THE EUROPEAN SOCIETY OF MEDICINE
Medical Research Archives, Volume 12 Issue 12

RESEARCH ARTICLE

Virus Retentive Filters - Effective Virus Removal in the Manufacturing Process of Biologicals

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OPEN ACCESS

PUBLISHED
31 December 2024

CITATION
Gröner, A., 2024. Virus Retentive Filters - Effective Virus Removal in the Manufacturing Process of Biologicals. Medical Research Archives, [online] 12(12). https://doi.org/10.18103/mra.v12i12.6084

DOI
https://doi.org/10.18103/mra.v12i12.6084

ISSN
2375-1924

ABSTRACT

In the manufacturing process of biologicals virus reduction steps – inactivation or removal – have to be implemented to assure a high margin of virus safety of these products. Orthogonal mechanisms of virus clearance should be integrated in the manufacturing process in order to inactivate / remove viruses/virus aggregates having been able to escape to a certain degree the reduction capacity of the previous virus clearance step. Virus retentive filters as an orthogonal virus clearance step are frequently implemented in the manufacturing process of biologicals as the virus removal capacity of virus retentive filters is based on size exclusion. Only the size of a virus impacts the removal capacity and not virus properties as enveloped/ non-enveloped or RNA / DNA viruses and their resistance to physiochemical treatment. Viruses larger than the mean pore size of a virus retentive filter are removed from the feed stream and the desired protein – if smaller than the pore size of the filter membrane – will pass the filter and can be collected in the filtrate without / with very low virus contamination. Depending on the filter pore size, virus retentive filters are grouped in large and small virus retentive filters i.e., filters removing large viruses as retroviruses and small viruses as picornaviruses and, especially, parvoviruses. Data of virus reduction factors from 89 publications, resulting in a total of close to 500 virus clearance studies for different viruses, product intermediates and large and small virus retentive filters are assessed. The virus clearance capacity of these filters can depend on the membrane layout and chemistry, the volumetric throughput of product intermediate as well as of buffer flush and transmembrane pressure including pressure/flow interruption and flow decay. These parameters, when disclosed in published data, show filter brand specific differences but, having the above-mentioned parameters for each filter optimised, effective virus removal could mostly be demonstrated in virus validation studies for each filter brand.

1 Introduction

Virus safety of biologicals derived from cell cultures (biotechnology products) or plasma (plasma-derived medicinal products (PDMPs)) is based on the three complementary approaches (i) selecting and testing the source and raw materials for the absence of undesirable infectious viruses, (ii) testing the product at appropriate steps of production to demonstrate the absence of contaminating infectious viruses, i.e., the starting material of these biologicals as unprocessed bulk of cell culture derived products and plasma pools for further manufacturing, and (iii) testing the capacity of the production process to inactivate and/or to remove viruses potentially present in the source and raw materials[1,2,3]. This clearance capacity of the manufacturing process is documented in virus validation studies resulting in virus reduction factors (LRF = reduction factor in $\log_{10}$). Dedicated virus clearance steps, i.e., manufacturing steps incorporated in the manufacturing process predominantly for virus clearance and not for the purification and/or concentration of the drug substance, demonstrate commonly an effective virus clearance capacity in such virus validation studies for a wide range of viruses. Generally, a manufacturing process contains two dedicated virus clearance steps with different mode of actions, so-called orthogonal steps, as solvent/detergent treatment, pasteurisation (heat treatment in aqueous solution at 60°C for 10 h), dry heat treatment of lyophilised product (commonly either 100°C of 30 min or 80°C for 72 hours), low pH treatment (only for plasma-derived immunoglobulins and monoclonal antibodies), or virus filtration.

In order to achieve a finished product with a sufficiently high margin of virus safety, the virus clearance capacity of the manufacturing process should definitely exceed the potential amount of viruses in the starting material. As pointed out in different guidelines[2,3] the potential virus load in that volume of the starting material required to produce one dose of product should be removed by an excess of 6 $\log_{10}$, i.e., less than one virus particle is to be expected in 1 million vials thus meeting the sterility assurance level also for pathogens not replicating in a cell-free environment. If the overall virus clearance capacity of the dedicated virus clearance steps, documented in virus clearance studies with inherent limitation of the LRF due to e.g., limit of the amount of virus to be added in the virus spike preparation and / or limit of the detection of the *in vitro* assay will not result in a sufficiently high LRF, further manufacturing steps for the purification and concentration of the drug substance, e.g., chromatography steps, will have to be validated for virus clearance capacity to achieve a high enough overall virus reduction factor.

Virus retentive filters are one of the dedicated virus clearance steps implemented in the manufacturing process of biologicals. The virus clearance capacity is based primarily on size exclusion, i.e., viruses larger than the mean pore size of the filter are retained[4,5,6]; the drug substance, the desired therapeutic protein, passes the filter with high yield when the size of the protein – not aggregated – is smaller than the virus to be removed. Detailed information on the use of virus retentive filtration can be found in the PDA Technical Report No. 41 (rev 2022)[7] and, as virus retentive filtration is an established method, in the ICH guideline Q5A(R2), Annex 5: Examples of prior knowledge including in-house experience to reduce product-specific validation effort[2]. Besides removing adventitious (exogenous) and endogenous (cell culture-derived) viruses from the drug substance during its production, virus retentive filters can also be employed to minimise the risk of virus contamination of cell cultures by viruses present in the raw material, e.g., cell culture medium and its compounds[8,9].

