Characterizing the Impact of Pressure on Virus Filtration Processes and Establishing Design Spaces to Ensure Effective Parvovirus Removal

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Virus filtration provides robust removal of potential viral contaminants and is a critical step during the manufacture of biotherapeutic products. However, recent studies have shown that small virus removal can be impacted by low operating pressure and depressurization. To better understand the impact of these conditions and to define robust virus filtration design spaces, we conducted multivariate analyses to evaluate parvovirus removal over wide ranges of operating pressure, solution pH, and conductivity for three mAb products on Planova™ BioEX and 20N filters. Pressure ranges from 0.69 to 3.43 bar (10.0–49.7 psi) for Planova BioEX filters and from 0.50 to 1.10 bar (7.3 to 16.0 psi) for Planova 20N filters were identified as ranges over which effective removal of parvovirus is achieved for different products over wide ranges of pH and conductivity. Viral clearance at operating pressure below the robust pressure range suggests that effective parvovirus removal can be achieved at low pressure but that Minute virus of mice (MVM) logarithmic reduction value (LRV) results may be impacted by product and solution conditions. These results establish robust design spaces for Planova BioEX and 20N filters where high parvovirus clearance can be expected for most antibody products and provide further understanding of viral clearance mechanisms. © 2017 The Authors Biotechnology Progress published by Wiley Periodicals, Inc. on behalf of American Institute of Chemical Engineers *Biotechnol. Prog.*, 33:1294–1302, 2017
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Introduction

During the manufacture of biotherapeutics produced from mammalian cell culture or plasma, it is critical that the downstream purification processes effectively remove actual and potential viral contaminants. Since virus removal by filtration is primarily a size-based mechanism, effective clearance can be achieved for a wide range of virus types, independent of their physicochemical characteristics. Virus filtration is also one of the few unit operations that is capable of achieving highly effective removal of small nonenveloped viruses, which are resistant to inactivation and may be difficult to remove by chromatography steps. Virus filtration has no impact on product quality, making it an ideal solution for enhancing the safety of most biological products, and owing to its high degree of effectiveness and robustness, virus filtration is one of the most commonly implemented unit operations.

As with other unit operations, the goal for developing and implementing a virus filtration step for a manufacturing process is to achieve a highly robust operation that ensures reliable performance with varying feed materials and provides process flexibility to minimize the likelihood of deviations.

Additional Supporting Information may be found in the online version of this article.

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For virus filtration, the main performance parameters for evaluating robustness are product capacity and viral clearance capability, while the major sources of variability are variations in feedstock, differences in filter lots, and ranges of operating parameters. Many case studies have demonstrated the robustness of virus filtration processes even with variation in solution conditions and under wide ranges of operating parameters.[1–5] For example, studies using Planova™ 20N and BioEX filters have shown high product capacity and virus removal over wide ranges of protein concentration, ionic strength and pH.[3,5] Reduced performance has typically been associated with product aggregates clogging the filter membrane and reducing product throughput and, in some cases, reducing viral clearance.[3,6–8] Impurities present in the virus spike also can play a significant role in filter fouling on certain filter types, although contract laboratories have made significant efforts to improve virus stock purity to lessen those effects.[9–11]

Operating pressure is one process parameter that has recently been observed to impact viral clearance. Prior to 2011, operating at high pressure was generally assumed to be the worst-case condition, and low pressure or depressurization events were not known to impact virus removal.[12] In 2011, two independent studies presented at the Parenteral Drug Association (PDA) Virus and TSE Safety Forum showed that low transmembrane pressure and depressurization events could negatively impact removal of parvoviruses by small virus filters.[5] Specifically, it was demonstrated that an increase in parvovirus in the filtrate could be observed immediately after a depressurization event for most filter types tested, but no impact was observed with a larger virus model.[13] At the same meeting, it was also demonstrated that Planova 20N filters operated at a very low pressure of 0.20 bar had increased parvovirus in the filtrate for certain solution conditions.[14] More recently, depressurization has been shown to impact parvovirus removal on most filter types to varying degrees and to be highly dependent on the specific product being filtered.[15,16] These product- and process-specific effects indicate that although a given filter type may not exhibit low pressure effects with a particular product under specific conditions, significant negative effects may be observed with other products and/or conditions. Therefore, it is critical to evaluate the low pressure and depressurization effects for each product and process on a case-by-case basis.

The mechanism by which reduced pressure influences parvovirus removal is not completely understood, but a well-supported hypothesis has been developed. That is, the convective force of fluid flowing through the filter entraps virus particles in regions of the filter where access is limited to a subset of pores, and the virus particles cannot pass farther through the filter. At lower flow rates, however, diffusion of the virus particles allows them to sample more pores and increases the probability that any given virus particle will encounter a pore that is large enough to pass through. This mechanism was originally proposed to explain flow rate-dependent elution of adenovirus from ion exchange columns under non-binding conditions.[17] Indeed, simulations have shown that this mechanism can produce the observed reduced clearance on virus filters,[18] and virus spiking studies in the presence of either polyethylene glycol or sucrose have confirmed that reducing virus diffusion by increasing solution viscosity can mitigate the impact of a process pause, as predicted by the hypothesized mechanism.[16,18] Additionally, confocal microscopy observations have confirmed that depressurization allows virus particles to pass farther into the filter and that only viruses that were trapped on the filter prior to the depressurization event are able to progress farther, while virus particles filtered following the pause are trapped in the same layer as those which experienced no pause.[19–21] These results are consistent with a mechanism in which previously trapped particles are released when flow through the filter is stopped and then have the ability to travel farther into the membrane when flow resumes. This mechanism is likely similar for constant low pressure processes, except that the virus particles may never be as effectively trapped as they are at recommended operating pressures and are thus more likely to sample more pores and pass through larger ones. The impact of product molecule characteristics and solution conditions, such as pH and ionic strength, on this mechanism are not understood at this time.

