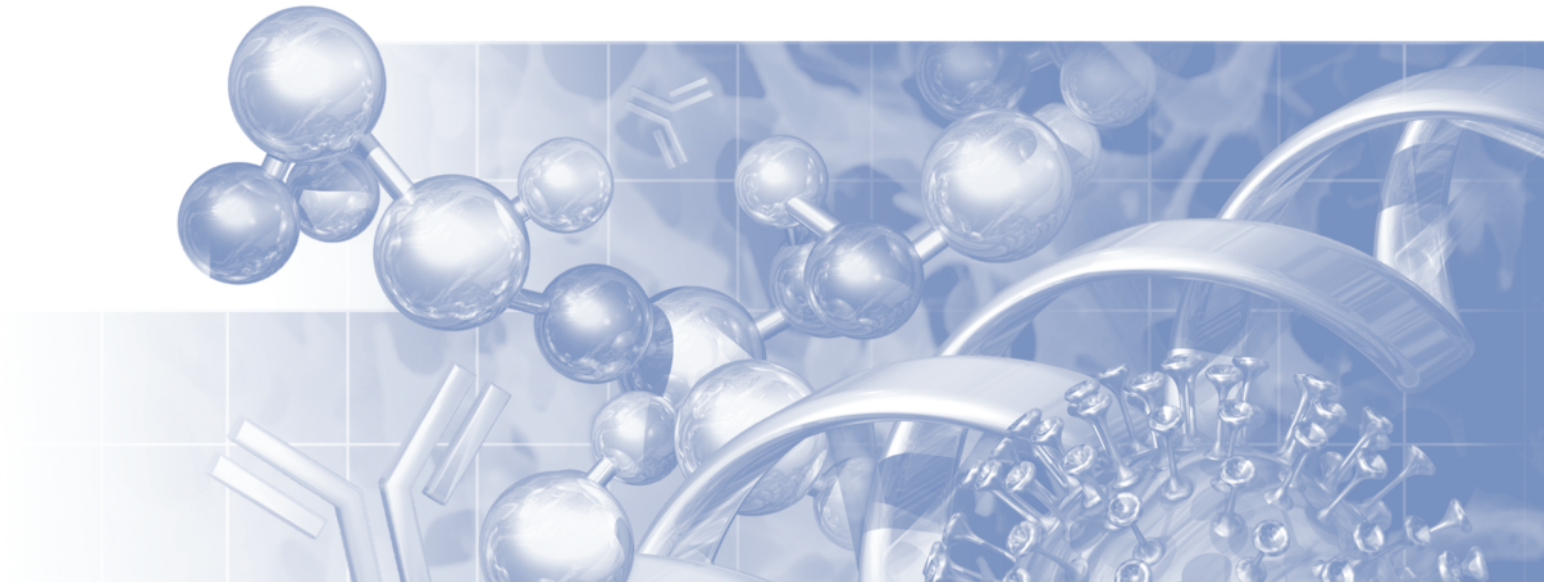
PALL Life Sciences

Application Note

USTR 2652

Filterability Testing and Virus Challenge of Pall® Minidisc Virus Removal Filter Capsules with Ultipor® VF DV20 Membrane



*Filtration. Separation. Solution.*SM

Contents

1. Introduction

The Pall Minidisc capsule is a pre-assembled disposable filter capsule assembly incorporating Pall virus filter membranes. It is an effective and simple-to-use device for all small-scale virus filter testing requirements. This application note will guide the user in the best approaches for filterability, protein transmission testing and virus challenges of Pall membranes in the Minidisc capsule.

Figure 1
Minidisc Capsules

Details of how to use Minidisc capsules are contained in the product's Instructions for Use (USD 2474)[1], which is provided in every box. Care should be taken to follow these instructions closely to ensure successful operation. The following are some key specifications that are relevant to planning experiments and analysing data generated with the Minidisc capsule.

