Vmax™ Constant Pressure Test for Sizing Viral Clearance Filters

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This document describes how to perform small scale virus filtration studies with Vmax™ filter sizing method, to assess the performance of any feed solution on a virus filter, and how to use the results of the small scale study to predict the virus filter area requirements for larger, production scale processes.

The Vmax™ filter sizing method assumes that size exclusion is the primary mechanism of particle retention and that decreases in flow during filtration are a consequence of gradual membrane pore blocking.

The Vmax™ filter sizing method is appropriate for sizing filters run under constant pressure such as Viresolve® Pro filters for virus removal in downstream processes.

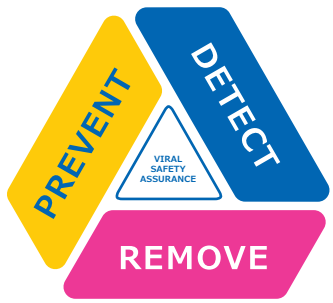
This protocol is not suitable to run validation studies.

Viral Safety Assurance: Prevent, Detect, Remove

Prevent viruses from entering the upstream process

Detect the presence of viruses

Remove or inactivate virus in downstream purification



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Technical Assistance

For more information or if you have any questions, please contact us:
EMDMillipore.com/virusexperts

The filter proposals suggested in this protocol are based on data from a first evaluation trial and should be taken for indication only. In order to enable a stable and robust filter scaling, the feasibility data have to be confirmed by intermediate and larger scale filtration trials using representative feedstock and controlled process parameters such as temperature.

Material

1.1 Feed stream

The feed stream used for sizing trials should be representative of the stream to be processed in terms of concentration, purification process etc. Ideally, fresh product should be used, however if the feed stream has previously been frozen, it is recommended to filter the feed stream over a 0.2 µm filter such as Stericup® filter units.

WFI and representative buffer are also required for prefilter/viral clearance filter preparation and equilibration prior to product filtration. Ensure that the buffer has been 0.2 µm filtered (e.g. with Stericup® Filter Units) before use.

The system can be cleaned and decontaminated using

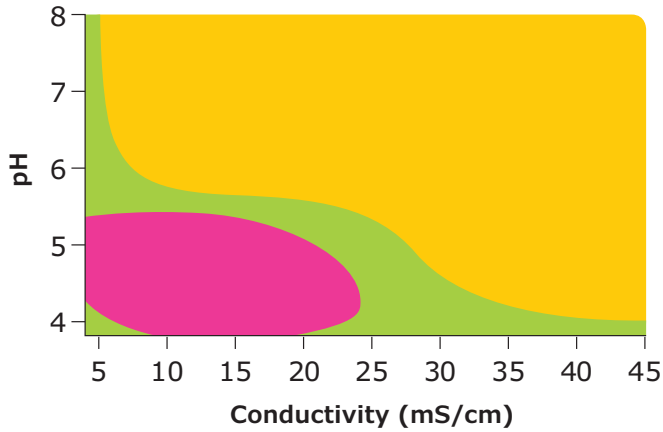
- A 0.1M NaOH solution: static soak overnight
- Or a 0.5M NaOH solution: for at least one hour

1.2 Filtration devices

- Viresolve® Pro micro devices (ref VPMNVALNB9)
- Viresolve® Shield Prefiltration micro device to improve the performance of the Viresolve® Pro filter.
  - Viresolve® Pro Shield: a cation exchange adsorptive membrane prefilter (ref VPMSKITNB9).
  - Viresolve® Pro Shield H: a mixed mode adsorptive membrane prefilter (ref VPMHKITNB9).

The prefiltration device is selected based on the conductivity and pH of the feed stream.