As low pressure can have a significant effect on parvovirus clearance, it is important to understand other parameters that can impact this effect in order to determine operating ranges in which effective clearance can be obtained. A previous study evaluated the effects of solution conditions on parvovirus removal from a human IgG product using Planova 20N filters.[14] Specifically, Hongo-Hirasaki demonstrated that filtration at 0.78 bar achieved complete clearance of porcine parvovirus (PPV) over a wide range of solution conditions. At the much lower pressure of 0.20 bar, effective PPV clearance was observed at high pH ($pH > 4.5$) or low salt (<100 mM NaCl), but the combination of low pH and high salt concentration resulted in a reduction in PPV logarithmic reduction value (LRV) to less than 4. These results illustrate the complex mechanism of virus removal during filtration and demonstrate the need to gain additional understanding of the mechanisms that affect virus clearance. In particular, it is important to define the ranges of pressure and solution conditions where effective parvovirus clearance can be expected. Additionally, determining whether trends observed previously for human IgG on Planova 20N filters can be applied to other products and/or filter types will provide a better understanding of this mechanism.

In this study, we perform multivariate analyses to test parvovirus clearance over wide ranges of pressure, pH, and ionic strength, using three different mAb products to define the design spaces for two virus filter types. We also investigate low pressure regions in order to understand the effects for different products and filter types and to identify trends in solution conditions. Finally, we discuss how low pressure and depressurization events can be managed during both manufacturing operations and during small-scale validation studies to ensure a highly robust virus filtration process.

Methods and Materials

Material preparation

Three different monoclonal antibody products were used for this study. The products were all IgG1 isotypes with various isoelectric points (Table 1). For each product, a process intermediate after Protein A capture chromatography was obtained from the manufacturing process at Janssen R&D and further purified at reduced scale through two additional polishing chromatography steps. Each product was then concentrated to 30 g/L using a 0.1 m^2 Pellicon 2 Mini Ultrafiltration Biomax-30 Module (Millipore Sigma), aliquoted and stored at $\leq -70°C$ until use.

Table 1. IgG1 Isotope Products Used in Design Space Filtrations

Product	Isoelectric Point (Range)	Isoelectric Point (Main Peak)
mAb A	7.05 – 7.50	7.2
mAb B	8.00 – 9.00	8.6
mAb C	8.70 – 9.18	9.0

Buffer solutions were prepared to 50 mM acetate, pH 4.0 and 5.5 and 50 mM Tris HCl, pH 8.0; NaCl was added to adjust conductivity. Combinations of high and low pH and high and low conductivity and a fifth condition at intermediate pH and conductivity as a center point condition were tested (Table 2). The solution parameters were chosen to represent a wide range of conditions, and pH was especially chosen to bracket the isoelectric point (pI) of Minute virus of mice (MVM; pI = 6.2) and that of at least one of the products (mAb A). Prior to each product filtration run, the product was thawed at 37°C and stored at 4°C until use. The product was then adjusted to 5 g/L using the appropriate buffer. If needed, the solution was then further adjusted to the desired pH and conductivity for that run using solutions of acetic acid or Tris base, and NaCl.

Preliminary studies were performed with all solution conditions for each product to ensure filterability and to verify the lack of cytotoxicity or viral interference in the MVM infectivity assay. Additionally, virus spiked into each product solution was demonstrated to not lose significant infectivity during a 6-h hold or during filtration through a Planova 35N filter, indicating that no gross virus aggregates were present at each condition (data not shown). Interestingly, MVM spiked into buffer alone with no protein did show significant removal on a Planova 35N filter for the pH 4.0 but not pH 5.5 or 8.0 solutions, suggesting that the presence of protein helps to mitigate virus aggregation at low pH. It is not known if this phenomenon impacts virus particles trapped in the filter during a buffer flush, but it is not believed to have impacted the results of this study since virus was observed in the buffer flush for some low pH runs.

Virus preparation

Minute virus of mice (MVM_p; ATCC, VR-1346) was propagated in A9 cells (ATCC, CCL-1.4) in Dulbecco's modified Eagle's medium (D-MEM) (11995–065 Gibco, Thermo Fisher Scientific) containing 10% fetal bovine serum (FBS) at 37°C. To obtain serum-free MVM, host cells were inoculated with virus, and the medium was exchanged with serum-free medium prior to the appearance of the cytopathic effect (CPE). The cell culture was centrifuged at 3,000 rpm for 20 min at 4°C to pellet the cells, and the supernatant was filtered through a 0.45 μm filter (50 mm diameter SFCA filter, Nalgene Nunc International). The serum-free MVM stock solution was centrifuged at 25,000 rpm for 4 h to pellet the MVM. The pellet was resuspended in PBS buffer (Nissui Pharmaceuticals) by sonication and vortexed. The concentration of the purified MVM preparations was approximately 8.50 $\log_{10}$ $TCID_{50}$/mL.

