

CUNO Zeta Plus® VR Filters for Viral Reduction in Biopharmaceutical Processes

Introduction:

The removal and/or inactivation to a high level of assurance of contaminating viruses from biotherapeutics is a requisite for ensuring product safety. Many biotherapeutic products are produced using mammalian cell culture techniques. Contaminating viruses can enter cell culture systems from a number of sources including animal derived nutrient additives, the cell line itself, or through adventitious contamination via human or animal process contact. 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 Application Brief presents:

- The use of CUNO Zeta Plus® VR filters to provide viral clearance from animal derived growth media feedstreams and from downstream process purification steps such as eluate from chromatography columns.
- The principal mechanism of viral clearance operative with CUNO VR filters is electrokinetic adsorption. Because Zeta Plus VR filters function in an ion-exchange like manner, they complement viral clearance steps such as inactivation and size exclusion filtration.
- CUNO Zeta Plus VR filters offer an effective means of prefiltration to membrane based viral retentive filters.



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

The Process:

Bioprocess manufacturing of cell culture derived therapeutic proteins involves upstream steps related to growth of recombinant mammalian cells followed by downstream processing steps including cell broth clarification and purification of the desired therapeutic protein. This process, including typical points of filtration, is illustrated in Figures 1 and 2. The growth of the desired mammalian cell line may require the addition of animal blood based nutrient feedstreams. Typically these growth medium supplements are well controlled by the supplier to be free of viral contaminants, however, the need for additional safety in manufacturing processes is always desirable. CUNO Zeta Plus VR filters offer an easy way to provide addi-

tional viral clearance assurance with a disposable, single use sterilizable filter cartridge. The downstream purification process of cell culture derived therapeutic proteins is illustrated in Figure 2. The purification process involves a number of unit operations including filtration and chromatography. Viral clearance may be provided by nano filtration and chromatographic steps. CUNO Zeta Plus VR filters can be used to complement these steps and to provide prefiltration to nano filtration single pass filter cartridges.