Figure 1. Working zones of Viresolve® Pro Shield and Viresolve® Pro Shield H as a function of pH and conductivity



More information:

Viresolve® Pro Shield and Shield H selection guide - lit.code TB1140EN | EMDMillipore.com/viresolvepro

1.3 Equipment

The following equipment will be required for the trials:

- Low Hold-up Volume Vmax™ Test Kit (ref VIRUSVMAX)
- Three Luer lock valves (2-way)
- A stopwatch
- Three disposable pipettes and pipette controller
- An equipment stand (Lab stand or retort stand)
- A 10 mL Luer lock syringe
- A balance (0.01 g accuracy if possible, otherwise 0.1 g accuracy)
- Receiving vessels to collect filtrate and vent output during trials
- Personal protective equipment (PPE): safety glasses, gloves and lab coat
- Printed copy of data collection sheet (Page 9)

1.4 Bench space utilities

The following utilities will be required for the trials:

- Around 2m linear bench space
- A source of compressed air (or nitrogen) with at least 2.2 bar / 32 psi outlet pressure
- Drain
- Electricity supply

Information and order

EMDMillipore.com/stericup
EMDMillipore.com/NaOH0.1
EMDMillipore.com/NaOH0.5
EMDMillipore.com/viresolvepro
EMDMillipore.com/vmaxtest

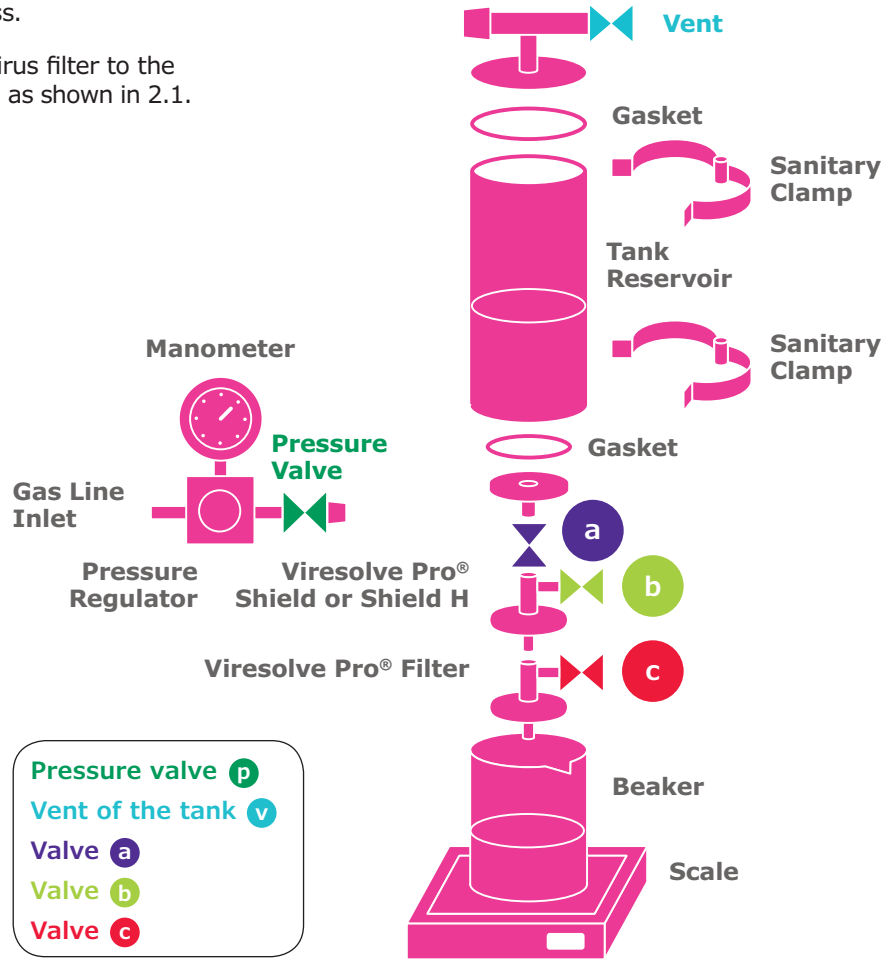
Test Method

The method below should be used in conjunction with the Viresolve® Pro Virus Filtration Solution User Guide found in every Viresolve® Pro device box.

- Familiarize yourself with the specifics of the method before you start the trial.
- Ensure that the equipment is sanitized with sodium hydroxide solution and cleaned before starting.
- Ensure that all the fluids (water, buffer and product) are at the expected run temperature, generally room temperature, before starting the experiment trial. Buffers and water should be filtered over 0.2 µm filters before use.
- After WFI wetting and buffer equilibration of the micro device, avoid introducing air into the system. Liquid should be introduced and removed using a pipette to ensure the bottom of the tank is kept wet. Any air introduced on the membrane may prevent from using all the filterable surface, and will lead to an oversized process.
- Connect the prefilter and virus filter to the tank containing feed stream as shown in 2.1.
- To avoid backpressure and potential membrane damage, do not use a valve downstream of the virus filter device.
- Prepare your data collection sheet prior to the trials (Table 1).
- During run, perform calculations as described in run sheet to monitor flow/flux decay.