The first commercially available virus retentive filter was produced by Asahi Kasei and launched in 1989: Planova 35N with mean pore sizes of 35 ± 2 nm, followed by Planova 15N with mean pore sizes of 15 ± 2 nm. These filters were evaluated to be implemented in the PDMP Factor IX and Factor XI with very good virus removal capacity and no detectable differences in the drug products associated with the virus filtration process.[10] The first commercial virus filtered product, licensed in Europe, was a four-factor human prothrombin complex concentrate (PCC); the Planova 35N filter removed all large viruses studied effectively, i.e., by more than 4 $\log_{10}$ whereas the small picornavirus poliovirus was not significantly removed.[11] Further virus retentive filters by Asahi Kasei as well as Millipore, PALL, and Sartorius are meanwhile on the market with different membrane composition and structures (Table 1). These filters are grouped in small and large virus retentive filters[7] based on the removal capacity for the bacteriophage PP7, a ~30 nm Pseudomonas phage, (> 4 LRF) for small virus retentive filters[12,13] and the bacteriophage PR 772, a ~64 to 82 nm E. coli phage, (> 6 LRF) for large virus retentive filters.[14]

Table 1: Relevant virus retentive filters covered in publications assessed

Manufacturer	Brand	Membrane Chemistry / Format	Mean Pore Size*	Max. Operating pressure [bar]
Asahi Kasei	Planova 15N Planova 20N Planova 35N	hydrophilic cuprammonium regenerated cellulose	15 ± 2 Small virus retentive filter	0.98
			19 ± 2 Small virus retentive filter	0.98
			35 ± 2 Large virus retentive filter	0.98
	Planova BioEX	hydrophilic PVDF# / hollow fibre	Approx. 20 Small virus retentive filter	3.43
Merck Millipore	Viresolve NFR	hydrophilic PES§ / membrane	Large virus retentive filter	5.5
	Viresolve NFP	hydrophilic PVDF# / membrane	Small virus retentive filter	5.5
	Viresolve Pro	hydrophilic PES§ / membrane	Small virus retentive filter	3.5
PALL	Ultipor DV50	hydrophilic PVDF# / membrane	Large virus retentive filter	3.0
	Ultipor DV20	hydrophilic PVDF# / membrane	Small virus retentive filter	3.1
	Pegasus SV4	hydrophilic PVDF# / membrane	Small virus retentive filter	3.1
Sartorius	Virosart CPV	hydrophilic PES§ / membrane	Small virus retentive filter	5
	Virosart HC	Surface modified PES§ / membrane	Small virus retentive filter	5

*Mean pore size [nm] or small and large virus-retentive filters based on size-based retention capacity[12,13,14]
Polyvinylidene fluoride
§ Polyethersulfone

Viruses to be removed by virus retentive filters have to be (at least slightly) larger than the mean pore size of a filter, i.e., small viruses will not be retained effectively by large virus retentive filters whereas small virus retentive filters will remove small and large viruses. The size of viruses employed in the referenced publications is shown in Table 2.

Table 2: Viruses used in spiking studies

Table 2a: Large viruses

Virus	Family (-viridae)	Genus	Genome	Envelope	Size [nm]	Shape
Large viruses						
Bovine herpesvirus	Herpes~	Varicellovirus	DNA	yes	120-200	Spherical
Cytomegalovirus (human herpesvirus 1) (CMV)	Herpes~	Cytomegalovirus	DNA	yes	120-200	Spherical
Human herpes virus (HSV)	Herpes~	Simplexvirus	DNA	yes	120-200	Spherical
Infectious bovine rhinotracheitis virus (IBRV)	Herpes~	Varicellovirus	RNA	yes	120-200	Spherical
Pseudorabies virus / suid herpesvirus-1) (PRV)	Herpes~	Varicellovirus	RNA	yes	120-200	Spherical
Human Immunodeficiency virus (HIV)	Retro~	Lentivirus	RNA	yes	80-110	Spherical
Murine leukaemia virus (MuLV)	Retro~	Gammaretrovirus	RNA	yes	80-110	Spherical
Parainfluenza 3 (PI3)	Paramyxo~	Respirovirus	RNA	yes	100-200+	Pleo/Sphere
Vesicular stomatitis virus (VSV)	Rabdo~	Vesiculovirus	RNA	yes	70 x 175	Bullet
Reovirus 3 (Reo3)	Reo~	Orthoreovirus	RNA	no	60-80	Spherical
Sindbis virus (SINV)	Toga~	Alphavirus	RNA	yes	60-70	Spherical
Semliki Forest virus (SFV)	Toga~	Alphavirus	RNA	yes	60-70	Spherical
Chikungunya virus (CHIKV)	Toga~	Alphavirus	RNA	yes	60-70	Spherical
Mayaro virus (MAYV)	Toga~	Alphavirus	RNA	yes	60-70	Spherical
Bovine viral diarrhoea virus (BVDV)	Flavi~	Pestivirus	RNA	yes	50-70	Pleo/Sphere
Japanese encephalitis virus (JEV)	Flavi~	Flavivirus	RNA	yes	50-70	Pleo/Sphere
West Nile virus (WNV)	Flavi~	Flavivirus	RNA	yes	50-70	Pleo/Sphere
Yellow fever virus (YFV)	Flavi~	Flavivirus	RNA	yes	50-70	Pleo/Sphere
Zika virus (ZIKV)	Flavi~	Flavivirus	RNA	yes	50-70	Pleo/Sphere
Hepatitis C virus (HCV)	Flavi~	Hepacivirus	RNA	yes	50-70	Pleo/Sphere
SV40	Papova~	Polyomavirus	DNA	no	40-50	Icosahedral
Hepatitis B virus (HBV)	Hepadna~	Orthohepadnavirus	DNA	yes	40-50	Spherical

