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Viral Filtration: A Review of Current and Future Practices in Bioprocessing

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August 9, 2021

Abstract

For drug products manufactured in mammalian cells, safety assurance practices are needed during production to assure that the final medicinal product is safe from the potential risk of viral contamination. Virus filters provide viral retention for a range of viruses through robust, size-based retention mechanism. Therefore, a viral filtration step is commonly utilized in a well-designed recombinant therapeutic protein purification process and is a key component in an overall strategy to minimize the risks of adventitious and endogenous viral particles during the manufacturing of biotechnology products. This review summarizes the history of viral filtration, currently available viral filters and prefilters, and viral filtration integrity test methods and study models. There is also discussion of current understanding and gaps with an eye toward future trends and emerging filtration technologies.

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Abstract

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For drug products manufactured in mammalian cells, safety assurance practices are needed during production to assure that the final medicinal product is safe from the potential risk of viral contamination. Virus filters provide viral retention for a range of viruses through robust, size-based retention mechanism. Therefore, a viral filtration step is commonly utilized in a well-designed recombinant therapeutic protein purification process and is a key component in an overall strategy to minimize the risks of adventitious and endogenous viral particles during the manufacturing of biotechnology products. This review summarizes the history of viral filtration, currently available viral filters and prefilters, and viral filtration integrity test methods and study models. There is also discussion of current understanding and gaps with an eye toward future trends and emerging filtration technologies.

KEYWORDS: Virus Retentive Filters; Downstream Bioprocessing; Viral Clearance Validation; Continuous Manufacturing; Mechanisms of Removal; Monoclonal Antibodies; Recombinant Therapeutic Proteins; Virus Diameter; Parvovirus; Sieving; Barrier Filtration of Media; Membranes; Hollow Fibers.

Introduction

Role of viral clearance in biotechnology

Biotechnology products (e.g. therapeutic proteins) are in large part produced in mammalian cell bioreactor cultures. Mammalian cells are susceptible to viral infection and propagation. Facility contaminations by viruses have occurred over the past 25 years, albeit on a very rare basis (Barone, Wiebe et al. 2020). Certain viruses seem to be more problematic than others, such as the parvovirus murine minute virus (MMV). MMV is very small (20-25 nm) and resistant to chemical inactivation, making it a concern for the biotechnology industry (Lubiniecki 2011, Barone, Wiebe et al. 2020). Other potential viruses of concern include Cache Valley Virus and Vesivirus, which have caused repeat facility contaminations (Lubiniecki 2011, Barone, Wiebe et al. 2020).

Regulatory background

Safety assurance practices are required to assure regulators and ultimately patients and the public that the final medicinal product is safe. In the 1990's, the International Conference on Harmonization (ICH) promulgated "Q5A: Viral Safety Evaluation of Biotechnology Products Derived From Cell Lines of Human or Animal Origin" (ICH 1998) which established a world-wide standard for viral safety. ICH Q5A describes a multi-tier scheme for testing and clearance validation to achieve this goal. The testing program focuses on cell banks, raw materials and bioreactor harvests, informed by a product risk analysis. Beyond testing, ICH Q5A mandates an evaluation of downstream purification, as a fail-safe in case of undetected contaminants upstream. The clearance validation assures that if any virus that evades the testing regime, it can be removed and/or inactivated before it ends up in the final medicinal product.

Context in overall viral safety program

In modern biotechnology manufacturing (or bioprocessing), there are typically three or four unit operations in the overall purification train that are capable of removing or inactivating viruses. These include certain chromatography steps (e.g. protein A or anion exchange), incubations with low pH or detergents, and virus retentive filters. Not all of these steps will be robust or even effective in removal or inactivation for all viruses. For example, low pH incubations are generally ineffective for non-enveloped virus inactivation (Miesegaes, Lute et al. 2010)) although quite robust for enveloped viruses (Brorson, Krejci et al. 2003). Under certain operating conditions, anion exchange columns may not bind and remove neutral isoelectric point viruses from product streams (Riordan, Brorson et al. 2009), but are effective for acidic viruses (Strauss, Lute et al. 2009). It is the combination of the three to four independent and orthogonal unit operations that together assure viral safety for biotechnology products.

It is commonly considered that a robust, effective and reliable process step will be able to remove or inactivate a substantial amount of virus (typically defined as 4 $\log_{10}$ or greater, where the log reduction value or LRV is calculated as the $\log_{10}$ of the total input divided by the total output virus). However, the LRV cannot be used as the single absolute measure of the effectiveness of a step. A robust, effective and reliable step should

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be easy to model, be relatively insensitive to changes in process conditions, and be effective for a range of viruses (WHO 2004). Virus filtration is generally agreed to be one such robust and effective process step and is a key component in an overall strategy to minimize the risks of adventitious and endogenous viral particles during the manufacturing of biotechnology products.

Viral filters are typically understood to function through a robust, size-based retention mechanism. Based on this robust mechanism of action, virus filters are more likely to provide predictable viral retention for a range of viruses than the chromatography steps. This is because filters are less likely to be influenced by differences in the physicochemical properties of different viruses, and the virus-resin interactions modulated by operating conditions. Therefore, a viral filtration step is commonly utilized in a well-designed recombinant therapeutic protein purification process(EMEA 1996) and also has proven to provide robust performance in the plasma processing industry (Roth, Dichtelmüller et al. , Junter and Lebrun 2017).

*Introduction of viral filters with a historical context*Virus filters are polymeric membranes with complex pore structures designed to provide high retention of 20—140 nm viral particles while allowing the smaller product molecules to pass freely. Due to the high selectivity required to distinguish closely sized viruses and molecules and the desire to perform at high flux and high throughput conditions, viral filter manufacture requires more stringent quality control relative to sterilizing grade filters and thus virus filtration can comprise one of the most expensive unit operations (Phillips, Bolton et al. 2007).

There are two types of viral filters typically used in bioprocessing. A recent ANSI accredited PDA standard classified filters into two categories, large virus retentive filters, which are designed to retain viruses larger than about 60 nm, and small virus retentive filters designed to retain viruses larger than 20 nm (Lute, Riordan et al. 2008, PDA 2021). In the last decade or so, new filters have been predominantly designed for parvovirus retention but can function as a retrovirus filter. The filters vary in their formats and materials of construction. The filters comprise either two or three layers of flat sheets, or consist of hollow fibers. The filters are made using one to three layers of the following hydrophilic polymers: polyvinylidene fluoride (PVDF), hydrophilic polyethersulfone (PES), or cuprammonium regenerated cellulose. The membranes may be symmetric or asymmetric in structure (Gefroh, Dehghani et al. 2014). Table 1 summarizes commercial viral filters available currently, although it is likely that additional filters may be developed in the future.