Table 1
Key Specifications of the Minidisc Capsule

Effective Filtration Area (EFA)	9.6 cm^2
Maximum Recommended Operating Pressure	3.1 barg (45 psig)*

**Temporary pressures up to 3.4 barg (50 psig) are acceptable, but the target operating pressure should not exceed 3.1 barg (45 psig) to allow for pressure fluctuations during testing. If tests at operating pressures exceeding 3.1 bar (45 psi) are required, a stainless steel disc holder and 47 mm membrane discs are available (Pall disc holder part number: FTK200; discs part number FTKDV2004705 and FTKDV20047025 for a box of 5 or 25).*

This application note is separated into two broad categories: Section 2 on filterability and protein transmission testing and Section 3 on viral challenge tests. Filterability and protein transmission trials are often conducted initially to determine the flow, capacity and protein passage through a virus filter with a product intermediate feed stream. The results show the potential throughput that a virus filter can provide during processing. Viral clearance validation is then required to confirm that the required virus retention can be achieved with this throughput.

2. Filterability and Protein Transmission Trials

2.1 How to Run a Filterability Trial

For the best results a filterability trial should be run using a calibrated balance (accuracy ≤ 0.1 g) to collect and measure the filtrate mass over time. 1 $g.mL^{-1}$ is a sufficiently accurate estimation of density for water and simple buffers, but the user should determine their product feed

density if this is believed to be significantly different from water. The temperature should be kept at the same level as the full-scale process where possible, in order to give the correct product viscosity.

A buffer conditioning step can be used if desired, to reduce the risk of aggregate formation at the water-product interface. Switch the feed from water to buffer as per Section 6 of the Instructions for Use for Pall Minidisc Virus Removal Filter Capsules (USD 2474)[1]. If buffer flux needs to be determined, discard the first 3 mL (i.e. in excess of the system hold-up volume) of filtrate before measuring the buffer flowrate over the next 10 minutes. As with water flow testing, ensure that there is sufficient buffer in the feed reservoir so that air does not get into the Minidisc capsule (see Section 3.5 of USD 2474). After the buffer flush, Section 6 of USD 2474 should be repeated again for the product feed.

We strongly recommend that filterability trials are run to full processing time for the most accurate estimation of performance. If time or product volume constraints are in place then the longest processing time possible should be used and results forward predicted using the V_{max} model to estimate throughput.

2.2 Forward Prediction of Throughput Using V_{max} Analysis

V_{max} is the estimated value of the maximum capacity of a membrane[2], i.e. the throughput that would be reached when the membrane is completely plugged if time and feed quantity were not restricted and the membrane fouls in line with the standard pore constriction model.

V_{max} is calculated from a plot of time over throughput (At/V) against time (t) and is the inverse of the gradient, as shown by the linear form of the standard blocking law equation:

Equation 1

Constant Pressure V_{max} Linear Equation

$$\frac{At}{V} = \frac{t}{V_{max}} + \frac{1}{J_0}$$

A = filtration area (m^2), t = time (h), V = volume (L), V_{max} = estimated maximum throughput capacity ($L.m^{-2}$), J_0 = initial flux ($L.m^{-2}.h^{-1}$).

The gradient should be determined from the linear portion of the graph only, as indicated in Figure 2. The initial flux should also be calculated from the same linear portion of the graph and is evaluated as the y-intercept of that linear data. This initial flux is not always as accurate as direct flux measurement, but will give the most accurate forward prediction. Equation 1 can then be rearranged to estimate the throughput achieved at a given time:

