

Zeta Plus™ VR Series Filters for Viral Reduction in Blood Plasma Fractionation Processes

Introduction

The removal and/or inactivation to a high level of assurance of contaminating viruses from blood derived therapeutics is a requisite for ensuring product safety. Screening methods to detect viral contamination are not adequate to ensure product safety due to limitations in assay sensitivity. For this reason process engineers must design into processes viral clearance steps to address adventitious contamination events. Regulatory requirements state that at least two viral clearance steps operating by different mechanisms should be employed in processes.

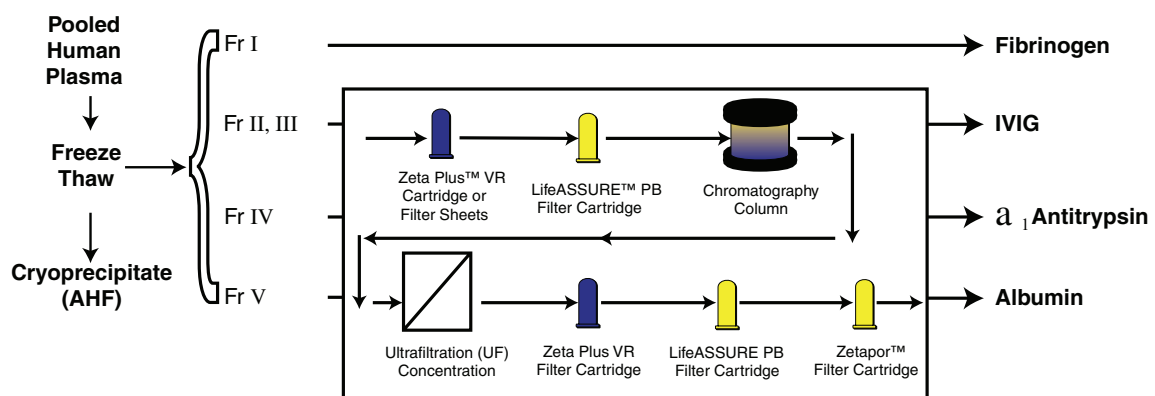
This Applications Brief presents:

- The use of CUNO Zeta Plus VR Series filters to provide viral clearance from blood fractionation processes.
- The principal mechanism of viral clearance operative with Zeta Plus VR Series filters is electrokinetic adsorption. Because Zeta Plus VR Series filters function in an ion-exchange like manner, they complement viral clearance steps such as inactivation and size exclusion filtration.
- CUNO Zeta Plus VR Series filters offer an effective means of prefiltration to membrane based viral retentive filters.

Industry experience with Zeta Plus VR Series filters has resulted in effective removal of enveloped and non-enveloped viruses from blood plasma and aqueous buffer systems. The typical log removal value (LRV) exhibited by Zeta Plus VR Series filters is 2 to 6 logs.

The Process

The purification of blood derived therapeutics is based on the successive fractionation method developed initially by Dr. Edwin J. Cohn. The products produced by this process include primarily albumin, immunoglobulins (IgG), blood coagulation factors and protease inhibitors. The initial stages of fractionation involve separation of plasma into precursor fractions for each of the major end products. CUNO Zeta Plus VR Series filters are often used at this stage. Once the initial separation has been completed, the various fractions are further purified to yield the end therapeutic products. An overview of the process is shown in Figure 1.



*CUNO Products not the subject of this paper but shown for reference - see appendix.

Figure 1 — Blood Plasma Fractionation Process

The Problem

The FDA requires a minimum of two viral clearance steps, operating by different mechanisms, to provide assurance of viral clearance. To be considered robust, a viral clearance step must be validated to consistently remove model viruses with at least 2 logs of clearance. Validation of filtration devices involves spiking studies with model viruses under actual process conditions. This involves spiking the desired virus into product representative of the process point at which the filter will be used, followed by filtration at the process flow rate, pH, temperature and volume, conditions representative of the actual process.

When filtration is employed to obtain viral clearance, the objective is to obtain effective viral clearance without affecting protein (product) loss. Both adsorption based and size exclusion based virus retentive filters can affect product yield. Size exclusion filters designed to remove small (20 nm) viruses, may have pores small enough to significantly retain globular proteins. Adsorptive filters are less likely to mechanically retain proteins, however, a small amount of protein (product) adsorption in addition to adsorptive virus retention may occur. For this reason it is necessary to test any viral clearance step for its ability to retain model virus and for possible effects on product yield and composition. Often with adsorptive processes, solvent parameters such as pH or ionic strength can be varied to obtain optimal viral clearance and minimal product loss.