The operation of virus retentive filters is independent of the size-based retention mechanisms. Earlier filters were designed to be run in tangential flow filtration (TFF) mode to reduce fouling (DiLeo, Allegrezza et al. 1992). Though many hollow fiber virus filters are capable of running in TFF mode, filters are now typically run in normal flow filtration (NFF) mode which is also referred to as direct flow filtration (DFF). Operation in NFF mode provides consistent process performance and eliminates the complexity of controlling feed and permeate flow rates required for TFF mode operation. The development of new parvovirus filters capable of NFF mode operation, which are robust, effective and reliable in the clearance of parvovirus, led the industry to widely migrate to the use of parvovirus retention filters and reduce usage of retrovirus-specific filters.

To improve virus filter throughput and economics, prefilters are often used in-line with the virus filter. These prefilters remove trace impurities that could otherwise foul virus filters, thereby increasing throughput and decreasing area requirements (Bolton, Spector et al. 2006, Brown, Bechtel et al. 2010). Initially prefilter options were limited to microfiltration membranes (e.g. 0.2 µm filters) and diatomaceous earth-based depth filters (Bolton, Spector et al. 2006). Diatomaceous earth-based depth filters have been shown to be effective, though they pose an increased leachables risk compared to other filter types. They are also known to release beta-glucans, which can interfere with endotoxin assays (Gefroh, Hewig et al. 2013). These risks can be mitigated with a water flush, buffer flush, or carbonate buffer flush. The carbonate flush has been shown to reduce beta-glucan levels in filter effluents (Holstein, Jang et al. 2021). Absorptive membranes utilizing ion exchange functionality were also developed to mitigate some of these challenges and more recently synthetic depth filters have been developed. Many virus filter manufacturers currently offer specialized prefilters to increase the capacity and throughput of their respective virus filters. Table 1 also includes some common prefilter options.

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Integrity Testing

A critical in-process viral filtration control is the integrity test. A filter integrity test is commonly performed to ensure the integrity of the viral filter membrane and overall unit. Some tests can be performed either before or after use while other tests may be destructive (e.g. gold particle) and are therefore performed post use. Integrity tests are commonly performed both by filter manufacturers and users. Viral filter vendors typically provide data to support that the integrity test is correlated to the virus retention of the device. However, some integrity tests only provide indications of gross defects.

Typical viral filter integrity tests comprise:

- Gold Particle
- Leak
- Diffusive Air Flow
- Pressure Hold
- Liquid-Liquid Porosimeter

Each of these tests are described in detail elsewhere (PDA 2008). The effects of the use of nitrogen and alcohol, as opposed to air and water, on air-liquid integrity tests are not discussed in detail in PDA TR41. The use of 30% isopropyl alcohol will alter the solubility and diffusivity of nitrogen and results in about an 8% reduction in diffusive gas flow, which does not significantly impact the ability to detect defects (Bolton, Cormier et al. 2006).

Filter vendors have described the use of binary gas filter integrity tests. These tests utilize filters with wet pores and a mix of a fast and slow diffusing gas. Any defects can be indicated by high flow (as indicated by high concentration downstream) of the slow diffusing gas. These tests are used successfully by filter manufacturers but less commonly used by filter users (Giglia, Caulmare et al. 2016).

Established robustness of viral filtration and existing knowledge gaps

As previously stated, the broadly accepted mechanism of virus removal by viral filtration is size based sieving which is relatively unaffected by physiochemical characteristics of the viral species. Decades of scale-down scientific studies with viral filters support the robust and effective removal of viruses under varying typical and worst-case process conditions. Parameters typically evaluated in viral filter studies include feed pressure, differential pressure, product loading, flow pausing, or solution conditions (e.g. pH, ionic strength, protein concentration, etc.). Existing published filtration studies are summarized in brief in sections below.

Effective clearance of large viruses:

The use of small retentive viral filters, also referred to as parvovirus filters, are considered industry standard as a dedicated virus removal unit operation. Removal of large viruses like murine retroviruses (80-110 nm) by both large (35-50 nm) and small (˜20 nm) virus retentive filters has been shown to be highly robust and effective (Lute, Brorson et al. 2005, Lute, Riordan et al. 2008, Chen 2014, Lefkowitz, Dempsey et al. 2017). However, for small virus retentive filtration, the pore size distribution can pose a challenge for retaining parvoviruses with diameters of 20-30nm. An FDA meta-analysis performed on the viral clearance data from monoclonal antibody (mAb) regulatory submissions from 1990-2015 demonstrated robust and complete clearance for larger viruses in all 112 large virus retentive filter records and 469 out of 471 records related to small virus retentive filters (Figure 1) (Miesegaes, Lute et al. 2010, Lute 2015). As noted in Figure 1, the two instances of reported non-complete X-MuLV clearance values with small virus retentive filters were determined to be study related. Additionally, only very rare instances of retrovirus breakthrough of small pore virus filters has been reported in the literature (Chen 2014, Stuckey, Strauss et al. 2014, Stanley, Holmes et al. 2021), with no clear explanation of whether the breakthrough was filter related or study related. This highlights that during virus filtration studies, care should be taken to avoid contaminating the filtrate samples which are expected to contain little to no virus. A more recent multi-company data review indicated parvovirus retentive filters provide effective (i.e. >4 $\log_{10}$) or below detection limit removal of a

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range of viruses beyond retroviruses (Stanley, Holmes et al. 2021), reinforcing the consensus that small virus filters provide robust retrovirus clearance (Mattila, Clark et al. 2016).

These studies provide a strong rationale for performing viral filtration validation studies with small viruses, such as parvoviruses. The parvovirus data could then represent worst-case to establish retention claims for larger viruses, such as retroviruses, in regulatory submissions (Mattila, Clark et al. 2016) (Gefroh, Dehghani et al. 2014, Stuckey, Strauss et al. 2014).

Pressure Impacts

Operating conditions that are considered worst-case for viral filtration are those that lead to break-through of the filter by a non-catastrophic failure such that the filter integrity test still shows that the filter is intact. The presumed failure mechanism for non-catastrophic failures is particle diffusion, where it is hypothesized that virus migrates through the membrane structure during flow disruptions by diffusion. Operating conditions that are considered worst-case (e.g. increase particle diffusion) include is low transmembrane pressure, transitions in pressure, low flux, or pausing of pressure/flow (Fan, Namila et al. , Brorson, Miesegaes et al. 2014, LaCasse, Lute et al. 2016, Strauss, Goldstein et al. 2017)."Virus movement within the membrane (e.g. particle diffusion) structure during flow disruptions was visualized using fluorescently labeled phi X-174 bacteriophage and polystyrene nanospheres."(Dishari, Micklin et al. 2015, Fallahianbijan, Giglia et al. 2017). In general, previous generation viral filters suffered a decline in viral retention at lower pressures or with flow pauses (Fan, Namila et al. , LaCasse, Lute et al. 2016), and log reduction values (LRVs) of MMV varied depending on the product and feed solution conditions (Strauss, Goldstein et al. 2017). In newer generation viral filters, clearance appeared to be robust despite variations in pressure and extended flow pauses (Mattila, Clark et al. 2016, Strauss, Goldstein et al. 2017). High pressure limits are typically set based on the structural integrity limits provided by the filter manufacturer or by system capabilities. High differential pressure operation within the filter limit is not known to lead to filter breakthrough by a non-catastrophic mechanism and, thus, high differential pressures across a membrane alone are unlikely to pose a risk to viral retention.