Table 2b: Small viruses

Hepatitis E virus (HEV)	Hepe~	Hepevirus	RNA	no	27-34	Icosahedral
Felines calicivirus (FCV)	Calici~	Vesivirus	RNA	no	27-40	Icosahedral
Torque teno virus (TTV)	Anello~	Alphatorquevirus	DNA	no	~ 30	Icosahedral
Bovines enterovirus (BEV)	Picorna~	Enterovirus	RNA	no	25-30	Icosahedral
Encephalomyocarditis virus (EMCV)	Picorna~	Cardiovirus	RNA	no	25-30	Icosahedral
Hepatitis A virus (HAV)	Picorna~	Hepatovirus	RNA	no	25-30	Icosahedral
Poliomyelitis virus (Polio)	Picorna~	Enterovirus	RNA	no	25-30	Icosahedral
Minute virus of mice (MVM)	Parvo~	Parvovirus	DNA	no	18-24	Icosahedral
Porcine parvovirus (PPV)	Parvo~	Parvovirus	DNA	no	18-24	Icosahedral
Canine parvovirus (CPV)	Parvo~	Bocavirus	DNA	no	18-24	Icosahedral
Bovine parvovirus (BPV)	Parvo~	Bocavirus	DNA	no	18-24	Icosahedral
Parvovirus B19 (B19V)	Parvo~	Erythrovirus	DNA	no	18-24	Icosahedral
PCV (porcine circovirus), a small DNA virus with a size of less than 20 nm, covered in one publication[15], was not assessed in this review as circoviruses are host-specific or have a narrow host range; furthermore, the majority of circoviruses infect avian species						

The virus removal capacity of the virus retentive filters was assessed in virus clearance studies, based on a valid downscale of the manufacturing process, by spiking the product intermediate with a defined amount of virus prior to filtration and assessing quantitatively the virus reduction capacity of the virus retentive filtration step as the difference in the spiked starting material and final sample (when a buffer flush is used according to the manufacturing process, the final sample is the pool of the filtrate plus the buffer flush). The amount of virus in the filtrate is quantified either employing an *in vitro* cell culture infectivity assay detecting virus replication due to infectious virus in the virus stock used for spiking or by a NAT (nucleic acid amplification test) such as PCR (polymerase chain reaction) detecting virus genome sequences in the filtrate. Polymerase chain reaction does not differentiate between infectious and non-infectious viruses (e.g.,[16,17,18]). Therefore, the size of the amplicon generated by PCR should be large enough to represent infectious virus in the virus stock.[19] Furthermore, prior to PCR assays, samples of the virus stock as well as of the filtrate to be employed in the PCR assay have to be treated by nucleases prior to capsid dissolution to remove free DNA and RNA, respectively.[20,21,22] When performing virus clearance studies, the volume added to the product intermediate should not exceed 10% according to guidelines in order not to change the properties of the intermediate too much. Furthermore, the virus spike should be of appropriate purity and monodisperse to (i) avoid blocking of the filter by impurities from the virus spike and (ii) document a too high virus clearance capacity due to the removal of virus aggregates by the virus retentive filter.[23,24,25,26] In order to remove impurities blocking the filter, either from the product intermediate itself or the virus spike, prefiltration of the feed stream is often applied; when the virus spike is of high purity, spiking the product intermediate is often performed after prefiltration.[27,28]

Virus filtration can be performed in two ways, either as dead-end filtration or tangential filtration; in the early days of virus filtration implementation, tangential flow was mostly used in order to avoid blocking of the filter (using the known principle of ultrafiltration); a disadvantage of tangential filtration is that a certain amount of product intermediate is lost in the system. In the meantime, commonly dead-end filtration is applied and a buffer flush at the end of the filtration is applied to recover most of the drug substance.

2 Materials and Methods

2.1. DATA COLLECTION

Publicly available data of virus reduction factors attained by virus retentive filters were compiled by searching PubMed for the terms “virus filtration, biologicals / virus retentive filters, biologicals / nanofiltration, biologicals” and the papers were checked for results of virus reduction factors. In the majority of publications, no detailed information on the virus filtration parameters used were disclosed as volume / filter area, pressure, intermediate composition as protein concentration, pH, conductivity, flux (with potential flux decay), flow interruption etc., but it was stated that the scaled-down laboratory system represents closely the manufacturing process.