Equation 2

Forward Prediction of Throughput at Constant Pressure

$$\frac{V}{A} = \frac{t}{\left(\frac{t}{V_{max}} + \frac{1}{J_0}\right)}$$

Figure 2

Data Analysis for Forward Prediction

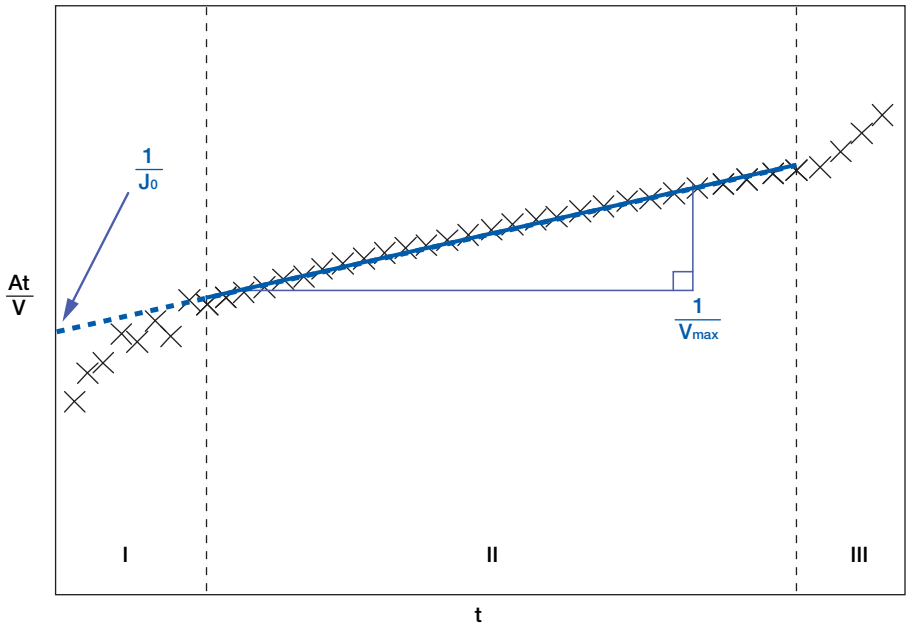


Figure 2 shows typical data collected during a Minidisc filterability run, showing three phases of data collection.

- I – Start up effects cause inaccurate and variable data due to the low flux decay relative to measurement accuracy and start time accuracy. The slope can be under or over- predicted and the time for this phase will vary between tests.
- II – The linear portion of the graph used to determine V_{max} and J_0 for forward prediction.
- III – End effects, only seen if the feed sample is filtered to completion and flow reduces to zero due to the feed running out.

Caution should be taken when forward predicting. The following conditions should be adhered to in order to minimize estimation errors:

- Forward predicted throughput no more than twice the measured throughput.
- Forward predicted throughput < 90% of the calculated V_{max}.
- Coefficient of determination (R^2 value) > 0.95.

The closer the raw data collection time is to the estimation time, the more accurate the estimated throughput will be. Fouling is more complex than a simple constriction mechanism, however the model is the most appropriate of all the traditional membrane fouling mechanisms[3] for small forward predictions of limited data sets. Using V_{max} to forward predict throughput relies on the assumption that the gradient measured from the At/V vs t plot remains constant up to the estimation time. The V_{max} value itself should therefore be quoted as a maximum capacity with caution, since this definition is based on the assumption that the gradient remains constant until complete blockage. This is often a long and potentially very inaccurate extrapolation for high capacity membranes such as Ultipor VF DV20.

In general, V_{max} values for Pall filters are very high and exceed the throughput that can be reached in typical processing times. Most of the time membrane performance (batch area requirement) is either independent or weakly dependant on V_{max} and batch area requirement is governed by the processing time and membrane initial flux. For high V_{max} values, we do not recommend performance comparisons using V_{max}, whereas for cases when the membrane is plugged V_{max} can potentially be quoted with caution as highlighted above.

Contact Pall for more advanced fouling analyses if you believe that the model does not fit the raw data.

2.3 Typical Filterability Results

For filterability testing, human immunoglobulin G (hIgG) solutions were made from lyophilized human gamma globulin powder (Seracare, MA) in pH 7.4 phosphate buffered saline (PBS) with 10 mM phosphate, 137 mM NaCl and 3 mM KCl. Prefiltration of IgG solutions with 0.1 µm Fluorodyne® II grade DJL filters is recommended to remove large undissolved aggregates.

Table 2 compares the average flux for Minidisc capsules (10MCFDV20) and 47 mm discs of Ultipor VF DV20 membrane (FTKDV20047) in stainless steel disc holders (FTK200). Testing was carried out in parallel using two hIgG lots with different fouling characteristics (Lot 1: 18% flux decay at 60 L/m^2, Lot 2: 38% flux decay at 60 L/m^2). These results verify that the Minidisc capsule with Ultipor VF DV20 membrane provides equivalent performance to 47 mm Ultipor VF DV20 membrane disc test equipment. Flat sheet Ultipor VF DV20 membrane discs demonstrate good IgG flux scalability factors to fanpleat construction Ultipor VF DV20 cartridges (scalability factor = 0.95) and laid-over pleat construction Ultipor VF Grade UDV20 cartridges (scalability factor = 1.04)[4].