Load conditions

Another parameter known to potentially affect viral filter performance are the conditions at which material is loaded onto the filter. A large number of studies have examined filter performance under wide ranges of loading conditions (such as product type, product concentration, pH, conductivity, and temperature) that are typical for the purification of therapeutic proteins (Marques, Roush et al. 2009, Hongo-Hirasaki, Komuro et al. 2010, Gefroh, Dehghani et al. 2014, Stuckey, Strauss et al. 2014, Strauss, Goldstein et al. 2017). Large virus (e.g., retrovirus, pseudorabies virus, reovirus 3) removal by small virus filters is robust regardless of the load conditions tested. This was consistent with the size exclusion mechanism and was corroborated by clearance results from more than 2000 validation studies spanning over two decades (Stanley, Holmes et al. 2021), as well as the experience from the plasma industry (Roth, Dichtelmüller et al. 2020). When small virus filters were used for parvovirus clearance, virus breakthrough has been reported. Significantly virus breakthrough and lower LRVs can occur as a result of a combination of low operating pressure and certain solution conditions (Strauss, Goldstein et al. 2017). However, at recommended operating pressure, no apparent correlation can be established between virus breakthrough and an individual feedstream condition (Stuckey, Strauss et al. 2014). This points to the importance of operating within the defined robust ranges to ensure effective parvovirus removal. In many cases, feedstream conditions can affect flux or cause filter fouling by modulating interactions of product, virus, and filter membranes (Marques, Roush et al. 2009, Hongo-Hirasaki, Komuro et al. 2010). Therefore, loading conditions can be optimized to improve filter flux and volumetric throughput.

Database studies supporting filter performance

In addition to the above discussed studies addressing specific filter parameters, database studies have been performed by industry groups, contract testing labs and regulatory agencies with the goal of gaining a cross-industry view of the robustness of virus filters in real-word manufacturing processes. These individual studies

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established robust viral clearance (at least 4 logs LRV) despite changes in pressure (flow pause), feedstream solution conditions, virus stock quality, or filter type. It was also demonstrated that newer generation small virus retentive filters may provide a slightly higher median clearance value (5.0 vs 5.67 LRV, combining all generational data) and less variability compared to first generation small virus retentive filters (Lute 2015)(Figure 2). In addition to these studies, several companies have reported viral filter performance in various combinations of process parameters from internal commercial processes and viral clearance studies. These datasets provided strong evidence of robust removal of both parvovirus and retrovirus species with various commercial viral filters in varying combinations of processes and product types (Gefroh, Dehghani et al. 2014, Stuckey, Strauss et al. 2014, Stanley, Holmes et al. 2021). Tables 2-4 summarize key findings from these industry database studies.

Considerations for Performing Viral Filter Clearance Studies" To understand the capability of a purification process to remove viral contaminants, viral filter clearance validation should be conducted in a laboratory equipped for virologic work on a qualified scaled-down model of the production process in accordance with the principles of good laboratory practices. Considerations for viral filtration scale-down models include filter load/feed material, virus spiking strategies, prefilter use, and the potential impacts of process parameters. Scale-down models should represent the commercial production procedure as closely as possible (EMEA 1996, ICH 1998). This is achieved by performing scale-down studies using feed materials and process parameters that represent the manufacturing or worst-case conditions. The small scale model is typically qualified for use by demonstrating comparable process performance with at scale operation, including yield, pressure (during constant flow operation), or flow (during constant pressure operation). Therefore, the scale down qualification experiments are typically limited to brief confirmatory runs.

Prefilters

Virus filters are prone to fouling thus often require the use of a prefilter to achieve the desired virus filter throughput. These prefilters also have limited options for scale-down devices (See Table 1 for suppliers). This presents a challenge for virus clearance studies, as some prefilters may contribute significantly to the removal of virus when used in-line with the viral filter. To avoid this issue, prefiltration may be performed independently and the resulting prefiltered feedstock can be spiked with virus and applied to the virus filter. In many cases operating the prefilter in-line with the virus filter is necessary to achieve optimal throughput and avoid the reintroduction of filter foulants. Therefore separating the filters may result in lower manufacturing throughput and higher costs. Alternative methods for spiking virus have been developed to introduce the virus spike after an inline prefilter using a highly accurate pump system (Lutz, Chang et al. 2011) to achieve the full benefit of the prefilter without impacting viral titer.

Feed Materials (Load Material and Virus Stock)

Feed materials should be representative of the in-process material from a manufacturing run in terms of product quality attributes and solution conditions. In cases where feed material property is affected by freezing and thawing, it is preferable to obtain feed material soon after a manufacturing scale run and use without freezing, or freshly prepare feed from the preceding unit operation at small scale, or filter thawed material to remove any potentially clogging aggregates or particles that result from freezing and thawing. This can result in not achieving the desired volumetric throughput in the validation study

Impurities present in a virus spike can significantly impact product mass or volume throughput in a virus clearance study (Slocum, Burnham et al. 2013, De Vilmorin, Slocum et al. 2015). Therefore, high purity and high titer virus stocks are desirable to evaluate virus removal while maintaining product filtration capacity. High viral titer can help reduce the required viral spike volume while allowing measurement of full viral removal capacity.

Virus spiking strategies

As discussed in ICH Q5A, to characterize the capacity of the unit operation to remove and/or inactivate viruses, the model virus should provide useful information about the performance of the unit operation.

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Of the viruses suggested in ICH Q5A, the parvovirus MMV represents a worst-case virus spike for small virus retentive filter validation studies. Thus, it is often used as a relevant model virus for scale-down viral filtration studies, along with other parvoviruses, such as PPV which can also be grown to high titers. It has been demonstrated that canine parvovirus (CPV) can pass through small virus retentive filters and can be construed as a worst case model (Nowak, Popp et al. 2019).However, the relevance of CPV to the manufacturing process should be taken into consideration, because, unlike MMV, CPV has not been a real-world contaminant in biomanufacturing.