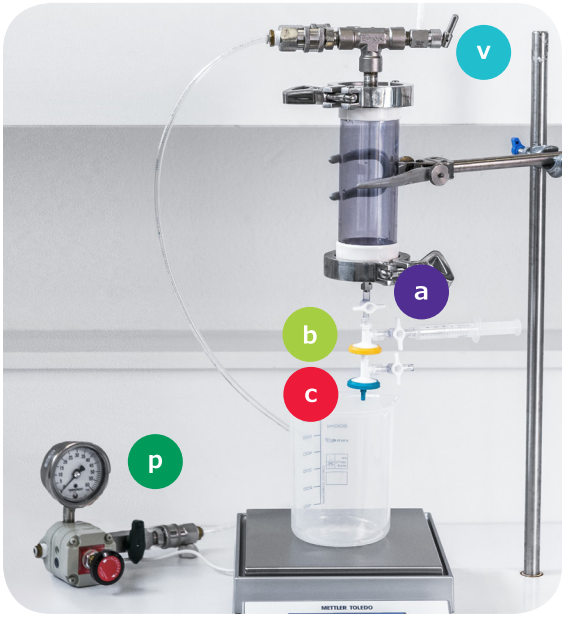
2.1 Equipment set up

- Connect the system to compressed air.
- Check that all valves of the system are closed



2.2 Prefilter and filter wetting with WFI

1. Introduce WFI in the tank. Close the lid.
2. Set pressure regulator to a pressure of 10 psi / 0.7 bar.
3. Slowly open the pressure valve (p).
4. Carefully vent the prefilter by first opening the prefilter vent valve (b), then the prefilter upstream valve (a). Tap on the side of the filter to ease the air release. Wait at least 10 seconds to ensure the prefilter is properly purged.
5. Fill a syringe with WFI, connect it to valve (b), and flush the valve to remove any trapped bubbles. Close first valve (a), then valve (b). Disconnect the syringe.
6. Open valve (c) and valve (a) to vent the virus filter. Tap on side of the filter to ease the air release. Wait at least 10 seconds to ensure the filter is properly purged. Close valve (a) and valve (c).
7. Set pressure regulator to a pressure of 30 psi / 2.0 bar (and check for leaks).
8. Open valve (a). Start the stopwatch when the first drop of fluid/filtrate enters the receiving vessel. Use the Vmax™ data collection sheet to collect data (see page 9).
9. Monitor the cumulative filtrate volume/weight every minute until a stable water flow is obtained (at 12 min, flow of ±0.1 mL/min). *Note:* if after 12 minutes, the flow is less than 2.1 mL/min, discard filters and start again with new ones.
10. Close valve (a). Stop stopwatch.
11. Close pressure valve (p).
12. Open the vent of the pressurized tank (v) to release the pressure.
13. Open the lid of the tank and empty the water of WFI with a pipette. Be sure to avoid introducing air into the filter. Leave sufficient water in the tank to cover the bottom to avoid introducing air into the filter.



Watch our tutorial

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2.3 Equilibration of filters with buffer

1. Introduce buffer in the tank. Close the lid.
2. Set pressure regulator to a test pressure of 30 psi / 2.0 bar.
3. Slowly open the pressure valve (p).
4. Open valve (a). Start the stopwatch when the first drop of buffer enters the receiving vessel. Use the Vmax™ data collection sheet to collect data (see page 9).
5. Monitor the cumulative filtrate volume/weight every minute until a stable buffer flow is obtained (±0.1 mL/min).
6. Close valve (a) to isolate the filter. Stop stopwatch.
7. Close pressure valve (p).
8. Open the vent of the pressurized tank (v) to release the pressure.
9. Open the lid of the tank and empty the buffer with a pipette. Leave sufficient buffer in the tank to cover the bottom to avoid introducing air into the filter.