Data for virus reduction factors from 89 publications, resulting in a total of close to 500 virus clearance studies for different viruses, product intermediates and virus retentive filters; 27 package inserts for plasma-derived medicinal products licensed by the FDA are not included in the assessment as commonly the filter type is not disclosed; for cell culture-derived products virus clearance factors are not required by the FDA and, thus, no virus clearance factors for this product class are disclosed in package inserts.

The virus clearance capacity of filtration processes were studied with the following product intermediates: Human immunoglobulins applied intravenously or subcutaneously, as well as hyperimmunoglobulin preparations and monoclonal antibodies, inhibitors include alpha-1-proteinase inhibitor, antithrombin III, and C1 esterase inhibitor, and the coagulation factor concentrates Factor VIII, IX, XI, XIII, thrombin, prothrombin complex concentrates, anti-inhibitor coagulant complex, von Willebrand factor, fibrinogen including the recombinant Factor IX and VIII, and a range of other intermediates as model proteins (e.g., human serum albumin (HSA), bovine serum albumin (BSA)), non-disclosed proteins and filters, different buffers, and some plasma- and cell culture-derived proteins.

Small and large virus retentive filters were employed in the production of the following products depending on the size of the desired protein to be filtered (Table 3) (data from package inserts not included). Since the principal mode of action by virus-retentive filters is size exclusion, the majority of studies were performed employing small virus-retentive filters; it is safe to conclude that small virus-retentive filters, able to remove parvoviruses (18 – 24 nm diameter), will also remove larger viruses effectively.[2,4,5]

Table 3: Product classes filtered

Poducts	Virus retentive filters	Number of studies
Immunoglobulins and monoclonal antibodies	Small virus filters	196
	Large virus filters	102
Coagulation Factors	Small virus filters	68
	Large virus filters	23
Inhibitors	Small virus filters	26
	Large virus filters	0
Further proteins (partly not disclosed)	Small virus filters	24
	Large virus filters	2
Buffers etc.	Small virus filters	31
	Large virus filters	25

2.2. STATISTICAL ANALYSIS

Viruses studied were grouped according to their size (Table 2). Virus reduction factors (LRFs) were analysed considering small and large viruses filtered through small and large virus retentive filters as well as filters used in series (2*15N and 2*20N). LRF were differentiated between no infectious virus or PCR signal detected in the filtrate (virus titre below the Limit of Detection (LoD) of the assays) and infectious virus or PCR signal detected in the filtrate (i.e., above LoD of the assays). Furthermore, under these conditions, the capacity of a virus filtration step to remove viruses was differentiated between a so-called effective step (LRF ≥ 4) and a manufacturing step contributing to the virus safety of a defined product (LRF < 4). As all LRF below LoD can not be defined appropriately (LRF ≥ 4.3 may be e.g., 4.4 or 7.9), all data with LRF below LoD were not used in a statistical analysis but only LRF above LoD.

An unpaired t-test was applied to compare equality of two means; prior to performing the t-test, the variances of both samples had to be equal / homogeneous and if the variance was not equal / homogeneous, the Welch's t-test was performed (unpaired samples with different variances).

3 Results

Small virus retentive filters (Planova 15N, 20N, BioEX, Viresolve NFP, Viresolve Pro, Ultipor DV20, Pegasus SV4, Virosart CPV, Virosart HC studied) are primarily applied to remove small viruses from the product intermediate; however, due to the capacity to remove small viruses, also large viruses are effectively retained. As stated in the ICH Q5A(R2) guideline, parvoviruses "may be used as single worst-case model virus for larger spherical/icosahedral viruses and enveloped viruses at validation of virus filters".[2]

3.1 SMALL VIRUS RETENTIVE FILTERS

Small virus retentive filters remove small viruses effectively in the order of 90% of all studies and all large viruses, as expected, effectively as shown in Figure 1 and 2. The detection of large viruses in the filtrate of small virus retentive filters, despite the fact that the removal capacity was effective (LRF ≥ 4), cannot be explained; e.g. Ajayi et al.[5] stated that no root cause for passing large viruses through small virus retentive filters could be identified. A cross-contamination of cell cultures used for quantification the virus load in the respective samples during handling the different filtrate fractions with virus or during the infectivity assays cannot be excluded.[29,5]

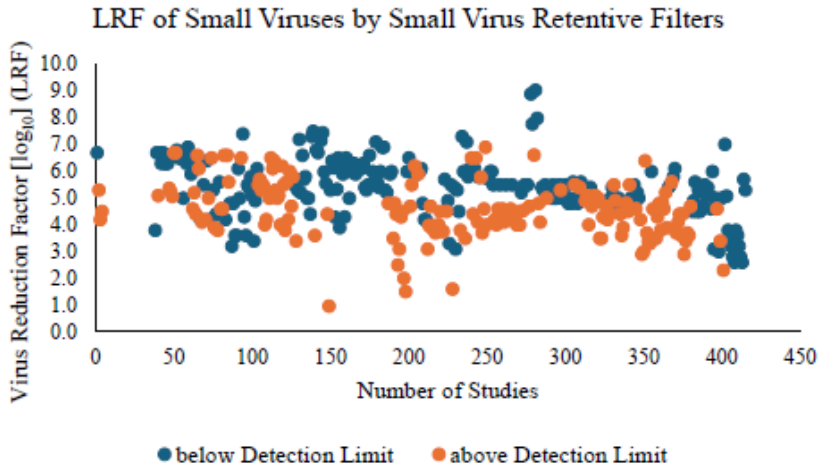
The studies showed that parvoviruses are within the group of small viruses the smallest viruses and, therefore, are a challenge for their removal even for small virus retentive filters. An assessment of the data published showed that no residual parvovirus vs. residual parvovirus in the filtrate could be detected in 47% vs 53% of all studies and for the other small viruses studied the relation was 92% vs. 8%.