When designing viral spiking strategies for viral filtration studies, several things should be considered including viral titer or purity of viral spike material. Studies have shown that some virus filters have a finite capacity to retain small viruses (Lute, Bailey et al. 2007). In these cases, an excessive virus spike e.g. 10^{14} viral particles per/m^2 would not be representative of typical manufacturing conditions, may lead to lower LRVs (Lute, Riordan et al. 2008), and should be avoided (Chen and Chen 2015). Newer generation filters may be more robust to higher spikes (Mattila, Clark et al. 2016, Strauss, Goldstein et al. 2017). High purity and low aggregate virus stocks are desirable to reduce the risk of filter fouling caused by virus spike (Khan, Parrella et al. 2009, Chen 2014).

Infectivity assays such as median tissue culture infective dose (TCID50) or plaque assays are the preferred assays to quantify virus clearance LRVs. Large volume testing is a common method to increase assay sensitivity and claimable LRVs. qPCR assays are rarely used currently due to the detection of residual free viral nucleic acids which may or may not be active viral particles. Detection of free nucleic acid that can pass through the virus filter resulting in artificially lower LRVs unless the buffer pH values pose the potential for inactivation.

For further information about viral spikes, the PDA Technical Report #47 may be consulted(PDA 2010).

Key Challenges/Gaps/Mitigations

Despite the overall robustness and effectiveness of virus retentive filters for the retention of large viruses (e.g. retroviruses) and small viruses (e.g. parvoviruses), the retention of small viruses can be highly variable and not always effective. While the newer generation small virus retentive filters appear to have less variability (Lute 2015) (Figure 2) there are still concerns with the occasional less effective clearance values. To address these concerns, the viral validation study is typically performed under challenging, or worst-case, conditions. The science seems to indicate that the target operating pressure, low pressure, a flow pause, and a buffer chase should be included during each scale down viral validation run. A pause typically occurs between the load and flush phase and is different from running at low pressure because liquid only flows through the filter during the low-pressure phase. There have been attempts to perfectly match the pressure ramp up and ramp down dynamics during viral filtration (Roush and Ma 2016), however it would be impossible to do a comprehensive experiment covering all ranges and combinations of pressure conditions and pauses that may occur during routine manufacturing. High pressure is typically not considered worst case as the limits are determined by filter structural robustness.

A variety of ways exist to set end point limit for a viral filter step. For some early model filters, flux decay due to pore plugging (Bolton, Cabatingan et al. 2005) and total virus loaded per filter area was reported to decrease virus retention (Lute, Bailey et al. 2007). Similarly, it has been reported that mass of protein per filter area (Soluk, Price et al. 2008, Chen and Chen 2015, Roush and Ma 2016, Kreil and Roush 2018) or alternatively, volume of protein solution per filter (Chen and Chen 2015, Roush and Ma 2016, Kreil and Roush 2018) may be a worst case condition. For instance, operating a low load concentration will provide the highest volumetric (liter/m^2) throughput and viral loading but the lowest mass (gram/m^2) throughput. Conversely, operating using a high load concentration will provide the lowest liter/m^2 throughput and viral loading but the highest gram/m^2 throughput, given the tendency for increased filter fouling at higher load concentrations, particularly when using scaled down filter membranes. While many ways fouling may occur and how end point limits are set, in-process controls are best described in terms of liters per square meter of filter area.

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Recent and emerging applications of existing technology

Barrier filtration

One potential source of viral contamination is from raw materials used in cell culture such as media or nutrient and glucose feeds. Biologically derived raw materials are at highest risk for containing viral contaminants. However, some feeds at high or low pH values that do not allow for viral propagation and, as such they may not require risk mitigation for viruses. To avoid potential viral contamination of cell culture components, viral filters, high-temperature short time (HTST) treatment, acid treatment, detergent treatment, ultraviolet inactivation, and gamma irradiation can be implemented. In theory these methods could be implemented by media vendors but the practicalities of shipping and receiving large volumes of liquid media makes implementation on site by the manufacturer more realistic. Viral filtration can be suitable for various manufacturing processing, including facilities that implement single-use, disposable manufacturing, including for newer products like cell and gene therapy. The advantage of viral filtration is that no new components are added to the media and there is minimal risk of damaging or precipitating growth factors or nutrients. Filters have recently been launched specifically for use in filtering cell culture media. Other filters typically used in downstream processing have been marketed by the vendors for use in filtering cell culture media as summarized in Table 5 below.

There are several challenges with implementing viral filtration of these raw materials. It is challenging to get complete assurance of viral removal as this would require viral filtration of all media used for cell line development, banking, and all cell culture scale up steps before the production reactor. Use of a viral filter may add capital cost, floorspace requirements and operational complexity. Just as with traditional viral filtration methods, the pre-use flushing and integrity testing of the filters must be considered. Clogging by some media components (e.g. shear protectants, anti-foam, etc.) should be avoided by heat treating them separately instead.(Liu, Carroll et al. 2000, Wolfgang, Mitterer et al. 2013, Carbrello, Perreault et al. 2017)

Another potential challenge in implementing viral filters as a barrier method is the potential interactions between media components (i.e. shear protectants like Pluronic F-68, block copolymers, antifoam, non-ionic surfactants, IgF, insulin, metals, glucose, amino acids, vitamins, lipids, fatty acids, trace elements, and antibiotics) and the viral filters. There must be assurance that any filter extractables/leachables do not affect the cell culture process or product quality or that critical media components are not retained by the filter. The impact of media viral filtration may require evaluation of cell culture bioreactors versus controls in scale-down studies.

Available literature does not describe methods of demonstrating viral clearance capabilities of these filters (Liu, Carroll et al. 2000, Kleindienst and Manzke 2016), therefore, demonstration of the filters viral retention performance is currently mostly studied by the vendor to support their products. Some media filters have been shown to provide virus and bacteria retention (Carbrello, Perreault et al. 2017, MerckMillipore 2017).

Study Models

As discussed previously in this review, validation and the development of viral filtration processes require scaled-down models studies. However, these studies can be laborious, costly, and time-consuming. Inadequate study design can translate into improperly sized filters. Under extreme circumstances, reprocessing may be triggered due to filtration failure or fouling at process scale. The advent of newer strategies to improve model design would reduce some of these hinderances to representative modeling at small scale. One such strategy is the use of high throughput process development (HTPD) proposed for resin screenings for viral filtration (Brown, Johnson et al. 2017, Brown, Burnham et al. 2018, Brown, Burnham et al. 2018). Filter plate technologies in combination with automated liquid and plate handling systems allow for high throughput analytics in filter process development. Screening of process conditions (e.g. load conditions) that may impact critical process parameters (e.g. flux decay) can be performed rapidly. Initial work by Tang et al (Tang, Ramos et al. 2020) provided a framework for HTPD filter plate screening with recombinant proteins. These tools can rapidly screen out failure mode conditions early in process development.