Table 2

Minidisc Capsule Performance Verification – Comparison of Average Fluxes for Minidisc Capsules with Ultipor VF DV20 Membrane (10MCFDV20) and 47 mm Ultipor VF DV20 Membrane Discs (FTKDV20047) in FTK200 Holders Measured at 21 °C and 2.1 bar (30 psi) (n = 6)

Test Solution	Average Minidisc DV20 Capsule Flux ($L.m^{-2}.h^{-1}$ at 4 hours)	Average 47 mm FV20/FTK200 Disc Assembly Flux ($L.m^{-2}.h^{-1}$ at 4 hours)
hIgG Lot 1	19.2 ± 3.6	19.3 ± 2.5
hIgG Lot 2	16.4 ± 3.9	16.4 ± 3.9

Figure 3 shows a typical flux profile for Ultipor VF DV20 membrane challenged with hIgG. The flux remains high relative to the initial rate throughout the experiment for both operating pressures. In contrast, triplicate competitor filter devices tested at their recommended operating conditions all completely blocked before processing 35 $L.m^{-2}$ (data not shown here). Increasing the operating pressure for Ultipor VF DV20 to 3.1 bar (45 psi) yields higher flux without significantly increasing flux decay. We therefore recommend using an operating pressure of 3.1 bar (45 psi) to achieve the maximum flux performance.

Figure 4 demonstrates the robust nature of the Ultipor VF DV20 membrane. The shaded blue area is the typical average flux expected for Ultipor VF DV20 membrane over a 4 hour process at an operating pressure of 2.1 bar (30 psi). The data is collected from feeds that present very variable filterability challenges: different protein types, multiple sources and various manufacturing processes. Due to the Ultipor VF DV20 membrane's strong resistance to plugging, there is a narrow range of process fluxes. The outlier, an example of an extreme filterability challenge, can only be effectively processed with membranes that are resistant to plugging, such as Ultipor VF DV20.

Figure 3

Increasing Performance at Higher Operating Pressure – Typical Flux Profiles for Ultipor VF DV20 Membrane Filterability Tests with hIgG Lot 1 in PBS at 21 °C and 2.1 bar (30 psi) or 3.1 bar (45 psi)