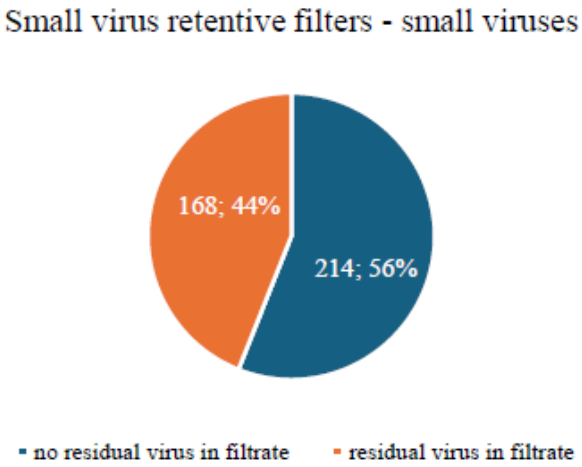
Serial filtration with 2 small virus retentive filters removed small viruses to a significant higher rate than a single filter (Table 4). The application of serial filtration employing 1:1 two filters or 2 filters parallel followed by one filter was also reported.[30]

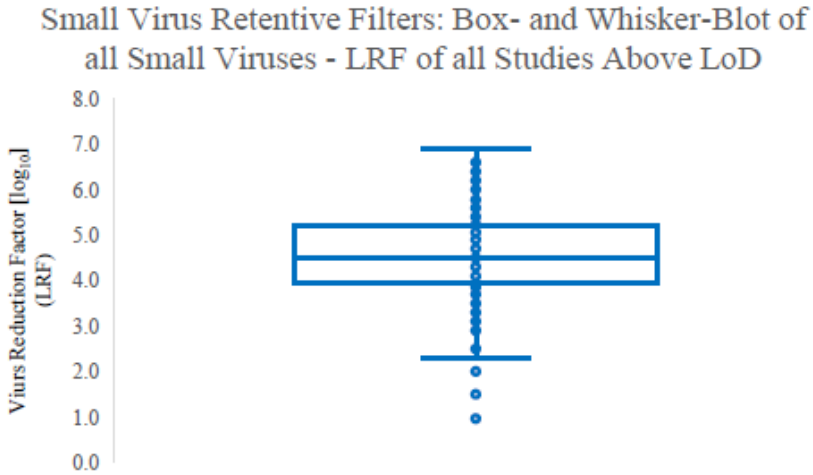
Table 4: Virus reduction factors for small viruses using single vs. serial filtration by small virus retentive filters

Filtration	Viruses	No. of Studies	Mean LRF	Difference in LRF
Single filters (Planova 15N excluded)	all small viruses studied	243	4.9 ± 1.2	Significant (p = 0.01)
2 filters in series (Planova 15N excluded)		29	5.9 ± 0.8	

Figure 1: Virus reduction capacity of small virus retentive filters for small viruses