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Other new technologies that can aid in refining filter process development include particle tracking technologies. These particle tracking technologies use gold or fluorescent particles, as mentioned previously, but can only track aggregate particle movement. These technologies can be used to model flow and the impacts of flow interruptions. One drawback is that these studies tend to have high material costs. The advent of single-particle tracking technologies such as the nanoparticles presented by Wu et al 2020 have been used in PVDF membrane filters. n these technologies, nanoparticles are continuously imaged, such as with an optical microscope, and tracking algorithms implemented to localize particle positions and generate trajectories (Wu and Schwartz 2020) which theoretically could be adapted to viral filtration flow studies.

Emerging manufacturing modalities

Newer manufacturing technologies (e.g. continuous manufacturing, gene therapy vectors) require special consideration when designing processes and scale-down viral clearance studies for viral filtration.

For very small gene therapy vectors, like AAV, it is conceivable to use large virus retentive filters in downstream manufacturing as a risk mitigation barrier for larger contaminating viruses. AAV is predicted to pass through these filters, while larger viruses like RVLPs are entrapped. While there are some studies assessing the use of viral filters in this field (Adams, Bak et al. 2020, Barone, Wiebe et al. 2020), this concept is being adopted on a case-by-case basis in this therapy class.

Inherent to continuous manufacturing is the seamless transition from one unit operation to the next, which presents a challenge for the viral filtration unit operation. Besides the unique spiking strategies, which are discussed below, integrated unit operations also have to consider the impacts of longer processing times, continuous but potentially low flow rates, and the higher level of impurities and product titer that will be experienced in an end-to-end processing unit operation linkage. One potential strategy to avoid the potential issues of clogging and filter overload is to implement a parallel switch-in and switch-out filtration scheme before the filters reach a validated total volumetric throughput. Monitoring of cumulative flow volume, in addition to traditional filtration pressure monitoring, would allow for control of switching from the first filter to the second once the established filter capacity is reached. Several groups have conducted proof-of-concept studies for such studies and showed equitable clearance values to traditional batch viral filtration (Patt, Dong et al. 2015, Swalec, Feng et al. 2015, Johnson, Brown et al. 2017).

The concept of "filter trains" for continuous viral filtration have also been posited as a potential strategy to allow for increased impurity clearance and improve overall viral filter lifetimes in the extended processing times (Arnold, Lee et al. 2019). Several filters are placed in series with no line or pressure breaks for clarification by depth filtration, TFF for product concentration, a charged membrane filter as a low pressure option for further impurity removal, and finally viral filtration for primary viral clearance. The train allows for reduced facility footprint as well as potentially faster processing times, but discussions on how to validate orthogonal viral clearance steps within these trains is required.

As discussed above, the development of the scale down models for integrated continuous viral filtration may pose a few challenges compared to batch viral filtration. These challenges include 1) performing virus filtration under constant flow, 2) extended volumetric throughputs and extended processing times, and 3) the potential for a dynamic product fluid stream potentially due to significant fluctuations in protein and buffer concentrations (Lute, Kozaili et al. 2020). While the use of constant flow may not be a difficult challenge, as this mode of operation can also occur in batch mode, proper modeling of the pump behavior may be required to avoid pulsations at small scale. A bigger challenge is how to perform virus spiking for the extended processing volumes and processing times. The traditional approach to virus spiking (bolus spike) may be prohibitive because the increased throughputs would require a higher virus spike concentration or a larger volume of virus. This could also lead to overloading of the filter with virus, which was previously discussed as a known concern for some virus filters (Lute, Riordan et al. 2008). Conversely, a low titer virus spike may be implemented to avoid overloading; however, this may lower the clearance values that can be achieved due to assay sensitivity and lowered linear range. Another challenge for virus spiking is maintaining the infectivity of the virus over the extended processing time. A bolus spike may experience a significant loss

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of infectivity over the course of the filtration study (David, Maiser et al. 2019, Lute, Kozaili et al. 2020). Alternative spiking strategies, have been proposed (Johnson and Roush 2018, Lute, Kozaili et al. 2020). Proposed bracketed spiking strategies involve spiking a high virus load in a small volume at the beginning and end of the filtration study, with either no virus or low titer virus spike for the majority of the process volume. This approach was able to achieve similar total virus loads and clearance values, for up to 2300 L/m^2, while avoiding potential virus overloading and loss of infectivity by only spiking virus at the beginning and end of the filtration study (Johnson and Roush 2018).

Another approach to avoiding a loss of infectivity is to have a fresh daily spike of virus throughout the filtration study. In this approach, a fresh spike can be applied to the filter every 24 hours for the duration of the experiment (Lute, Kozaili et al. 2020). To avoid overloading of the filter with virus, the total virus spike should be determined prior to the study and back calculated for a reasonable load titer per day. Care should be taken to seamlessly integrate the fresh load and avoid the introduction of air bubbles or pressure fluctuations that may negatively impact filtration study. This approach was able to demonstrate an LRV of $>6 \log_{10}$ for up to 4 days and 2900 L/m^2(Lute, Kozaili et al. 2020).

One final spiking approach mixed the virus stock with the feed solution to desired viral titer, then prefiltered the final feed solution through a sterile 0.1μm filter to mimic the practice of prefilter usage for aggregates. The feed was then pumped at a continuous rate through the viral filter(s) with a constant flux of $0.3L/m^2$/hour for up to 72 hours. Load samples were taken twice a day ($\log_{10}$ Day) and overnight without fresh spike being added to the feed solution. To account for potential loss of viral titer, the study calculated LRVs as equal to the $\log_{10}$of the daily load titer minus the $\log_{10}$ daily pool sample titer (David, Maiser et al. 2019).

These studies have provided frameworks for spiking strategies and provide strong supportive evidence that valid models for continuous viral filtration exist. Though the extended processing times in these scale-down models do not appear to affect viral reduction, other aspects like filter leachables need consideration.

Emerging technology

Virus removal filters, due to the nature of their use, were originally designed as single use systems, and can be easily adapted to newer modular, disposable facility systems. One generally accepted drawback to current virus filter technologies, however, is the higher cost.

Here, we discuss potential technologies, pre-commercial or established, that have the potential to be eventually adapted as an orthogonal option to traditional virus filters.