Quantification of virus

We determined the virus concentration in the feed and filtrate samples by the 50% tissue culture infectious dose ($TCID_{50}$) method using 324K cells (Peter Tattersall, Yale University). We prepared 10-fold serial dilutions of samples and then inoculated them into round-bottom 96-well plates containing cells. After incubation at 37°C for 10 days, the infected wells were detected by hemagglutination (HA).[22] $TCID_{50}$ was calculated using the Reed and Muench method.[23] The detection limit of the method was 0.80 $\log_{10}$ $TCID_{50}$/mL.

Table 2. Design of Experiment Conditions Tested

Parameter	Low	Center	High
Conductivity (mS/cm)	3.0	7.0	20.0
pH	4.0	5.5	8.0
Pressure* (bar)			
Planova 20N	0.50	0.78	1.10
Planova BioEX	0.69	2.76	3.43

*Additional pressure settings were tested to determine effects outside of the design space.

The pool MVM LRV of the grab samples, and unless otherwise indicated, the buffer flush was calculated as shown in Eq. (1),

$$\text{MVM LRV} = \log_{10}\left[\frac{c_l \times v_l}{\sum (c_f \times v_f)}\right] \quad (1)$$

where c_l and v_l are the MVM titer and volume of the load solution, respectively, and c_f and v_f are the MVM titer and volume of each filtrate pool, respectively.

Filtration procedure

All virus filtration runs were performed on 0.001 m^2 Planova 20N and BioEX filters (Asahi Kasei Medical Co., Ltd.). Each mAb product was prepared with the target buffer pH and conductivity, filtered through a 0.1 μm vacuum filter (Nalgene RapidFlow, Thermo Fisher Scientific) spiked to 1.0% (v/v) with MVM stock, and then filtered again through another 0.1 μm filter (Millipak20, Millipore Sigma). The load solution was then filtered on Planova 20N and BioEX filters using pressurized air at constant pressure until 50 or 200 L/m^2 throughput was achieved for each respective experiment. For each run, once the target load throughput was reached, the pressure was released, the feed was switched to buffer solution matched to the load solution, and after a 15 min pause, the run was resumed for a 5 L/m^2 buffer flush at the same pressure as was used for the load filtration. Load, filtrate, flush, and grab samples were each diluted 1:2 in cell culture media based on previous cytotoxicity evaluations and stored at −80°C until assayed.

Initial filtration runs were performed to 50 L/m^2 at worst-case conditions to identify the putative Low Pressure Limit (LPL) for both filter types and under varying conditions to define the design space. These runs were conducted at operating pressures of 0.19, 0.30, 0.40, 0.50, 0.60, 0.78, and 1.10 bar for Planova 20N filters and at 0.69, 2.76, and 3.43 bar for Planova BioEX filters.

LPL confirmation runs were performed at worst-case solution conditions to 200 L/m^2 at operating pressures of 0.40 and 0.50 bar for Planova 20N filters and at 0.69 bar for Planova BioEX filters. These runs were also performed at center point pressures and conditions for each filter type. For each run, "grab samples" of 1 mL each were collected at 4, 25, 50, 100, 150, and 200 L/m^2. The grab samples provide instantaneous measures of virus titers during the filtration run and are used to evaluate the profile of virus passage through the filter, providing a more detailed comparison of

the virus retentive capability. The acceptance criteria used to define the LPL were (1) MVM LRV $\geq$ 4.0 for runs with worst-case solution conditions and (2) MVM titers in all grab samples at corresponding throughputs for both worst-case and center point filtrations are within 1 log $TCID_{50}$/mL.

Results

Design space experimental strategy

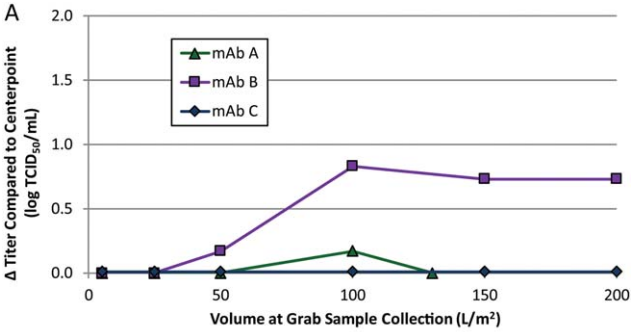
We evaluated the impact of operating pressure, solution pH, and solution ionic strength on parvovirus removal for multiple IgG1 mAb products on Planova 20N and BioEX filters. mAb products covering a range of pI (Table 1) were selected in order to evaluate potential electrostatic effects of protein products on parvovirus clearance. The mAb concentrations were held constant at 5 g/L for all products and all runs. Significantly higher mAb concentrations can generally be processed on these filter types under optimized filtration conditions, but testing broad ranges of solution conditions with acceptable flux levels was found to be challenging at higher concentrations, and reduced mAb concentrations were therefore used. The solution pH and conductivity ranges (Table 2) were set broadly with the intent to bracket most mAb production process ranges. MVM spiking was performed to a 1% (v/v) ratio, resulting in load titers of approximately 6 log $TCID_{50}$/mL. This viral challenge level is sufficient to demonstrate effective virus removal (*i.e.*, virus LRV $\geq$ 4) and is consistent with many viral clearance validation studies while not being so high as to result in spiking artifacts that may occur at high spiking challenge levels.