Figure 1. - Upstream Process

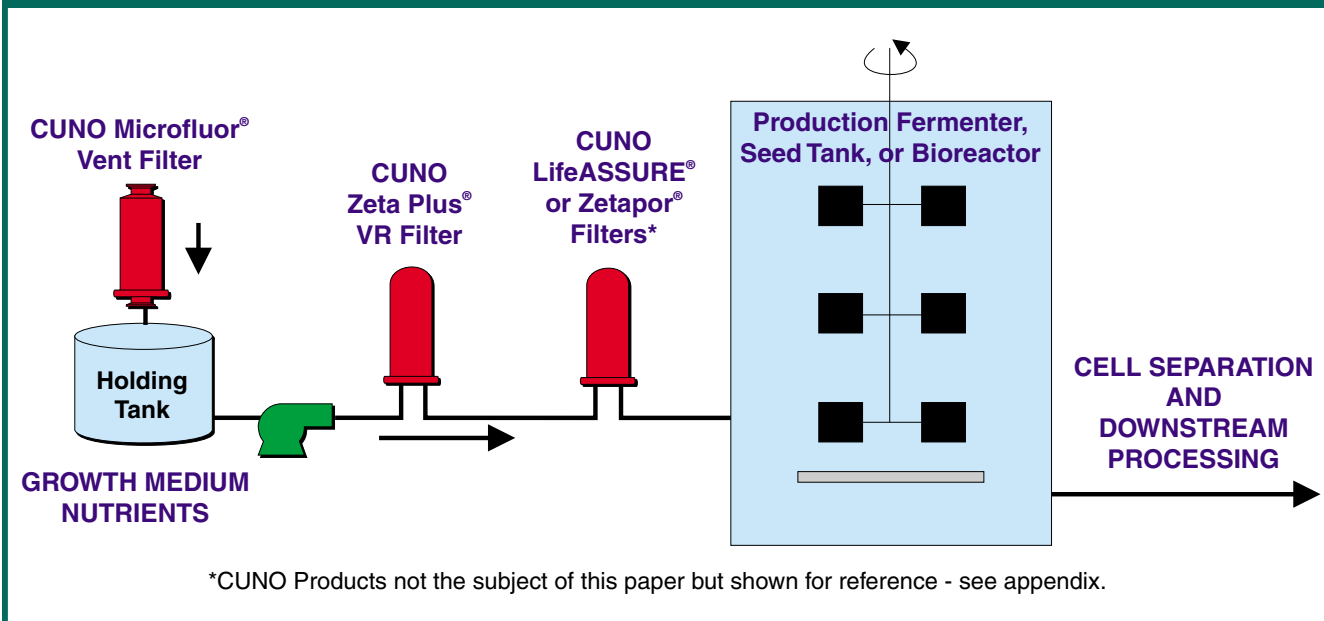
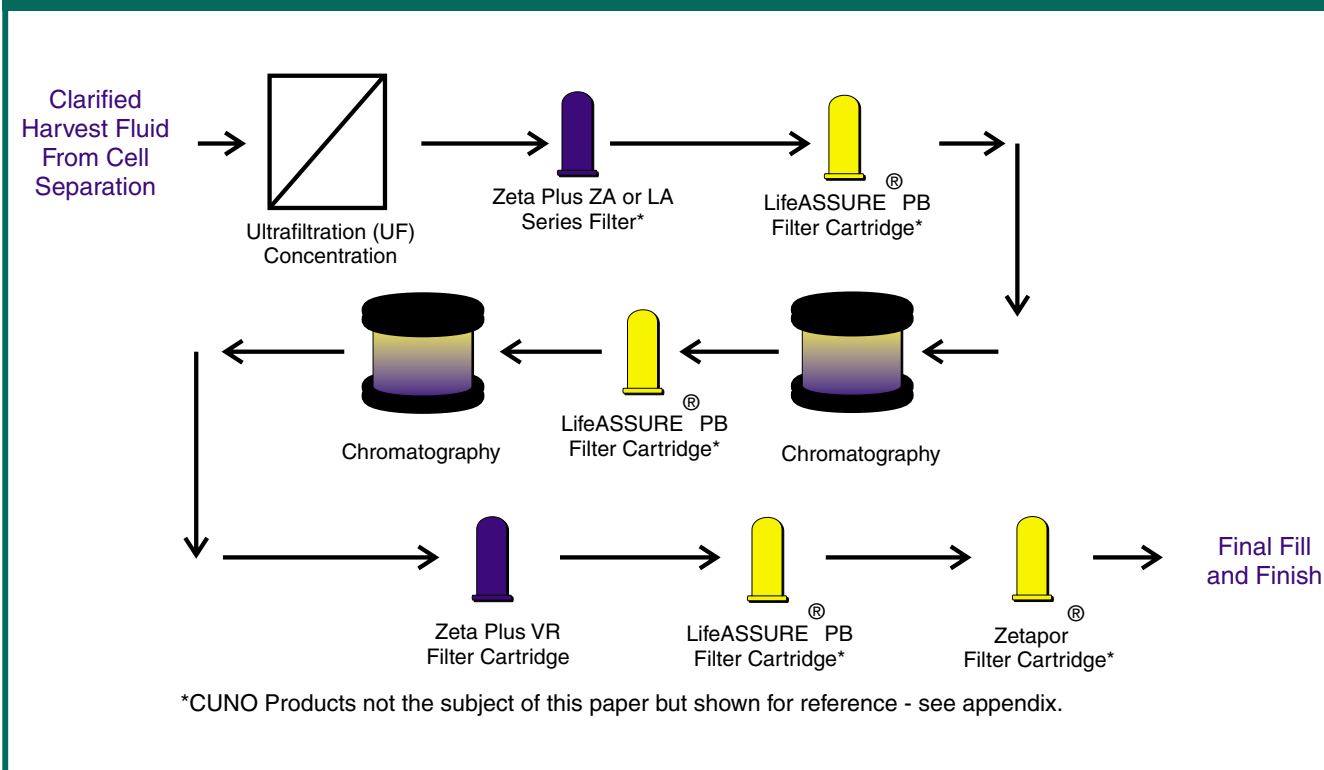