Depth filters, as previously discussed, have been used as prefilters for viral filtration. However, due to the batch-to-batch variety in diatomaceous earth derived depth filters, they are rarely claimed for viral clearance. Recent advances in synthetically derived depth filtration media show increased process performance and batch-to-batch consistency. Due to these improvements, the newer depth filters have been posited as orthogonal viral clearance components to the traditional nanofilters used for viral filtration. In general, depth filters have already been adapted for high-density continuous culture systems (Oh, Choi et al. 1994) and for the higher load titers. The newer synthetic depth filters also show higher clearance levels of host-cell derived impurities (Khanal, Singh et al. 2018, Nguyen, Langland et al. 2019). Therefore, these newer single-use depth filters offer an orthogonal option to viral clearance especially in a continuous process with longer processing times and increased impurity and overall titer profiles. This could also provide longer lifetimes for viral filters and extend their ability to economically filter more products, a bonus for a continuous flow schematic. Studies are needed, however, to show viral clearance capabilities for these newer depth filter technologies. In addition, vendors are building data sets to more strongly establish viral clearance capabilities.

Beyond traditional filtration technologies, there are many exciting technologies in pre-commercial stages that could be developed and adapted to process scale. These include the use of electrospun nanofibers (Zeytuncu, Ürper et al. 2018), crystalline cellulose nanofibers (Metreveli, Wågberg et al. 2014), ceramic capillary membranes (Bartels, Batista et al. 2019), or isoporous self-assembled block copolymer films (Shethji,

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Dorin et al. 2019). The advent of adsorptive hybrid filters (Singh, Arunkumar et al. 2017) could allow for two-step purification schematics for biotechnology products. Issues, however, of orthogonality for viral clearance validation purposes, could arise with their use.

Another potential cost-saving emerging technology are "filter papers" derived from cellulose that can be applied at the point-of-use and are easily scalable both in filter size and pore-size distribution (Gustafsson, Lordat et al. 2016). Several groups have demonstrated the capability of these nanocellulose filter papers to remove bacteriophage, XMuLV, and MMV (Asper, Hanrieder et al. 2015, Gustafsson, Lordat et al. 2016, Gustafsson and Mihranyan 2016, Gustafsson and Mihranyan 2017, Gustafsson, Gustafsson et al. 2018) as well as the feasibility of use in typical bioprocess fluids e.g. cell culture media (Manukyan, Li et al. 2019, Manukyan, Padova et al. 2019).

While more work is needed until commercial use, many of these technologies offer potential orthogonal and possibly economical options to traditional viral filtration.

Conclusions

Viral filtration is commonly utilized in a well-designed recombinant therapeutic protein purification process and is a robust and effective component in an overall strategy to minimize the risks of adventitious and endogenous viral particles during the manufacturing of biotechnology products. .

Key takeaways include:

- There is a consensus based on decades of data that parvovirus filters robustly remove both small and large viral species in various processes and product types providing a strong rationale for performing viral filtration validation studies with small viruses as worst-case to establish retention claims for larger viruses in regulatory submissions.
- Worst-case conditions for virus retention include low transmembrane pressure, pressure transitions, pauses in pressure or flow, and low flux based on recent data.
- For scale-down models of viral filtration, key considerations include:
- Filter load/feed material, virus spiking strategies, prefilter use, and the potential impacts of process parameters such as the target operating pressure, low pressure, a flow pause, and a buffer chase should be included.
- The viral validation study should be performed under challenging, or worst case, conditions using representative feed materials.
- High purity and high titer virus stocks are desirable to evaluate virus removal while maintaining product filtration capacity.
- Virus filters often require prefilters to achieve the desired virus filter throughput. Prefilters may remove virus when used in-line with the viral filter, necessitating novel spiking strategies.
- Emerging viral filtration technologies to consider include:
- Viral filtration of media for various manufacturing processing, including facilities that implement single-use, disposable manufacturing or newer products like cell and gene therapy.
- New viral filter development strategies include HTPD and filter plate technologies in combination with automated liquid and plate handling systems. Particle tracking technologies can be used to model flow and the impacts of flow interruptions.
- Viral filtration in continuous manufacturing requires new spiking models such as switch-in and switch-out filtration, filter trains, and bracketed or daily spiking.
- Depth filters, electrospun nanofibers, crystalline cellulose nanofibers, ceramic capillary membranes, filter papers derived from cellulose, or isoporous self-assembled block copolymer films are potentially new viral filtration modalities that require further development for commercial application.

Viral filtration will continue to be relied upon to provide robust and effective viral clearance as the technologies and industry-wide process knowledge continue to improve.

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Tables and Figures

Table 1. Commercially available virus filters and common prefilters associated with virus filtration

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Manufacturer	Model	ANSI/PDA Target Category[a]	Pore symmetry and format
Virus Filters	Virus Filters	Virus Filters	Virus Filters
MilliporeSigma	Viresolve NFR	Large virus	Asymmetric, triple layer pleated
	Viresolve NFP	Small virus	Asymmetric, triple layer pleated
	Viresolve Pro	Small virus	Asymmetric, double layer flat sh
Sartorius Stedim Biotech	Virosart CPV	Small virus	Symmetric, double layer pleated
	Virosart HF	Small virus	Asymmetric, single layer hollow
Pall	Ultipor VF grade DV50	Large virus	Symmetric, triple layer pleated s
	Ultipor VF grade DV20	Small virus	Symmetric, double layer pleated
	Pegasus grade SV4	Small virus	Symmetric, double layer pleated
	Pegasus grade Prime	Small virus	Pleated sheet
Asahi Kasei	Planova 35N	Large virus	Asymmetric, single layer hollow
	Planova 20N	Small virus	Asymmetric, single layer hollow
	Planova 15N	Small virus	Asymmetric, single layer hollow
	Planova BioEX	Small virus	Asymmetric, single layer hollow
Common Prefilters	Common Prefilters	Common Prefilters	Common Prefilters
MilliporeSigma	Viresolve® Shield	N/A (0.2 µm)	Flat sheet
	Viresolve® Shield-H	N/A (0.2 µm)	Flat sheet
	Viresolve® Prefilter	N/A (0.1 µm nominal)	Depth filter
Sartorius Stedim Biotech	Virosart Max	N/A (0.1 µm)	Pleated sheet
Pall	Pegasus Protect	N/A (0.2 µm)	Pleated sheet
	Pegasus grade UL6	N/A	Pleated sheet
Asahi Kasei	Planova 75N	N/A (75 nm)	Asymmetric, single layer hollow

Polyethersulfone (PES), Polyvinylidene fluoride (PVDF);[a](PDA 2021);[b] (Gefroh, Dehghani et al. 2014)

Table 2. Parvovirus removal by small virus filters: LRV and operating conditions reported in the literature. Parameters that are not reported are marked as "**NA**" , not available.