For each filter type and product, initial experiments were first conducted to determine the putative LPL, or the pressure above which effective parvovirus clearance is obtained for each product in each solution. These runs were performed using low pH and high conductivity solution conditions, which were confirmed to be worst-case in this study, and using a volumetric throughput of 50 L/m^2. Having identified the putative LPL from 50 L/m^2 runs, the LPL was then confirmed with runs performed to 200 L/m^2. Full factorial design spaces were then defined for all solution condition ranges and pressure ranges between the LPL and a high pressure set at or above the manufacturer recommended maximum forward differential pressure for each filter type (Table 2). Flux decay was minimal for most filtration runs and was less than 50% on all runs, except for mAb A at low pH on the Planova BioEX filter (Supporting Information Figures 1–3). For all runs, a process pause with complete depressurization for 15 min was included prior to a 5 L/m^2 buffer flush.

Planova BioEX filter design space

In this study, we tested Planova BioEX filters over the pressure range of 0.69 to 3.43 bar (10.0 – 49.7 psi), extending the lowest pressure tested far below the recommended range and setting the highest pressure tested at the maximum recommended pressure. Initial experiments suggested 0.69 bar, the lowest pressure tested on this filter type, as the LPL, and comparison of runs to 200 L/m^2 at low pressure and worst-case conditions to center point conditions confirmed 0.69 bar as the LPL. Pool MVM LRV for the 200 L/m^2 filtrations was 4.8, 4.2, and $\geq$5.1 for mAb A, B, and C, respectively, and all grab sample titers were within 1 log $TCID_{50}$/mL of those from center point runs (Figure 1). These results

A



B

Product	Pre-Pause Pool LRV	Total Pool LRV
mAb A	4.8	4.8
mAb B	4.6	4.2
mAb C	≥5.1	≥5.1

Figure 1. Low Pressure Limit (LPL) Confirmation for Planova BioEX Filters.

(A) Differences in MVM titer results are shown between runs performed at 0.69 bar and worst-case solution conditions (pH 4.0, 20.0 mS/cm) and center point runs at 2.76 bar and moderate solution conditions (pH 5.5, 7.0 mS/cm). Processes for mAb B and C were performed to 200 L/m^2, while the process for mAb A was stopped at 130 L/m^2 due to filter plugging. (B) MVM LRV results for these runs are shown for the pre-pause pool, which does not include the buffer flush, and the total pool, which does include a 15-min pause followed by a 5 L/m^2 buffer flush.

indicate that 0.69 bar (10.0 psi) is the LPL for Planova BioEX filters and that this filter type is expected to achieve effective MVM clearance at pressures between this level and the recommended maximum pressure.

The Planova BioEX design space was then evaluated by testing all combinations of solution conditions for 0.69 to 3.43 bar for all three products for product filtration to 50 L/m^2 with a 15 min process pause and a 5 L/m^2 buffer flush. MVM LRV for all conditions tested was observed to be at or near complete clearance (Figure 2). Overall, these data indicate that Planova BioEX filters are capable of providing effective viral clearance results for multiple mAb products over the recommended pressure range of 1.96 to 3.43 bar (28.4–49.7 psi) and at lower pressures, independent of solution conditions. These results illustrate the robustness of this filter type with respect to parvovirus removal at low pressure conditions.

Planova 20N filter design space

We first tested Planova 20N filters over a pressure range of 0.19 to 1.10 bar (2.8–16.0 psi), evaluating operating pressures from the lowest achievable pressure to a pressure higher than the recommended limit. Initial studies to 50 L/m^2 under the worst-case conditions of pH 4.0 and 20.0 mS/cm showed that for all three products MVM LRV above 4 was maintained from 0.40 to 1.10 bar but fell below that level at lower pressures (Figure 3). Of the operating pressures tested, 0.40 and 0.50 bar were further evaluated to determine the LPL. The LPL confirmation runs identified 0.50 bar as the LPL for the Planova 20N filter based on pool MVM LRV for the 200 L/m^2 filtrations of 4.0, 4.4, and 4.5 for mAb A, B, and C, respectively, and all grab sample titers were within 1 log $TCID_{50}$/mL of those from center point