2.4 Filtration of feed stream

1. Introduce feed stream into the tank first with a pipette. Then – carefully, down the sidewall, to avoid creating bubbles.
2. Close the lid.
3. Place a clean beaker or bottle below the filtration set-up to collect the filtrate.
4. Set pressure regulator to a test pressure of 30 psi / 2.0 bar, or at the chosen working pressure.
5. Slowly open the pressure valve (p).
6. Open valve (a). Start the stopwatch when the first drop of product enters the receiving vessels. Use the Vmax™ data collection sheet (see page 9).
7. Calculate flow at 5 mins (volume processed over time) this corresponds to Q_0.
8. Stop the test when 90% of flux decay is reached (see data collection sheet on see page 9).
9. Close valve (a). Stop the stopwatch.
10. Collect the filtrate and take a sample according to the sampling plan.
11. Remove the test devices from the system and discard.
12. Close the pressure valve (p).
13. Open the vent of the pressurized tank (v).
14. Once all the pressure is released, open the lid.
15. Place a beaker or a bottle under the pressurized tank, open valve (a) and recover the product. Clean the equipment.

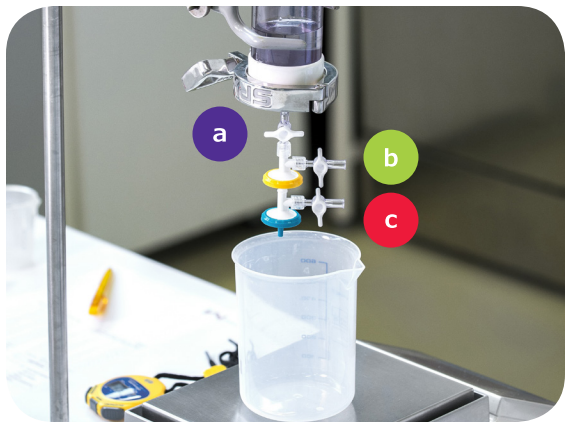


Table 1. Vmax™ constant pressure test for virus filtration: data collection sheet

Product:		**Date:**	
Test pressure (bar/psi):		Test temperature:	**Room temperature**

Prefilter:		Filter:	**Viresolve® Pro Filter**
Catalog number:		Catalog number:	**VPMNVALNB9**
Lot number:		Lot number:	
Filtration area (cm²):	**3.1**	Filtration area (cm²):	**3.1**

1. Wetting (WFI)

Time (min)	Volume (mL)	Flow rate (mL/min)	Comment
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
...			

Stop when flow is stable (±0.1 mL/min).
If flow < 2.1 mL/min, discard filters and restart.

2. Buffer equilibration

Time (min)	Volume (mL)	Flow rate (mL/min)	Comment
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
...			

Stop when flow is stable (±0.1 mL/min).

3. Product filtration

Time (min)	Volume (mL)	Flow rate or Q_{inst} (mL/min)	Flow decay (%)	Comment
1				
2				
3				
4				
5			**0%**	**Q_0**
6				**% flow decay (FD) = [1-(Q_{inst}/Q_0)] x 100%**
7				
8				
9				
10				
12				
14				
16				
18				
20				
25				
30				
35				
...				

Stop experiment at 90% of flow decay. Please contact us if you do not reach a 90 % flow decay.
EMDMillipore.com/virusexperts

Data analysis and sizing

3.1 Data analysis

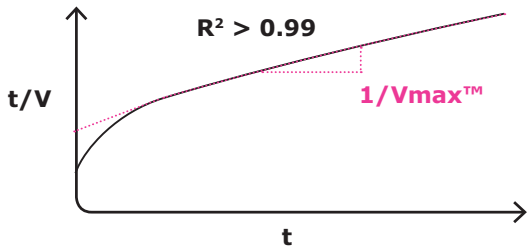
The units used in all calculations are:

Volume (V):	Liters (L)
Filtration area (A):	Square meters (m²)
Time (t):	Hours (h)

To determine the filter sizing, the data is analyzed using a gradual pore-plugging model as follows:

1. Enter all the time (hours) and volume (in liters) into an Excel spreadsheet.
2. Plot t vs t/V.
3. Add a linear trendline to the data.