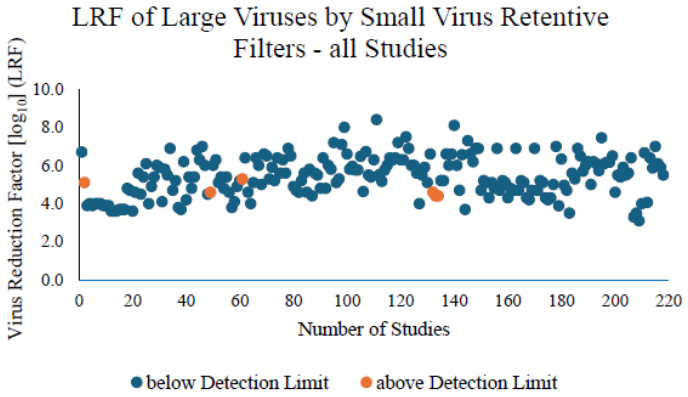


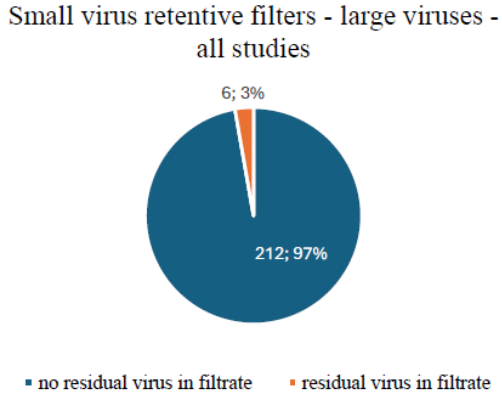


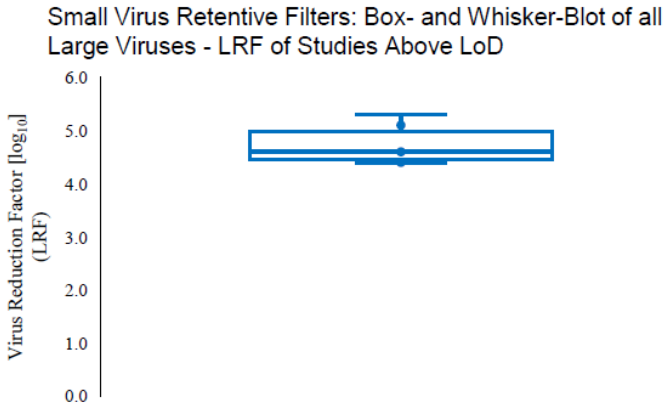


Summary of Virus Clearance Capacity of Small Virus Retentive Filters for Small Viruses		
Virus Clearance capacity	Number of studies	% of studies
No residual virus in filtrate	214	56
Effective clearance (LRF ≥ 4)	126	33
LRF < 4	42	11

Figure 2: Virus reduction capacity of small virus retentive filters for large viruses







Summary of Virus Clearance Capacity of Small Virus Retentive Filters for Large Viruses		
Virus Clearance capacity	Number of studies	% of studies
No residual virus in filtrate	212	97
Effective clearance (LRF ≥ 4)	6	3
LRF < 4	0	0

3.2 LARGE VIRUS RETENTIVE FILTERS

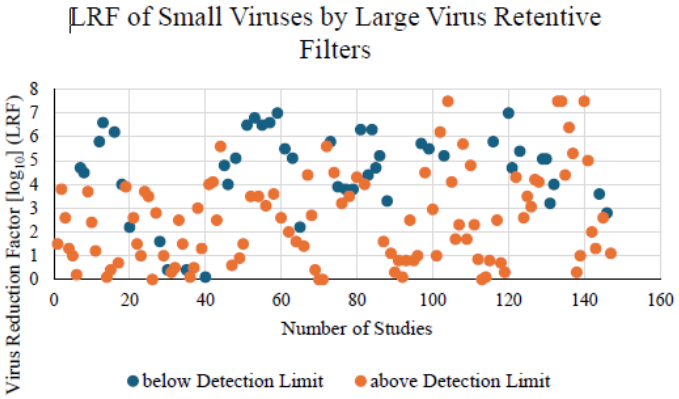
Due to the pore size of the large virus retentive filters, it is expected that small viruses are not retained to a high rate by these filters; Figure 3 shows the detail of studies; in 47% of all studies an effective virus reduction capacity for small viruses (LRF > 4) could be stated. As the distribution of the virus reduction factors for small viruses is very high, a reason for this effect was assessed. As immunoglobulins and, partly, monoclonal antibodies (mAbs) bind to viruses enlarging their size considerably,[31,32] this enlarging effect was assessed for small viruses and large virus retentive filters. There is no difference in the virus reduction capacity for small virus retentive filters in immunoglobulin intermediates compared to all studies covering small viruses; therefore, for small virus retentive filters enlarging the size of the virus by binding to immunoglobulins / mAbs is not relevant. However, the effect of enlarging a virus particle by the binding of antibodies is considerable when assessing large virus retentive filters. The effect of antibodies binding to the viruses studied [bovine parvovirus (BPV), parvovirus B19 (B19V), hepatitis A virus (HAV), hepatitis E virus (HEV) (stripped from the quasi-envelope)[33], and poliovirus] versus not binding to the viruses studied [minute virus of mice (MVM), porcine parvovirus (PPV), HEV with quasi-envelope, and encephalomyocarditis virus (EMCV)] is shown in Table 5; in addition, the binding / non-binding effect to different parvoviruses was also assessed.

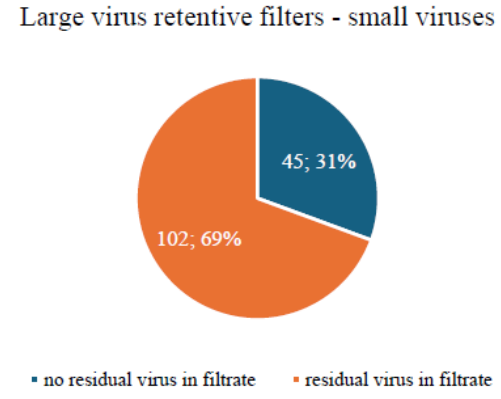
All studies with residual infectivity in the filtrate of large virus retentive filters were mid-size viruses (BVDV, WNV, YFV, TBEV, HCV, SINV, ZIKV, CHIKV, MAYV, SV40, Reo3, and HBV – compare Table 2a) whereas the large viruses (HIV, MuLV, PI3, PRV, HSV, and VSV) were removed to below the limit of detection of the infectivity assay with the exception of 2 publications detecting HIV in the filtrate[34]; the reported removal of BVDV, Reo3, SV40 and even BPV under the same conditions with no residual infectivity in the filtrate indicate a cross contamination of the cell culture with HIV during the experiment. In the second publication[35] in one of ten studies employing HIV this virus was detected in the filtrate.