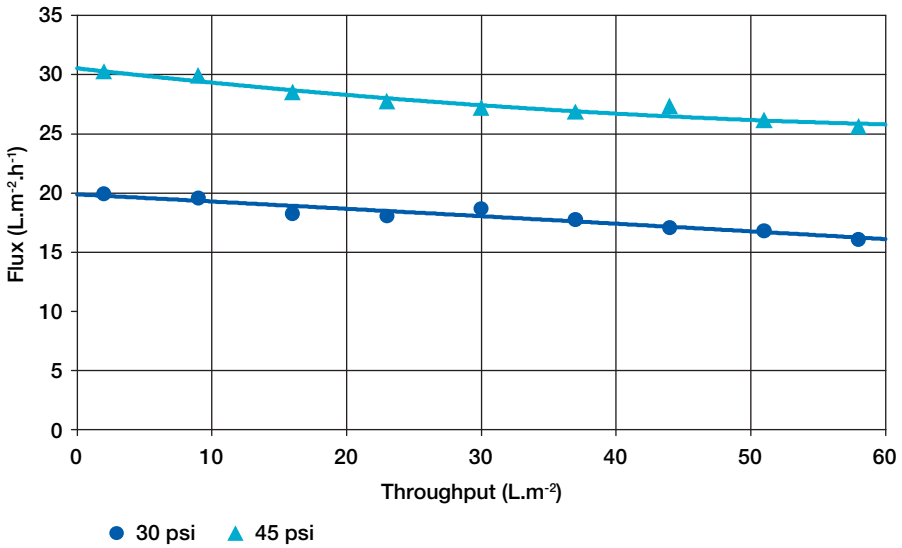


Figure 4

Ultipor VF DV20 Robustness

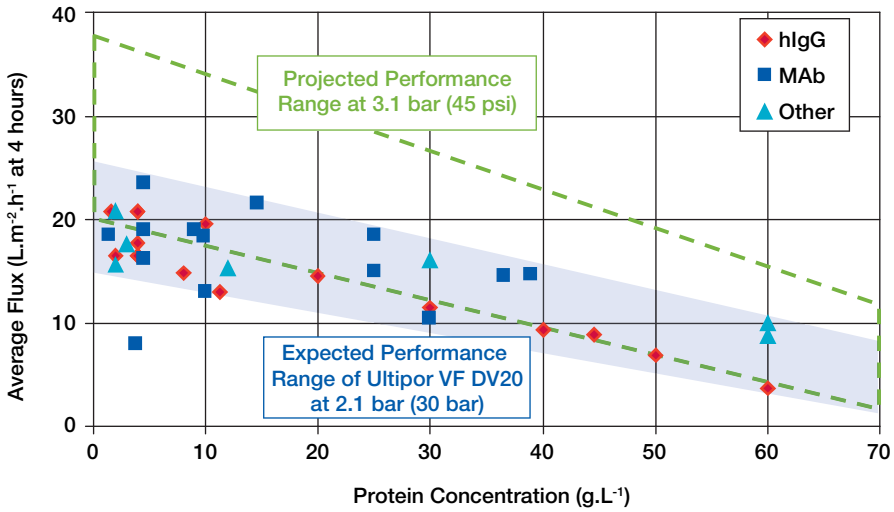


Figure 4 shows a summary of data collected for a variety of feed types and concentrations during internal and external testing at 2.1 bar (30 psi) operating pressure. The blue shaded area represents the expected performance range of Ultipor VF DV20. The green dashed box represents the projected performance range when operated at 3.1 bar (45 psi).

2.4 Constant Flow Operation

We recommend that small-scale tests be carried out at constant pressure. Difficulties in maintaining a constant flow and accurately measuring a fluctuating test pressure will generate more experimental noise than a constant pressure test and the results will be less reliable. Therefore constant pressure testing will always be preferable.

If required, the key to successful constant flow operation is a pump that is capable of supplying the required flowrates accurately up to 3.1 bar (45 psi) pressure. The pump can be used at each point in the instructions when the pressure would otherwise be applied. When pumping out a fluid from the system, the Minidisc capsule should be closed off from the other equipment and the pump can be used in a priming mode or at a manually set higher flow.

2.5 Protein Transmission

Protein transmission studies can be conducted concurrent with filterability testing by collecting samples of feed solution before and after filtration and subjecting them to protein assays. Where the target protein is the major protein species, either a generic protein assay or a target protein-specific assay can be applied. Other assays may be employed on the filtrate to assess conformation, biological or enzymatic activity, as appropriate.

3. Virus Challenges

3.1 General Protocol Recommendations

For virus challenges with volumes up to our recommended Minidisc capsule challenge volume of 50 mL (50 $L.m^{-2}$), the filtrate should be collected in two aliquots of equal volume. Combine equal sample volumes from each aliquot to form a pool of sufficient volume to carry out the virus titer assay. Retain the remaining filtrate aliquots. Analyze the pooled sample first. If the pooled sample meets the required viral titer reduction (log reduction value, LRV), this is sufficient to establish a viral clearance claim, and additional virus assays will not be necessary. If the pooled sample does not meet the required viral LRV, then assay the individual aliquots to evaluate the filter performance as a function of the two throughput volumes.

For example, if a 50 mL (50 $L.m^{-2}$) virus challenge is required and 10 mL is needed for virus titer determination, then the following procedure is recommended:

- Collect 2 x 25 mL consecutive aliquots
- Take out equal 5 mL sample volumes from each aliquot and mix to generate a 10 mL pooled sample, representative of the entire pooled filtrate at 50 mL (50 $L.m^{-2}$) throughput.
- Label the remaining aliquots and store appropriately.
- Assay the prepared pooled sample.
- If LRV of the pooled sample is less than target (typically > 3 or > 4 log), then assay the individual stored aliquots to assess initial performance

For virus challenges greater than 50 mL (> 50 $L.m^{-2}$), two initial 25 mL aliquots should be taken and treated as above. The subsequent filtrate volume above 50 mL should be split into an appropriate number of additional aliquots of equal volume and the same pooling approach should be carried out for analysis. This will give two pooled samples: one up to 50 mL and one from 50 mL onwards. Assay these two pooled samples first (or a single pooled sample prepared from all the aliquots), followed by the relevant intermediate aliquots as required. This approach limits the number of expensive assays, but still retains the option of investigating intermediate aliquots if required.

Collection of aliquots should be in individual graduated sterile containers. The time taken to collect each aliquot should be recorded in order to calculate the flux.

Virus clearance is measured by the log titer reduction (LTR) or log reduction value (LRV), which is the base-10 logarithm of the ratio of feed input concentration (C_{feed}) to filtrate concentration ($C_{filtrate}$).