4. Using the trendline function, determine slope and correlation coefficient (R^2).
   - If $R^2 > 0.99$, the Vmax™ model can be applied (i.e. gradual pore plugging is the main fouling mechanism).
   - If $R^2 < 0.99$, contact us (EMDMillipore.com/contactPS).
5. If $R^2 > 0.99$, divide 1 by the value of the slope. This is the Vmax™ value (maximum volume in liters that can be filtered through the test device used in this study).



6. Divide the Vmax™ value by the membrane surface area (in m²) of the Viresolve® Pro micro device (0.00031m²).

   This Vmax™ value in L/m² represents the maximum volume in L that can be filtered through 1m² of filter area.
7. Divide the initial flow rate Q_0 by the area of the test device (in m²) to obtain the initial flux J_i (in L/h/m² or LMH):
   - $J_i = Q_0 / 0.00031$
8. Calculate the predicted throughput at V_{90} (in L/m²) as follows:
   - $V_{90} = 0.68^* V_{max}$
9. Calculate the minimum area (for a given batch size and process time) using the equations in Section 3.2.

3.2 Sizing calculations

Either a specific **batch size**:	Or a **volume in a specific time**:
$A_{min} = V_b/V_{90}$	$A_{min} = V_b/V_{90} + V_b/(J_i * t_b)$

Where:
A_{min} is the minimum filtration area (m²)
V_b is the batch volume (L)
V_{90} is predicted throughput at V_{90}, determined previously (L/m²)
J_i is the initial flux (L/h/m²)
t_b is the processing batch time (h)

To size Viresolve® Pro filters, we recommend using the V_{90} value calculated in Section 3.1. One of the following formulas, dependent on whether the process is defined by (a) batch volume or (b)processing time, can be used to determine the minimum area needed for viral clearance filtration.

To allow for variability due to feed, and process conditions, a safety factor is typically included to define a required filtration area. The required filtration area for a process will be the minimum area for a average performance multiplied by a safety factor:

$A = A_{min} * SF$

Where:
A is the final process area (m²)
A_{min} is the minimum process area calculated (m²)
SF is the safety factor (Table 2)

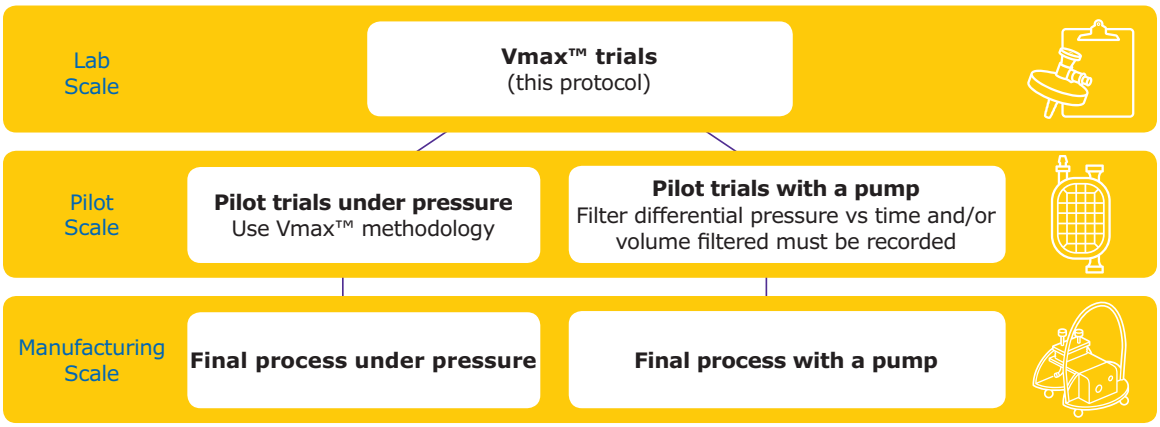
Use the same surface area for Viresolve® Pro Shield or Shield H prefilter than for Viresolve® Pro filter.

Table 2. Typical range of safety factors

Application	Typical safety factor range	Initially recommended safety factor (without detailed information)
Virus filtration	1.4-1.8	1.5

** Information can be found in Herb Lutz, Journal of Membrane Science 341 (2009) p268–27*

The pilot trial should be a scaled-up version of the system recommended based on Vmax™ testing. When possible, the batch volumes, flow rates, temperatures, etc. should be simulated. The recommended scale-up strategy is as follows:



For more information on Vmax™ sizing, please read application notes AN1512EN00 and AN1025EN00.

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