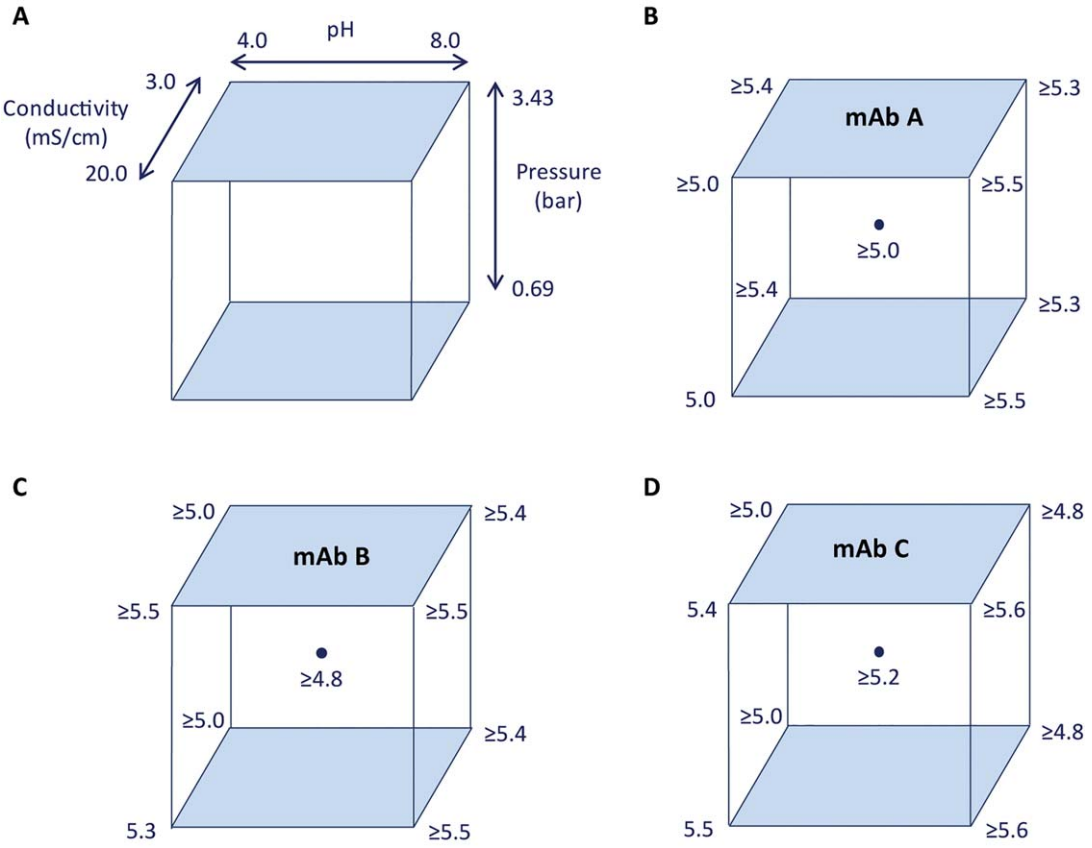


Figure 2. Planova BioEX Viral Clearance Design Spaces.

(A) Designation of the ranges of pressure, pH, and conductivity evaluated in the design space. Each filtration run consisted of a 50 L/m^2 product load, followed by a 15 min pause and a 5 L/m^2 buffer flush. Using the same format as (A), the MVM LRV results obtained at the specific design space conditions are shown for (B) mAb A, (C) mAb B, and (D) mAb C. The MVM LRV result in the center of each cube is for the center point condition (performed at 2.76 bar, pH 5.5, and 7.0 mS/cm).

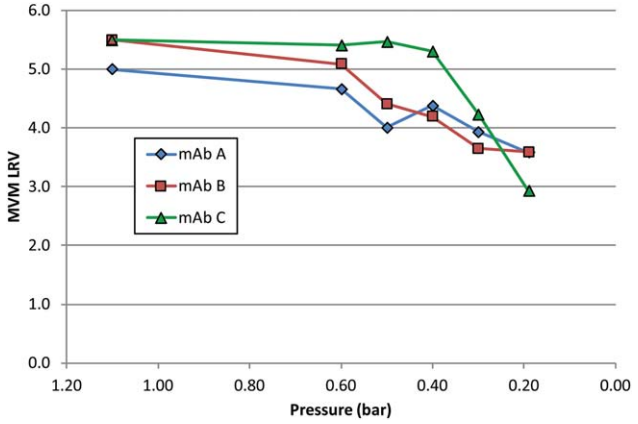
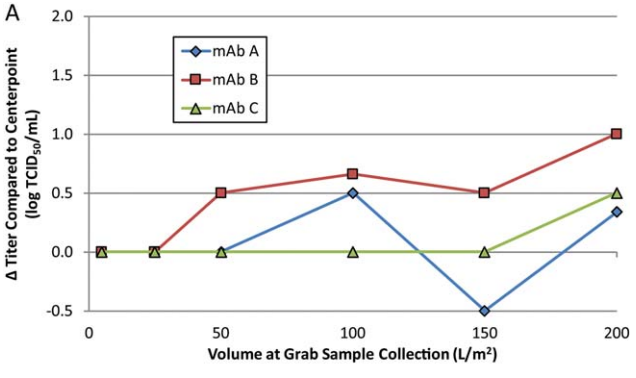


Figure 3. Effects of Reduced Pressure on MVM Clearance by Planova 20N.

MVM LRV results are shown for 50 L/m^2 Planova 20N processes ranging from 1.10 bar (16.0 psi) to 0.19 bar (2.8 psi) with three different monoclonal antibodies. Worst-case solution conditions (pH 4.0, 20.0 mS/cm) were used for all runs.



B

Product	Pre-Pause Pool LRV	Total Pool LRV
mAb A	4.5	4.0
mAb B	4.5	4.4
mAb C	4.7	4.5

Figure 4. Low Pressure Limit (LPL) Confirmation for Planova 20N.