Filter	Model virus[3]	Load virus Titer/spike conc.	TMP (psi)	Volumetric through-put (L/m2)	Feedstream protein type	Feedstream protein conc. (g/L)	Feedstream pH / conductivity	Flow pause (min)	LRV[1,2]
Planova 20N	PPV	6.0-6.5 $log_{10}TCID_{50}$ /mL (0.5% spike)	11.4	NA	Polyclonal antibody, pI 6.8 - 10	1 - 50	pH 5, 100 mM NaCl	NA	[?] 5.42
	PPV	0.1 % single spike	14 ±2	100-200	mAbs, therapeutic proteins, and bispecific antibodies (9 molecules)	3.1 - 13.0	pH 4.9 - 8.3, various buffers with 50-300 mM NaCl	Yes	2.86 – 7.15

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Filter	Model virus[3]	Load virus Titer/spike conc.	TMP (psi)	Volumetric throughput (L/m2)	Feedstream protein type	Feedstream protein conc. (g/L)	Feedstream pH / conductivity	Flow pause (min)	LRV[1,2]
		0.1% mixed spike with 2.5% MMV		110-190	mAbs (6 molecules)	4.2 – 13.0	pH 4.9 - 8.3, various buffers with 50-200 mM NaCl		3.59 – 5.19
	PPV	1:50 single spike	manufacturer recommendation ˜13	50	Prothrombin complex concentrate solution	2.6	pH 6.5 sodium citrate buffer and 15 mM NaCl	2	[?]6.0
		Mixed spike with CPV and MMV							[?]5.8
	MMV	NA	NA	200-400	23 mAbs (IgG1 and IgG2)	NA	pH 5.0 – 7.5	NA	Approx. [?]3.9 – [?]6.9
	MMV	Approx. 6.0 $Log_{10}TCID_{50}$ /mL, 1% (v/v) spike	7.3 – 16.0	50	3 mAb molecules (IgG1), pI 7.2-9.0	5.0	pH 4.0 – 8.0, conductivity 3.0-20.0 mS/cm	15	4.0 - [?]5.5
			2.8	NA					2.9 - [?]5.0
	MMV	1:50 single spike	manufacturer recommendation ˜13	50	Prothrombin complex concentrate solution	2.6	pH 6.5 sodium citrate buffer and 15 mM NaCl	2	[?]7.3

Filter	Model virus[3]	Load virus Titer/spike conc.	TMP (psi)	Volumetric through-put (L/m2)	Feedstream pro-tein type	Feedstream pro-tein conc. (g/L)	Feedstream pH / con-duc-tivity	Flow pause (min)	LRV[1,2]
		1:50 mixed spike with CPV and B19V	2.9			1.7			[?]5.7
	CPV	5.5 – 8.5 $\log_{10}$ $CCID_{50}$/ 0.001m^2, 1:50 single spike	manufacturer recom-menda-tion ~13	50	Prothrombin com-plex concen-trate solution	2.6	pH 6.5 sodium citrate buffer and 15 mM NaCl	2	3.8 ± 0.7
		1:50 mixed spike with MMV and B19V	2.9			1.7			2.9
	B19V	1:50 single spike	13	50	Prothrombin complex concen-trate solution	1.7	pH 6.5 sodium citrate buffer and 15 mM NaCl	2	[?]4.4
		1:50 mixed spike with CPV and MMV	13						[?]4.3
		1:50 mixed spike with CPV and MMV	2.9						[?]6.8

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Filter	Model virus[3]	Load virus Titer/spike conc.	TMP (psi)	Volumetric through-put (L/m2)	Feedstream pro-tein type	Feedstream pro-tein conc. (g/L)	Feedstream pH / con-duc-tivity	Flow pause (min)	LRV[1,2]
Viresolve Pro	MMV	0.1% spike	NA	904-1042	mAb	NA	pH 5.0, 50 mM acetate	NA	[?]4.68
		NA	10 - 50	125 - 500	mAb	5-40	pH 4.5, or pH 7.5, with 0-500 mM NaCl	NA	5.20 - [?] 6.10
		6.47-8.22 $\log_{10}$ copies/mL by QPCR	NA	NA	8 mAbs	NA	NA	NA	3.69 - [?]5.45
		NA	NA	NA	mAbs	NA	NA	NA	[?]4.2
		NA	NA	NA	Not specified (1 molecule)	NA	NA	NA	[?]6.44
	PPV	NA	NA	NA	Not specified (2 molecules)	NA	NA	NA	5.72 - [?]6.96
Virosart CPV	PPV	NA	NA	NA	Not specified (1 molecule)	NA	NA	NA	6.00
Planova BioEx	MMV	Approx. 6.0 $\text{Log}_{10}\text{TCID}_{50}$ /mL, 1.0% spike	10.0 – 49.7	50	3 mAb molecules (IgG1), pI 7.2-9.0	5.0	pH 4.0 – 8.0, conductivity 3.0-20.0 mS/cm	15	[?] 4.8 - [?] 5.6
Viresolve NFP	MMV	0.1% spike	NA	904-1042	mAb	NA	pH 5.0, 50 mM acetate	NA	4.07

Filter	Model virus[3]	Load virus Titer/spike conc.	TMP (psi)	Volumetric throughput (L/m2)	Feedstream protein type	Feedstream protein conc. (g/L)	Feedstream pH / conductivity	Flow pause (min)	LRV[1,2]
Various small virus retentive filters (Planova 20N, Planova BioEX, Viresolve Pro, Viresolve NFP, Ultipor DV20, Pegasus SV4, Pegasus Prime, Virosart CPV, Virosart HF, Virosart HC)	CPV	1:50 mixed spike with MMV and PPV	Manufacturer recommendation	28.5-50	Prothrombin complex concentrate solution	1.7	pH 6.5 sodium citrate buffer and 15 mM NaCl	No pause	0.7 - [?]6.9
		1:100 mixed spike with MMV		126-900	Human serum albumin solution	4.0	pH 5.5 MES buffer and 225 mM NaCl		0.1 - [?]6.8

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Filter	Model virus[3]	Load virus Titer/spike conc.	TMP (psi)	Volumetric throughput (L/m2)	Feedstream protein type	Feedstream protein conc. (g/L)	Feedstream pH / conductivity	Flow pause (min)	LRV[1,2]
	MMV	1:50 mixed spike with CPV and PPV	Manufacturer recommendation	28.5-50	Prothrombin complex concentrate solution	1.7	pH 6.5 sodium citrate buffer and 15 mM NaCl	No pause	3.5 - [?]7.0
		1:100 mixed spike with CPV		126-900	Human serum albumin solution	4.0	pH 5.5 MES buffer and 225 mM NaCl		2.5 - [?]7.2
	PPV	1:50 mixed spike with CPV and MMV	Manufacturer recommendation	28.5-50	Prothrombin complex concentrate solution	1.7	pH 6.5 sodium citrate buffer and 15 mM NaCl	No pause	[?]3.9 - [?]5.7
Various nominal pore size 15-20 nm filters (Planova 15N, Planova 20N, Planova BioEX, Pall DV20, Virosart HC, Viresolve NFP, Viresolve VPro)	parvoviruses (B19V, BPV, CPV, MMV, PPV)	NA	4-50	0-1200	Plasma proteins (Inhibitors, immunoglobulins, coagulation factors, and others)	NA	pH 4.8-8.4, conductivity 0.48-48 mS/cm	NA	Approx. 3.9 - [?] 8.9 when intended for parvovirus removal; Approx. 1.5 to [?]7.5 if not specifically targeted for parvovirus