The CUNO Solution:

CUNO Zeta Plus™ VR Series filters can be used in sheet or cartridge configurations during the initial stages of separation in the fractionation process. In final purification stages, Zeta Plus VR Series filters can be used upstream of nano-filtration virus retentive membranes. In both instances, Zeta Plus VR Series filters complement alternative viral clearance steps such as heat inactivation, solvent detergent and size exclusion filtration as the primary mechanism of virus retention by Zeta Plus VR Series filters is adsorption. The data below support the adsorptive mechanism of virus retention by Zeta Plus VR Series filters.



Adsorptive Mechanism of Virus Retention by Zeta Plus VR Series Filters.

Zeta Plus VR Series filter media are a family of cellulosic depth filtration media designed to retain contaminants by ion exchange adsorption. They are composed of high area process filter aids embedded in a cellulose fiber depth filter matrix. During the manufacturing process, a cationic charge modifier is chemically bound to the matrix component, forming a permanent, interconnected, rigid depth filter with positively charged electrokinetic capture sites. The resulting porous depth filter structure is a tortuous network of adsorptive flow channels capable of retaining contaminating viruses by an ion exchange adsorption. The range of nominal pore size for the Zeta Plus VR Series of depth filters is 200 nm to 800 nm. The smallest mammalian viruses are on the order of 20 nm.

Reports from end users and in the literature demonstrated that Zeta Plus VR Series depth filters are effective in retaining various mammalian viruses. Because the nominal pore size of Zeta Plus VR Series depth filter media is significantly larger than the smaller mammalian viruses, it was anticipated that ion exchange capture mechanisms predominate. In order to test this hypothesis, a series of experiments to evaluate retention by different Zeta Plus VR Series depth filter media of bacteriophage Phi X-174 suspended in different ionic strength buffers were conducted. Bacteriophage Phi X-174 was chosen as a model for small mammalian viruses. Phi X-174 has a diameter of 28 nm and small mammalian viruses such as Parvovirus B19 and poliovirus are 18-24 nm and 20 nm in diameter, respectively. Table 1 shows the retention of bacteriophage Phi X-174 by different Zeta Plus VR Series filters. Retention is expressed as log reduction value (LRV).

Table 1 — Bacteriophage Phi X-174 Log Reduction Value (LRV) By Zeta Plus VR Series Filters

Zeta Plus VR Series Filter Type	LRV in Phosphate Buffer	
	20 mM Phosphate	20 mM Phosphate +150 mM NaCl
VR 05	2.7	0.7
VR 07	3.1	2.2
Average	2.9	1.45

The results in Table 1 show LRV (log reduction value) obtained for both types of Zeta Plus VR Series filters is lower at higher ionic strength buffer conditions. At higher ionic strength buffer conditions, competition for Zeta Plus VR media adsorption sites increases and as a result, viral log reduction value decreases. These results support a primary retention mechanism of ion exchange adsorption.

Reports have also demonstrated the effectiveness of Zeta Plus VR Series filters in achieving significant viral clearance from blood based protein solutions. At the IBC Second International Symposium on Viral Clearance in June 1998, D. Revie, of Nabi in Boca Raton, Florida, presented the data in Table 3 regarding clearance of several mammalian viruses in a paper titled “Novel Validation Approaches to Obtain Maximum Viral Clearance from an Immunoglobulin Production Process”.

Table 2 — Viral Clearance from a Blood Based Protein Solution

Process Step	Cumulative Virus Titer Reduction (Log ₁₀)				
	BVD	EMC	HIV	PPV	PRV
Solvent Detergent	> 4.3	–	> 5.3	–	> 7.3
Supernatant III	1.4	4.3	6.1	4.7	3.8
Zeta Plus VR 03 Depth Filtration	4.8	4.5	4.7	3.7	5.4
Total Cumulative Reduction	> 10.5	8.8	> 16.1	8.4	> 16.6

The results in Table 2 show viral clearance for a number of process steps. In all cases, the viral log clearance observed with Zeta Plus VR Series Depth filters is significant.

Comparison of Zeta Plus VR Series Adsorptive Depth Filters to Recognized Viral Clearance Options.

The data above support the adsorptive mechanism of viral clearance by Zeta Plus VR Series filters. As stated earlier, the FDA recommends that multiple viral clearance steps, operating by different mechanisms, be employed in biopharmaceutical processes. Table 3 lists several recognized viral clearance steps and their relation to Zeta Plus VR Series filters with respect to mechanism of viral clearance.

Table 3 — Comparison of Zeta Plus Series VR Adsorptive Depth Filters to Alternative Viral Clearance Options

Viral Clearance Option	Mechanism of Clearance	Relationship to Zeta Plus VR Series Filters
Nanofiltration	Size exclusion	Complements
Solvent Detergent	Inactivation	Complements
Pasteurization	Inactivation	Complements
Extreme pH	Inactivation	Complements
Anion Exchanger	Adsorptive	Competes

Table 3 shows that Zeta Plus VR Series filters can be effectively utilized in conjunction with several alternative viral clearance technologies to provide complimentary, multiple step viral clearance.