Equation 3

Log Titer Reduction (LTR) or Log Reduction Value (LRV)

$$LTR,\ LRV = \log_{10}\left(\frac{C_{feed}}{C_{filtrate}}\right)$$

3.2 Typical Virus Challenge Results

Table 3 and Table 4 summarize the typical retrovirus and parvovirus retention obtained by users of Ultipor VF DV20 membrane. The test pressures for these virus challenge results ranged from 2.1 bar (30 psi) to 3.1 bar (45 psi). Minidisc capsules with Ultipor VF DV20 membrane demonstrate robust effective virus retention across a range of pressures. We recommend using 3.1 bar (45 psi) for validation and process conditions to achieve the maximum flux performance and robust viral clearance.

Table 3

Effective Parvovirus Reduction – Typical Parvovirus Reduction by Ultipor VF UDV20 Filters. PPV = Porcine Parvovirus, MMV = Mouse Minute Virus, B19 = Human Parvovirus

Product Type	LRV		
	PPV	MMV	B19
mAb 1	6.3	–	–
mAb 2	–	6.2	–
mAb 3	–	> 5.8	–
mAb 4	–	5.1	–
mAb 5	> 5.8	–	–
mAb 6	> 5.0	–	–
mAb 7	4.2	–	–
hIgG 1	> 4.8	–	–
hIgG 2	–	–	> 5.0
hIgG 3	> 6.6	–	–

Table 4

Effective Reduction of Large Viruses – Typical Retrovirus Reduction by Ultipor VF UDV20 Filters. X-MuLV = Xenotropic Murine Leukemia Virus, A-MuLV = Amphotropic Murine Leukaemia Virus, HIV = Human immunodeficiency Virus, BVDV = Bovine Viral Diarrhea Virus

Product Type	LRV			
	HIV	BVDV	X-MuLV	A-MuLV
mAb 1			> 5.6	–
mAb 2			> 5.3	–
mAb 3			> 5.1	–
mAb 4			> 5.7	–
mAb 5			–	> 6.0
mAb 6			> 5.4	–
mAb 7			> 4.9	–
mAb 8			–	> 5.7
mAb 9			> 5.4	–
mAb 10			> 4.6	–
hIgG 1	> 5	> 5		
hIgG 2	> 6.2	> 5.4		
hIgG 3	> 5	> 4.6		
hIgG 4	> 4.6	> 6.1		

3.3 Crude Virus Preparations

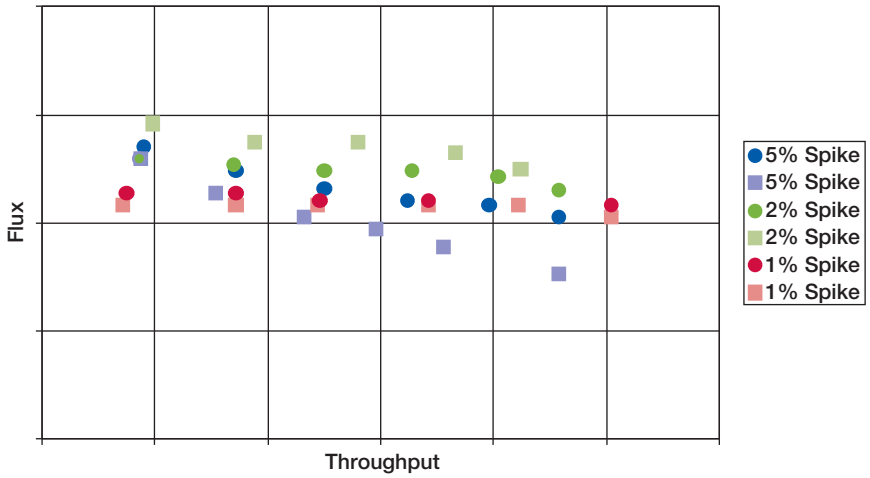
When spiking crude (unpurified) virus preparations into feed solutions, cell culture-derived contaminants are introduced that can potentially block some virus filters. Figure 5 shows how virus spike levels of up to 5% have been used with Ultipor VF DV20 membrane while still maintaining sufficient flux to process the required viral validation throughputs.

This demonstrates that complex virus spike purification and non-standard spike challenge procedures are not required with Ultipor VF DV20 filters in order to generate high viral clearance results, especially with retroviruses which are typically retained 100% to the limit of detection. This is because of the Ultipor VF DV20 membrane's high capacity and robustness to plugging by spike contaminants such as cell debris, large proteins and protein aggregates.