(A) Differences in MVM titer results are shown between runs performed at 0.50 bar and worst-case solution conditions (pH 4.0, 20.0 mS/cm) and center point runs at 0.78 bar and moderate solution conditions (pH 5.5, 7.0 mS/cm). Processes were performed to 200 L/m^2. (B) MVM LRV for these runs are shown for the pre-pause pool, which does not include the buffer flush, and for the total pool, which does include a 15-min pause followed by a 5 L/m^2 buffer flush.

runs (Figure 4). Comparable runs performed at 0.40 bar (5.8 psi) did not consistently meet all criteria (data not shown). These results indicate that 0.50 bar (7.3 psi) is the LPL for Planova 20N filters and that this filter type is expected to achieve effective MVM clearance at pressures between this level and the recommended maximum pressure.

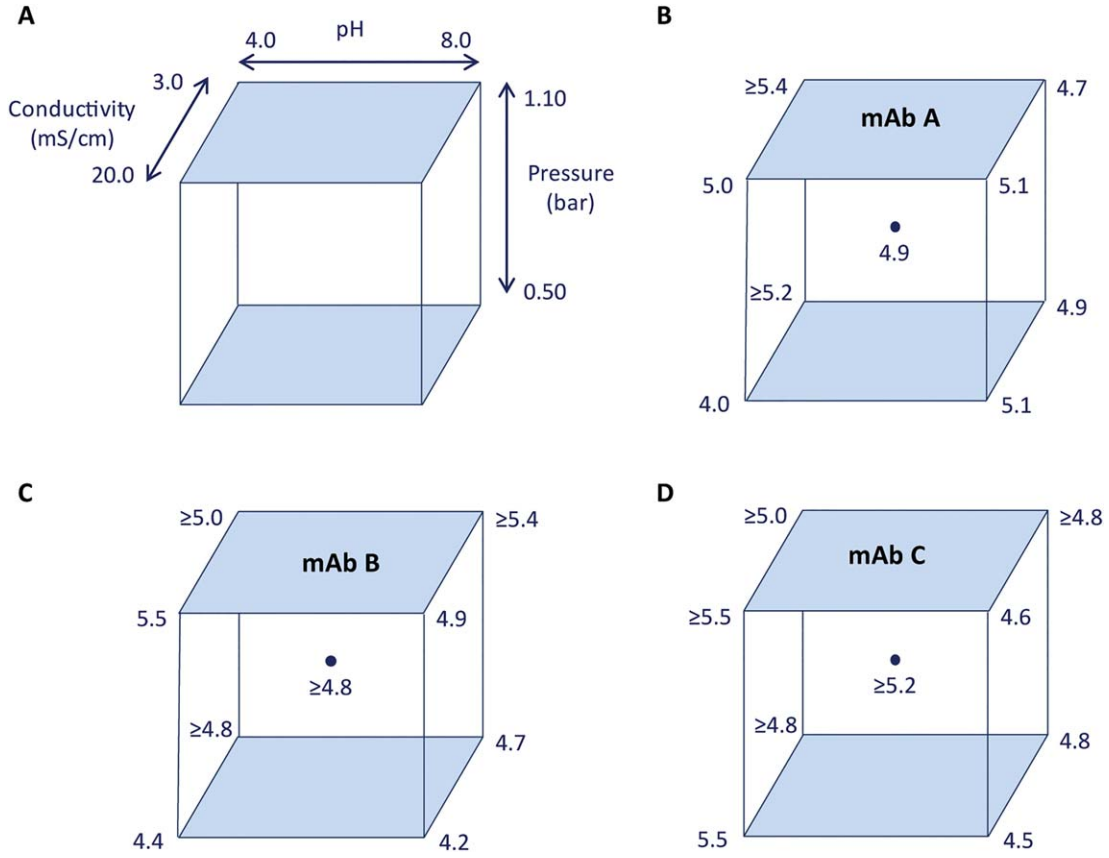


Figure 5. Planova 20N Viral Clearance Design Spaces.

(A) Designation of the ranges of pressure, pH, and conductivity evaluated in the design space. Each filtration run consisted of a 50 L/m^2 product load, followed by a 15 min pause and a 5 L/m^2 buffer flush. Using the same format as (A), the MVM LRV results obtained at the specific design space conditions are shown for (B) mAb A, (C) mAb B, and (D) mAb C. The MVM LRV result in the center of each cube is for the center point condition (performed at 0.78 bar, pH 5.5, and 7.0 mS/cm).

The Planova 20N design space was then evaluated for parvovirus clearance for all combinations of solution conditions for 0.50–1.10 bar for all three products with product filtration to 50 L/m^2 with a 15 min process pause and a 5 L/m^2 buffer flush. MVM LRV from all conditions tested was observed to be effective with LRV of 4.0 or greater (Figure 5). Overall, these data indicate that Planova 20N filters are capable of providing effective viral clearance results for multiple mAb products over the pressure range from 0.50 to 1.10 bar (7.3–16.0 psi). Solution conditions can impact clearance in this region, but even under the worst-case conditions of low pH and high ionic strength, effective parvovirus clearance can be expected. These results illustrate the robustness of this filter type within typical operating pressures, but they also underscore the importance of understanding and controlling operating pressure.

Table 3. MVM Clearance on Planova 20N Filters Below the LPL*

Conductivity (mS/cm)	pH	Product	MVM LRV
3.0	4.0	mAb A	5.0
		mAb B	≥5.0
		mAb C	≥5.0
	8.0	mAb A	3.5
		mAb B	4.4
		mAb C	4.2
20.0	4.0	mAb A	3.5
		mAb B	3.6
		mAb C	2.9
	8.0	mAb A	4.3
		mAb B	3.9
		mAb C	3.0

*Studies were performed at 0.19 bar (2.8 psi) which is below the recommended low pressure limit (LPL) of 0.50 bar (7.3 psi).