Testing Zeta Plus VR Series Filters

As shown above, the primary mechanism of viral retention by Zeta Plus VR Series filters is adsorption. In order for adsorptive retention of viruses to be effective, Zeta Plus VR Series filters must possess a positive charge. Zeta Plus VR Series filters are tested and certified on a lot release basis for the presence and magnitude of positive charge. During validation of Zeta Plus VR Series filters, users should verify viral removal is adequate for their process volume. This testing verifies adsorptive sites will not be saturated during use.

In addition to affirming positive charge capacity, Zeta Plus VR Series filters can be tested to ensure the production filter assembly is properly installed and does not provide opportunity for fluid bypass (see LITTDIQ). This test is performed by wetting the filter assembly with an aqueous fluid followed by pressurizing the upstream filter side. An integral Zeta Plus VR filter assembly will not allow the passage of air at the specified test pressure. This test method enables users to verify proper filter installation.

Bench Scale Evaluation of Zeta Plus™ VR Series Filters

Because the mechanism of viral clearance by Zeta Plus VR Series filters is adsorptive, process variables such as pH or buffer ionic strength can affect the level of viral clearance obtained. For this reason it is necessary to first screen Zeta Plus VR filter clearance followed by validation of performance using optimal clearance conditions. Zeta Plus VR Series filters are available in a range of configurations from

13 mm discs to full size cartridges. The most common size for screening and validation studies are Zeta Plus BC 30 capsules containing approximately 30 cm² effective filtration area. The recommended flux for Zeta Plus VR Series filters is 0.25 ml/cm²/min in order to allow sufficient residence time to obtain optimum adsorption. Based on the BC 30 filter area, the recommended flow rate for viral clearance studies is 7.5 ml/min. For process design purposes, the typical throughput for Zeta Plus VR Series filters is >100 l/m². Scaling down to the 27 cm² BC 30 filter area, the throughput required to evaluate viral clearance to the end point of filtration would be approximately 350 ml.

Conclusion and Summary

A number of reports from end users and experiments conducted at CUNO demonstrate that Zeta Plus VR Series filters offer an effective means to provide viral clearance from blood plasma fractions.

The observation that Zeta Plus VR Series filters provide significant virus log removal with a pore structure that is greater than an order of magnitude larger than retained viruses supports an adsorptive mechanism of viral clearance. The experiments demonstrating reduced viral clearance as a function of buffer ionic strength further supports the adsorptive mechanism of viral clearance.

Based on FDA requirements to employ multiple stages of viral clearance operating by different mechanisms in purification processes, Zeta Plus VR Series filters offer a complement to viral clearance steps involving size exclusion filtration and viral inactivation methods. Specifically, Zeta Plus VR Series filters can be used to provide incremental viral log clearance to heat, solvent/detergent, or pH inactivation steps as well as size exclusion nano-filtration membranes. The larger pore size of Zeta Plus VR Series filters ensures adequate process flow rates (> 2.7 lpm/m²) and minimizes product (protein) loss due to mechanical retention. Zeta Plus VR Series filters are convenient to use and validate. Process scale Zeta Plus VR Series filters are designed as single use, integrity testable filters. This eliminates the need for cleaning validation studies and the integrity testable feature provides a means to ensure filter performance following installation and before use. Zeta Plus VR Series filters are available in a variety of configurations for viral clearance evaluation at bench scale. These small area Zeta Plus VR filters are constructed using the same materials as full-scale production Zeta Plus VR filters. This ensures viral clearance validation studies are applicable to full scale production size Zeta Plus VR Series filters.

APPENDIX

LifeASSURE™ PB Cartridge and Capsule Filters are CUNO's latest advance in membrane filter technology. Encompassing two leading-edge processes, FlexN membrane manufacture and MaxMedia pleating construction, the LifeASSURE PB Series of filters offers unmatched protection of final membrane filters, as well as exceptionally long service life. Designed with pleated Nylon 6,6 membrane in an all-polypropylene cartridge construction, LifeASSURE PB filters are ideally suited for a wide range of prefiltration and clarification applications in the pharmaceutical, biological, and bioprocess industries. For more information see CUNO literature LITCLAPB1.

Zetapor™ SP Sterilizing Grade Cartridge and Capsule Membrane Filters — CUNO pioneered the development of charge modified Nylon 6,6 filters for the pharmaceutical industry. Zetapor sterilizing grade filters and capsules are validated for absolute bacteria retention and provide reliable sterile filtration performance. In addition to a fixed bacteria retentive pore structure, Zetapor membrane is charge modified to provide enhanced removal of negatively charged biological contaminants such as endotoxin, virus and nucleic acid fragments. The combination of a validated bacteria retentive membrane, together with enhanced removal of negatively charged contaminants, make Zetapor membrane an ideal choice for pharmaceutical and biopharmaceutical sterilizing applications. For more information see CUNO literature LITCZR020SP

