurance delivered by the Viresolve® Pro solution has been further strengthened by the addition of the Binary Gas Test to our multi-tier approach.

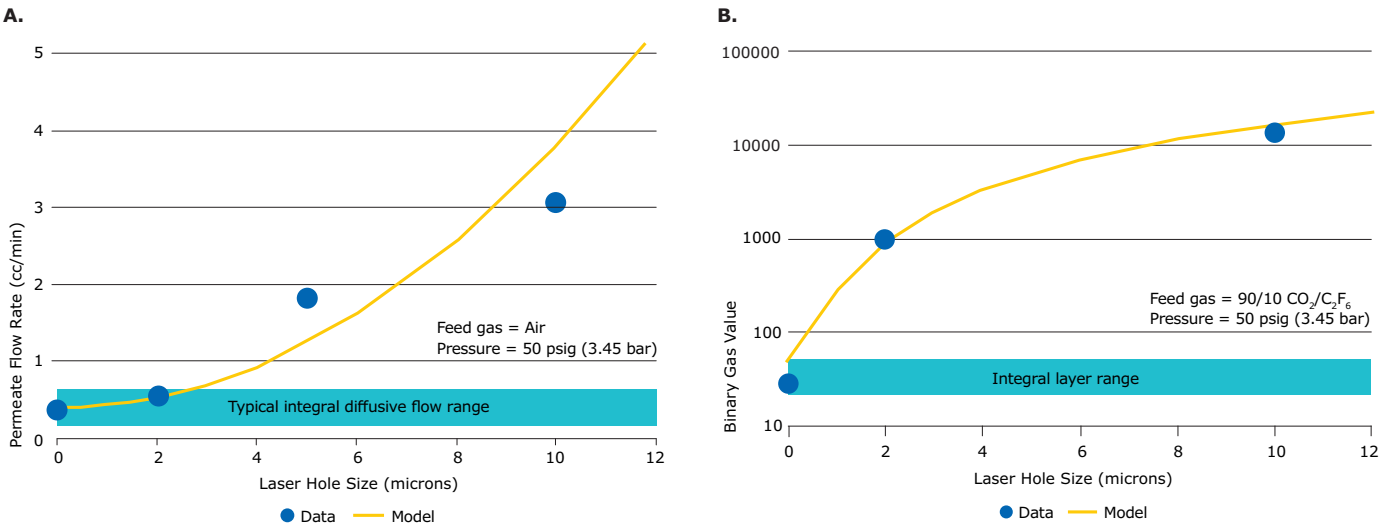


Figure 3.

A. Defect detection by air diffusion test.

B. Defect detection by Binary Gas Test.

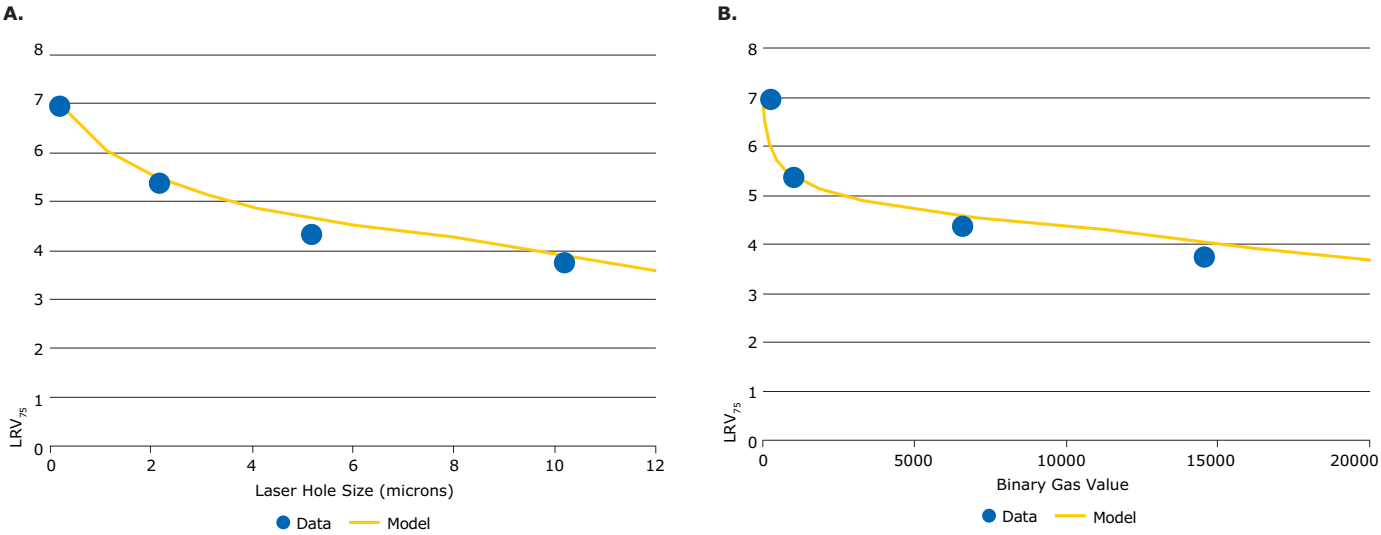


Figure 4.

A. LRV loss as a function of defect size.

B. Relationship between LRV_{75} and permeate binary gas composition.

MilliporeSigma
400 Summit Drive
Burlington, MA 01803

To place an order or receive technical assistance

Please visit
EMDMillipore.com/contactPS

For additional information, please visit
EMDMillipore.com

Lit. No. AN1033EN00 Ver. 2.0
2017 - 03221
03/2018