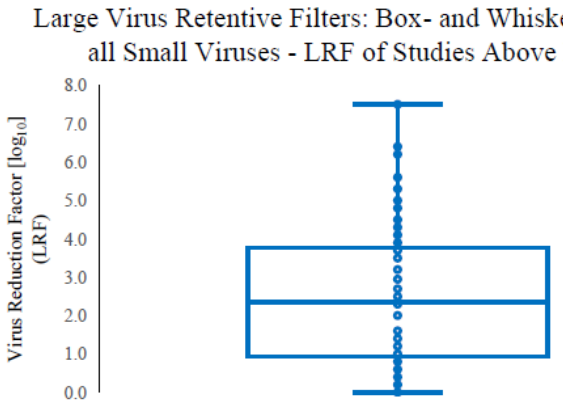
Table 5: Virus reduction factors for small viruses binding vs. non-binding to the product intermediates immunoglobulin / monoclonal antibody passing large virus retentive filters

Product intermediate	No. of Studies	Mean LRF	Difference in LRF
Not binding to small viruses	22	1.7 ± 1.3	Significant (p = 0.01)
Binding to small viruses	53	5.1 ± 1.2	
Not binding to parvoviruses	12	1.8 ± 1.4	
Binding to parvoviruses	25	5.9 ± 1.1	

Figure 3: Virus reduction capacity of large virus retentive filters for small viruses







Summary of Virus Clearance Capacity of Large Virus Retentive Filters for Small Viruses		
Virus Clearance capacity	Number of studies	% of studies
No residual virus in filtrate	45	31
Effective clearance (LRF ≥ 4)	24	16
LRF < 4	78	53

4 Discussion

In several publications data, partly not published, are compiled demonstrating for plasma-derived medicinal products as well as biotechnological products derived from cell cultures that virus retentive filtration removes viruses depending on the pore size of the respective filters highly effectively and robust. The volume per filter area, operating pressure and total protein concentration had had no significant impact on the efficacy of the virus removal capacity within the studied ranges.[6] A multicompany collaboration with data compiled from CROs demonstrate that large viruses (MuLV, PRV, Reo3) were removed (primarily) by small virus retentive filters very effectively: No virus detected in the filtrate was reported for 97.3% of all runs (2311 runs in total), effective virus removal was reported for 99.1 % and only 0.9% of all runs resulted in a LRF < 4.[36] Authors from CDER/FDA published data,[37] extracted from the CMC section of IND and BLA applications, documenting that for the large virus MuLV the LRF was always above 2 and in only 1% of all studies the LRF was < 3 for small and large virus retentive filters. The removal of parvoviruses by small virus retention filters was stated to be filter type specific with one filter type (not disclosed) resulting in low LRFs. A further publication compiled studies from 10 biotechnology companies having employed small virus retentive filters of different manufacturers categorised into PES (polyethersulfone) and RC (regenerated cellulose).[38] In all studies employing retro- and herpesviruses, no residual infectivity could be detected in the filtrate; the mid-size Reo3 was removed always effectively with only one experiment with residual infectivity in the filtrate and a LRF of 6.1. Parvoviruses were removed effectively by both, PES and RC filters (LRF of 5.9 (PES filters) vs. 5.0 (RC filters). The data analysis showed that the virus load per filter area has a considerable effect on the virus clearance capacity / virus infectivity in the filtrate for RC filters. Passage of the phage PP7and ΦX-174 into the filtrate of small virus retentive filters (Viresolve NFP, Virosart CPV, Ultipor DV20 and Planova 20N) occurred in each filter type, particularly when overloaded with phage.[39] The authors also reported brand-specific differences in flux decay due to phage overload and concluded that small virus retentive filters should not be viewed as absolute in their capacity to clear virus and they should not be viewed as interchangeable between brands.

The effect of overloading virus filters with viruses was studied intensively with the Planova 20N filter (regenerated cellulose) resulting in the fact that non-infectious MVM, i.e., also empty particles, can cause an overload of the filters resulting in a breakthrough of (infectious) viruses. It was concluded that a total particle number of more than approx. 12 $\log_{10}/m^2$ filter area should not be used in virus validation studies,[40] just to achieve a very high LRF using an unrealistic high virus load. Compilation of data assessing also the so-called second generation of small virus retentive filters (Planova BioEX, Viresolve Pro) showed that these improved filters were able to remove e.g., parvoviruses to a higher degree as the so-called first generation of small virus retentive filters (Planova 15N, Planova 20N, Viresolve NFP, Virosart CPV, Ultipor DV20) and appeared to have less variability in the reported LRFs.[5] In the meantime further small virus retentive filters were developed, e.g., Planova S20N, prepared, as Planova 20N, also from regenerated cellulose but with increased thickness of the membrane structure of the hollow fibre withstanding a higher membrane pressure and the Virosart HC, consisting of two asymmetric membranes oriented in opposite direction.