Low pressure and process pause effects

As low pressure filtration and process pause have been shown to adversely impact virus removal, we additionally evaluated the impact of solution conditions on Planova 20N filters at 0.19 bar (2.8 psi), which is below the LPL. Even at this low pressure, effective parvovirus clearance with MVM LRV of 4.0 or greater was achieved for half of the filtration runs (Table 3), but some combinations of solution conditions significantly impacted the clearance. Specifically, MVM LRV was less than 4.0 for the high conductivity and low pH solutions for all products tested, as well as at high pH for some products. Overall, these data illustrate that acceptable parvovirus clearance can be achieved on Planova 20N at very low pressure, but these results depend on the product and the solution conditions.

All Planova BioEX and 20N runs in this study included a 15-min pause prior to the buffer flush and the MVM LRV data used to evaluate the design space have included the buffer flush. This indicates that effective parvovirus reduction can be readily achieved in the presence of flow interruption. To examine the effect of a process pause on parvovirus clearance, we compared MVM LRV from the pools prior to the pause and for the pools including the buffer flush

following the 15-min pause (Figures 1B and 4B). Pool MVM LRV was impacted by a process pause for one of the three products with Planova BioEX filters and for all three products with Planova 20N filters. Importantly, the impact on MVM LRV was significantly less than 1 $\log_{10}$ and effective clearance was obtained even with a process pause. These results demonstrate that although a process pauses can have an impact on parvovirus reduction, the effect is manageable and the resulting processes often provide effective viral clearance.

Discussion

Recent studies have identified the potential for low pressure and depressurization events to impact parvovirus removal.[5,15,16,24] Given the importance of pressure on the performance of this critical virus removal unit operation, we sought first and foremost to define the operating regions in which these two virus filters provide robust parvovirus clearance. We have identified operating pressure ranges for Planova BioEX filters from 0.69 to 3.43 bar (10.0–49.7 psi) and for Planova 20N filters from 0.50 to 1.10 bar (7.3–16.0 psi) where effective removal of parvovirus is achieved for multiple monoclonal antibody products independent of solution conditions. These results demonstrate that both filter types exhibit robust operating ranges and that typical operating ranges for these filters are capable of providing effective removal of small viruses. Specifically, the data show that Planova BioEX filters operated within the recommended range of 1.96–3.43 bar (28.4–49.7 psi) and Planova 20N filters operated within a typical operating range of 0.80–0.98 bar (11.6–14.2 psi) are both well within the defined design space regions. Therefore, notwithstanding the complex interactions of conditions and parameters discussed below, this study demonstrates that the operating conditions and control strategies generally used for these unit operations are appropriate for most products and processes to provide the expected viral clearance capabilities. In support of this conclusion, a recent publication showed that Planova 20N filters with average flow rates at about 50% of normal showed no negative impact on clearance of B19 parvovirus and runs with pauses of up to 2 h still provided complete clearance of MVM.[25]

One clear outcome of this study is the complex nature of parvovirus clearance when operated under low pressure conditions, since we observed clear effects due to filter types, products, and solution conditions. For instance, on Planova 20N, all of the products showed some reduction of clearance due to low pressure and process pauses under certain conditions, while the performance of Planova BioEX was generally more robust, showing no significant effect due to low pressure and only a slight reduction of MVM LRV on one product (mAb A) due to the process pause. Solution conditions were also observed to have a significant impact on MVM removal at very low pressures, especially on Planova 20N filters where variations in solution conditions alone resulted in variation in MVM LRV from $\geq$5.0 to 3.0. Importantly, the results illustrate the difficulty of extrapolating data from one product and one set of conditions to other products and conditions when operating below the LPL. For example, evaluation of Planova 20N filtration at low pH and low conductivity conditions achieved complete MVM clearance at all pressures tested. Testing at only that condition could lead to the inaccurate conclusion that parvovirus removal by that filter type is not impacted by low pressure. Given this complexity, evaluation of the effects of low pressure events on a particular filter type using only one product or process solution could lead to significant underestimation of their impacts. It is therefore critical that each product and process be evaluated on a case-by-case basis to ensure acceptable performance over the intended operating pressure range.

Table 4. Electrostatic Charges of Potential Interacting Components

Component	pI	pH 4	pH 8
Products			
mAb A	7.2	+++*	-
mAb B	8.6	+++	+
mAb C	9.0	+++	++
Virus			
MVM[27]	6.2	+++	–
Membrane Material of Planova Filters†			
Regenerated cellulose membrane (P20N)	Low	—	—
Hydrophilized PVDF membrane (PBioEX)	Low	—	—

*Relative charges are based on the difference between the pH and pI.
†The regenerated cellulose filter is known to be negatively charged under the range of pH.[3] The hydrophilized PVDF membrane has not been directly evaluated, but it is believed to be negatively charged as well.