Table 5 shows the corresponding parvovirus clearance values (LRVs) generated from the tests shown in Figure 5. At the spike level of 1% there was insufficient virus concentration to validate clearance above an LRV of 4.0. The high spike levels of 2% and 5% had sufficient numbers of viruses in the feed to enable higher LRVs to be demonstrated. The ability to effectively process crude virus preparations gives more flexibility for virus challenges and increases the potential to validate a higher viral clearance claim.

Figure 5

Resistance to Plugging by Crude Virus Preparations Spikes



Ultipor VF DV20 membrane flux profiles during duplicate viral challenge tests at different virus spike levels. The data are duplicate results from three separate monoclonal antibody solutions that required a variety of virus spike specifications.

Table 5

Effective Virus Reduction at High Spike Levels – Parvovirus Reduction by Ultipor VF DV20 Membrane at Different Virus Spike Concentration Levels

Product	Spike Concentration	Virus	Pressure	LRV*
Plasma Factor	1%	PPV	2.1 bar (30 psi)	> 4.0
MAb 1	2%	MMV	3.1 bar (45 psi)	6.7, 6.3
MAb 2	5%	MMV	3.1 bar (45 psi)	5.9, 6.2

** Test in duplicate*

4. Calculating Throughputs and Fluxes

4.1 Accounting for Minidisc Capsule Hold-up Volumes

During normal operation there will be a small volume of liquid within the Minidisc capsule that remains in the system when switching from one process fluid to the other. The total hold-up volume is 2.1 mL. The downstream hold-up volume (after upstream liquid displacement through the membrane with air or nitrogen) is 0.7 mL.

Where the Minidisc capsule is first flushed with water or buffer, the flush liquid will make up the first 2.1 mL of the initial filtrate collected from the filter. For virus challenges, the effect on retention will be negligible, with an error of 0.02 in the LRV at our recommended test volume of

50 mL. For any small grab sample taken directly from the capsule and not the pooled with the subsequent filtrate, the recovery will be accurate if taken after the first 2.1 mL of filtrate. We recommend that the throughput at which these retentions and recoveries are quoted should be adjusted to account for hold-up volume and this is detailed in Table 6.

Table 6
Volume to Throughput Conversion

Volume Collected (mL)	Throughput (L/m^2)	Adjusted Throughput (L/m^2) to Account for Hold-Up Volumes (For Protein Transmission/ Viral Retention)
1.0	1.0	–
2.1	2.2	0.0
5.0	5.2	3.0
10.0	10.4	8.2
15.0	15.6	13.4
20.0	20.8	18.6
25.0	26.0	23.9
30.0	31.3	29.1
40.0	41.7	39.5
50.0	52.1	49.9
75.0	78.1	75.9
100.0	104.2	102.0
x	$\frac{x}{0.96}$	$\frac{(x-2.1)}{0.96}$

4.2 Estimating Flux

When aliquots are taken, the flux ($L.m^{-2}.h^{-1}$) for that aliquot is simply the total unadjusted throughput ($L.m^{-2}$) divided by the time (h). If continuous data is collected using a balance, then the flux can be charted throughout the experiment. Many different options for calculating flux from continuous data exist with varying complexity. We recommend that the flux at a given data point should be calculated as the slope of the throughput and time data up to 5 minutes either side of the data point. This provides an accurate estimate of flux because of Ultipor VF DV20 membrane's low rate of fouling relative to other filters. Calculating the instantaneous flux between every time point collected can lead to significant variation in the calculated flux, especially when collecting data over small time intervals, due to the discrete nature of the filtrate drops and instability of the balance reading.

Contact Pall if you require further advice on flux and throughput analysis or any other aspect of Minidisc capsule operation.

5. References

1. USD 2474 – Instructions For Use For Pall® Minidisc Virus Removal Filter Capsules.
2. F. Badmington, E. Honig, M. Payne and R. Wilkins. *Pharmaceutical Tech*. 19 (1995), pp. 64–76.
3. J. Hermia. Trans. *Inst. Chem. Eng*. 60 (1982), pp. 183–187.
4. USTR 2612 – Scalability of Ultipor® VF Grade DV20 and UDV20 Virus Removal Filter Cartridges.

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