These review publications support the data accumulated from the published data reviewed here documenting a robust and effective clearance of viruses by virus retentive filters. It has to be considered, however, that certain parameters of the filtration procedure may impact the virus clearance capacity of such filters as pressure, flow decay and flow interruption, volumetric throughput of product intermediate and buffer flush. The impact of volume / filter area, protein load and operating pressure on the virus clearance capacity was negligible, documented for plasma-derived medicinal products.[6] Flow decay due to blocking filters (e.g., fouling[41]) should be considered a relevant parameter as under such conditions the virus clearance capacity may be reduced;[39,42,43,44,45,46,47] therefore, based on virus clearance studies, a minimum flow rate (LMH – litre per m^2 and hour) for production conditions should be defined. A flow decay to zero LMH may occur when switching from product feed stream to buffer flush; this flow interruption is known to impact considerably the overall virus clearance capacity assessing the pooled filtrate of the product intermediate and the buffer flush to recover as much product as possible. This flow-interruption associated virus breakthrough is because viruses may migrate into deeper membrane layers,[48,49,50,51] partly based on the membrane specific pore interconnectivity.[52]

The ICH Q5A(R2) guideline[2] defines potential critical parameters in virus filtration as volumetric throughput of product intermediate as well as of buffer flush and pressure including pressure/flow interruption due to prior knowledge / in-house experience. As summarised here, these parameters are important for distinct filter types and have to be controlled. Therefore, a change of filter brands, especially in a post-approval change has to be carefully assessed also regarding these parameters.

The size-exclusion mechanism of (small) virus retentive filters is also able to remove prions, the causative agent of TSEs (transmissible spongiform encephalopathies) as (variant) Creutzfeldt-Jakob disease ((v)CJD) to a high degree. In the extremely unlikely situation that prions would be present in the product intermediate of plasma-derived medicinal products, the infectious prion material is a multimeric protein aggregate[53] – not the monomeric protein – and this material can be removed. The challenge of prion removal data are the physicochemical properties of the prion spike material as the nature of the infectious agent in blood, if present, is currently not known.[54] Different spike preparations were used in prion evaluation studies with a considerable removal capacity.[55,56,57,58,59,60,61,62,63,64,65]

Continuous manufacturing is increasingly applied in the manufacturing process of biologicals in order to reduce costs and the footprint of the equipment used throughout the production facility. Challenges for virus filtration studies are discussed in general in the ICH guidelines

Q5A(R2)[2] and Q13.[66] Virus filtration under constant flow, commonly at low pressure, extended volumetric throughputs and processing times, and, especially, product feed for the virus retention filter with fluctuations in protein and buffer concentrations have to be addressed properly.[67,68,4] Furthermore, inline virus spiking has to be performed.[4,67,69] In order to avoid significant variations in the feed stream, especially post chromatography steps, surge tanks for temporarily hold of the continuous stream can be considered; this approach will than mimic a classical batch process for virus filtration. Under these conditions, replacing filters during the process would be simpler; such filter replacement may be required during the long duration of the continuous process to avoid blocking of the filter and other, including unexpected, process events.

Implementation of virus retention filters in the manufacturing process of biological products reduces effectively the potential presence of viruses in the starting material. For cell culture derived products the contamination of the bioreactor with adventitious viruses is primarily caused by components of the cell culture medium, especially animal derived components as foetal bovine serum and to a minor degree by the operator.[70,71] Therefore, pre-treatment of the cell culture medium by appropriate methods to inactivate and/or remove potentially present viruses would mitigate such contamination risk (especially relevant for ATMPs / cell therapy products as, commonly, virus clearance steps cannot be integrated in its manufacturing process). Such upstream virus clearance methods are high temperature, short time (e.g.,[72,73]), UV treatment (e.g.,[74] (besides gamma irradiation of FBS[75])), and virus filtration (e.g.,[76,8]). The challenge of this approach for the virus retentive filters is the very large volume of the feed stream, a long duration of filtration time and, potentially, multiple process interruptions; these parameters result in a considerable flow decay. Also under these conditions, filter specific virus reduction factors can be observed,[8] and, after selection of the most suitable filter for this approach, an effective virus reduction capacity can be achieved.

5 Conclusion

Published data on virus clearance by virus retentive filters demonstrate that virus removal is based on size exclusion, i.e., large viruses are very effectively removed by small virus retentive filters. Therefore, the LRF demonstrated for small viruses as parvoviruses can be applied to large viruses as retroviruses.[2] Parameters considered potentially critical in virus filtration according to the ICH Q5A(R2) guideline[2] are volumetric throughput of product intermediate and buffer flush as well as pressure including pressure/flow interruption. The presented data confirm, depending on the filter brand, this assessment which should include also flow decay. These brand specific differences[39,46] show that brands cannot be changed (post-approval change) without appropriate validation. Each filter brand effectively removes viruses based on the pore size of the filter, i.e., large virus retentive filters remove large viruses and small virus retentive filters remove small and large viruses effectively having implemented the above-mentioned parameters for high virus removal capacity of the respective virus retentive filters, assessed in virus validation studies. The assessment of these parameters, preferably in a Design of Experiment approach covering virus clearance, are one of the bases of the specification of these critical process parameters ("Established Conditions"[77]) resulting in a platform validation approach when the process step is predictable and robust in virus removal capacity based also on prior knowledge.[2]

6 Conflict of Interest

No conflict of interest to be disclosed

7 Funding Statement

No funding to be reported

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