Although the primary focus of this study was the evaluation of the virus filtration knowledge space and definition of the operating design space, the results also provide some information on the mechanism of action of virus removal under low pressure conditions. For instance, the impact of pH and ionic strength suggest that electrostatic interactions may play some role in parvovirus clearance at low pressures. Possible components that may play roles in these interactions include the filter itself, the virus particles, the product, and unknown impurities introduced by either the product or the virus spike. Considering the isoelectric points of the known components and their relative charges at the various pH levels (see Table 4), it is possible to consider potential interactions. At low salt conditions, where electrostatic interactions are stronger, the high MVM LRV results observed at the low pH condition may be due to attractive interactions between the filter membrane and either the product or the virus. However, at high pH, where mAb A has a repulsive interaction with the membrane, the MVM LRV seen for mAb A filtration is reduced considerably compared with the other products. This result suggests that attractive interactions between the product molecule and the membrane surface may potentially cause a slight reduction in pore sizes, increasing virus LRV. Such an interaction between the product and membrane would explain the filter type-specific and product-specific effects observed here and elsewhere. It is important to note, however, that other unknown impurities may play a role in these interactions rather than the known components shown here, and additional data will be needed to fully understand which components are most important for these effects. Further, these interactions only appear to play a role at very low pressures, since no effect on parvovirus removal due to pH and ionic strength have been observed at typical pressures for the Planova 20N filter.[3]

Given the importance of pressure for parvovirus removal, it is critical to ensure that this operating parameter is well-controlled and validated. Such a strategy is not new to biomanufacturing as pressure has already been considered a

critical process parameter for this unit operation and as such, it is required to be well-controlled and well-understood. Our results suggest that for the filter types tested here, broad operating ranges are available. For example, the design space determined here for Planova 20N is 0.50–1.10 bar (7.3–16.0 psi). A control strategy with a constant target operating pressure of 0.90 bar (13.1 psi) and an acceptable range of 0.80–0.98 bar (11.6–14.2 psi) can easily be maintained using either a pressure vessel or a pump system. As with any critical process parameter, any deviation outside of this range would need to be supported with data regardless of filter type. Based on our results for these two filter types, it is likely that effective clearance may be obtained at lower pressures for many processes, although the specific virus LRV obtained would vary based on the filter type, product, and solution conditions. Intentional decreases in pressure, such as process pauses, should be avoided in virus filtration operations whenever possible. This can be achieved for processes with a buffer flush step or multiple feed tanks by using appropriate valves and instantly switching from one tank to the other. Fortunately, the data obtained here demonstrate that processes that do include a pause prior to the buffer flush can nonetheless achieve effective parvovirus clearance results. Longer duration pauses or low pressure events which occur due to unforeseen events such as electrical or other facility issues should be avoided at GMP facilities whenever possible.

Validation studies of virus filtration processes should consider the effects of pressure on virus removal. Consistent with previously reported results, low pressure was observed to have an impact on parvovirus removal and this condition should be considered to provide a worse-case scenario. Although the products used in this study all show a worst-case condition at low pH and high ionic strength, the effects of these parameters show some product dependency, and additional data is needed to determine the worst-case condition for other products and processes. Regarding validation of process pause, it is recommended to include a pause in the validation study for any process which includes a pause. Since longer pause duration has been shown to be a worse-case condition, the pause should have a similar or slightly longer duration than that expected to occur during actual manufacturing activities. For processes which do not contain a pause, it is best to validate the process without any pause. As a rule, deviations such as low pressure events should not be included in validation studies, but rather, a study should be performed separate from the validation study to determine whether such an event impacts parvovirus LRV. If a pause is determined to have minimal impact on the parvovirus LRV, this data can support the process in the event of a deviation, while if the pause is determined to have a significant impact on the parvovirus LRV, other options such as reprocessing the batch or switching to a new filter may be considered. Finally, to ensure accurate parvovirus validation results, it is important to avoid overspiking of the filtration load solution during virus spiking studies. Challenging virus filters with large quantities of parvoviruses has been shown to impact virus LRV and has been associated with increasing the impact of process pauses.[24,26] The virus titer with which a virus filter could be challenged during actual manufacturing operations is limited due to in-process tests which detect contamination events when they occur and by earlier purification processes which remove contaminants prior to the filtration step. As such, the increased impact of process pauses observed at high spiking levels are not representative of actual manufacturing conditions and may lead to an underestimation of the true ability of the unit operation to remove virus particles. Performing virus filtration studies using load titers of approximately 10^6 $TCID_{50}$/mL is usually sufficient to demonstrate effectiveness of the process while minimizing the impact of virus spiking artifacts.

Conclusion

A design space has been identified with operating pressure ranges for Planova BioEX filters from 0.69 to 3.43 bar (10.0–49.7 psi) and for Planova 20N filters from 0.50 to 1.10 bar (7.3–16.0 psi) where effective removal of parvovirus is achieved for multiple monoclonal antibody products independent of solution conditions. Thus, for typical operating pressures, we have identified a large operating region for each filter type and product tested in which robust parvovirus clearance is obtained. However, a failure mode of low transmembrane pressure during virus filtration processes has been shown in this and other studies to potentially reduce parvovirus clearance. At pressures below the LPL, this phenomenon is dependent on many different factors, including filter type, product, and solution conditions such as pH and ionic strength, illustrating the need to independently evaluate the impact of low pressure events for each product and process. Nevertheless, at low pressures, many conditions are still able to produce adequate clearance results. This report demonstrates that typical control strategies for virus filtration operations are generally appropriate to achieve robust viral clearance validation results and to ensure removal of potential viral contaminants in the manufacturing process.

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