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Filter	**Model virus**[3]	**Load virus Titer/spike conc.**	**TMP (psi)**	**Volumetric through-put (L/m2)**	**Feedstream pro-tein type**	**Feedstream pro-tein conc. (g/L)**	**Feedstream pH / con-duc-tivity**	**Flow pause (min)**	**LRV**[1,2]
Not specified	MMV	0.5 – 1.0% spike	9-14	200-2500 g/m2	mAb >100 data points	7-17	pH 6.7-7.1, salt 0-150 mM	NA	>4
Not Speci-fied (3 com-mercial small virus filters)	MMV	5.33-7.60 $\times$ 10^6 PFU/mL	Various pres-sure reduc-tion schemes	20 - 325	mAb (IgG1), pI 8.9	10	25 mM ac-etate, 175mM NaCl, pH 5.2	Various schemes	3.1 - [?] 6.6

[1] LRV values with ">" or "[?]" indicate that no virus was detected in the filtrate. When viruses were detected in the filtrate, LRV values were reported as numbers without ">" or "[?]".

[2] LRVs with "approx." were approximate values estimated from pictorial data. Numerical LRVs were not reported in the cited reference. When a number of different molecules were tested, or when multiples runs were performed for the same molecule, LRVs are presented as a range of values.

[3] Abbreviations: **CPV** , Canine parvovirus;**MMV** , Minute virus of mice; **PPV** , Porcine parvovirus;**MuLV** , Murine Leukemia Virus; **Reo-3** , reovirus type 3;**PRV** , pseudorabies virus; **B19V** , human parvovirus B19;

Table 3. Virus (other than parvovirus) removal by small virus filters: LRVs reported in the literature

Filter
Viresolve Pro
Viresolve NFP
Virosart CPV
Planova 20N
Pall DV20

Filter
Pall DV50
Various nominal pore size 15-20 nm filters (Planova 20N, Planova BioEX, Viresolve Pro, Viresolve NFP, Ultipor DV20, Peg
Various small virus retentive filters (not specified)

[1] LRV values with ">" or "[?]" indicate that no virus was detected in the filtrate. When viruses were detected in the filtrate, LRV values were reported as numbers without ">" or "[?]".

[2] LRVs with "approx." were approximate values estimated from pictorial data. Numerical LRVs were not reported in the cited reference. When a number of different molecules were tested, or when multiples runs were performed for the same molecule, LRVs are presented as a range of values.

[3] Abbreviations: **xMuLV** , Xenotropic Murine Leukemia Virus**; HAV,** Hepatitis A virus; **BEV** , Bovine enterovirus; **EMCV** , Encephalomyocarditis virus; **PEV** , Porcine enterovirus; **HPV-1** , Human poliovirus 1; **TMEV** , Theiler's murine encephalomyelitis virus; **FCV** , Feline calicivirus; **SV40** , simian virus 40; **BVDV** , bovine viral diarrhea virus; **WNV** , West Nile virus; **SINV** , Sindbis virus; **SFV** , Semliki Forest virus; ; **HIV** , Human immunodeficiency virus; **VSV** , vesicular stomatitis virus;**Reo-3** , reovirus type 3; **PRV** , pseudorabies virus;**HSV-1** , Human herpesvirus 1 (herpes simplex virus);**IBRV** , Bovine herpesvirus 1 (Infectious bovine rhinotracheitis virus); **BPV** , Bovine parvovirus;

Table 4. Virus removal by large virus filters: LRVs reported in the literature

Filter	Model
Various large virus retentive filters with nominal pore size 35- 50 nm (Planova 35N, PALL DV50, Viresolve NFR)	MuLV Parvovi picorna flavi- ar retro-,

[1] LRV values with ">" or "[?]" indicate that no virus was detected in the filtrate. When viruses were detected in the filtrate, LRV values were reported as numbers without ">" or "[?]".

[2] LRVs were approximate values estimated from pictorial data. Numerical LRVs were not reported in the cited reference. When a number of different molecules were tested, or when multiples runs were performed for the same molecule, LRVs are presented as a range of values.

Table 5. Characteristics of cell culture media viral filters

Filter	Manufacturer	Type	Sanitization	Maximum Pressure (psi)	Parvovirus LRV
Viresolve® Barrier	Millipore	Dual layer flat sheet polyethersulfone	Pre-sterilized	60	> 3

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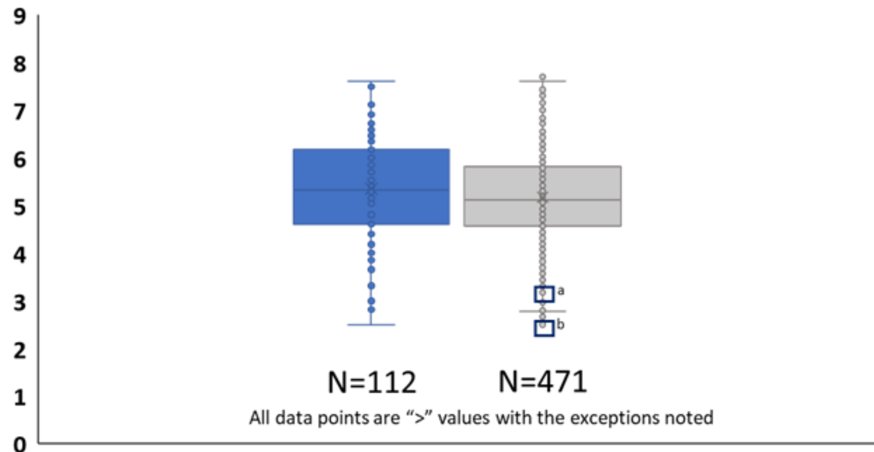
Filter	Manufacturer	Type	Sanitization	Maximum Pressure (psi)	Parvovirus LRV
Virosart® Media	Sartorius	asymmetric polyethersulfone hollow fiber membrane	Pre-sterilized	73	> 4
Planova 20 N*	Asahi Kasei	asymmetric cellulose hollow fiber membrane	Pre-sterilized	14	> 4
Planova BioEX*	Asahi Kasei	asymmetric PVDF hollow fiber membrane	SIP/Autoclave	50	> 4

* Traditional downstream virus removal filter that has been promoted by the manufacturer as suitable for use in barrier applications.



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