Figure 2. - Downstream Process



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 upstream in cell culture processes to provide additional assurance of virus removal from serum-based growth supplements. For downstream viral clearance, Zeta Plus VR filters can be used at the final stages of purification before sterile filling. In both instances, Zeta Plus VR filters complement size exclusion membrane filters and viral inactivation steps, as the primary mechanism of virus retention by Zeta Plus VR filters is adsorption. The data below support the adsorptive mechanism of virus retention by Zeta Plus VR filters. The data also show viral clearance performance of Zeta Plus VR filters from serum and from downstream purification steps involving an affinity chromatography column eluate sample.

Adsorptive Mechanism of Virus Retention by Zeta Plus VR 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 anion exchange adsorption. The range of nominal pore size for the 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 VR Series depth filters are effective in retaining various mammalian viruses. Because the nominal pore size of 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 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 describes the results obtained with different VR Series filters.

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

In a second series of experiments two types of VR filters, VR05 and VR07, were evaluated for clearance of Xenotropic Murine Leukemia Virus (XMuLV, 90 nm) and Porcine Parvovirus (PPV, 30 nm). These studies were conducted in conjunction with a major US West Coast biopharmaceutical manufacturer and BioReliance. The study involved spiking a Protein A affinity column eluate with the virus samples. The column eluate solution consisted of a partially purified monoclonal antibody solution in pH 5, 20 mM sodium acetate buffer at 20 degrees C. The results are shown in Table 2.

Table 2. Protein A Affinity column Eluate Log ₁₀ Removal Values (LRV)		
Filter Type	Virus Type	
	XMuLV	PPV
VR05	> 4.8 +/- 0.4	0.9- 1.1 +/- 0.8
VR07	> 4.8 +/- 0.4	1.4- 2.0 +/- 0.5

The results in Table 2 demonstrate a higher clearance value for Murine Leukemia virus as compared to Porcine Parvovirus. Despite the lower level of Porcine Parvovirus clearance, the fact that both virus types are significantly smaller in diameter than the typical pore size of the VR filters supports their removal by an adsorptive rather than size exclusion mechanism.

Reports have also demonstrated the effectiveness of CUNO 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, Dan 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 3. 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 VR03 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 3 show viral clearance for a number of process steps. In all cases, the viral log clearance observed with CUNO VR Series filters is significant. The ability to provide viral clearance from blood based solutions supports the use of Cuno VR Series filters for effective viral clearance from serum based, cell culture nutrient feed streams.

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

The data above support the adsorptive mechanism of viral clearance by Zeta Plus VR filters. As stated earlier the FDA recommends that multiple viral clearance steps, operating by different mechanisms, be employed in biopharmaceutical processes. Table 4 lists several recognized viral clearance steps and their relation to VR Series filters with respect to mechanism of viral clearance.

Table 4. Comparison of Zeta Plus VR Adsorptive Depth Filters to Alternative Viral Clearance Options		
Viral Clearance Option	Mechanism of Clearance	Relationship to VR Series Filters
Nanofiltration	Size exclusion	Complements
Solvent Detergent	Inactivation	Complements
Pasteurization	Inactivation	Complements
Extreme pH	Inactivation	Complements
Anion Exchanger	Adsorptive	Competes

Table 4 shows that VR Series filters can be effectively utilized in conjunction with several alternative viral clearance technologies to provide complimentary or multiple step viral clearance.

Testing Cuno VR Series Filters

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

In addition to affirming positive charge capacity, CUNO VR Series filters can be tested to insure the production filter assembly is properly installed and does not provide opportunity for fluid bypass. This test is performed by wetting the filter assembly with an aqueous fluid followed by pressurizing the upstream filter side. An integral 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 VR filter clearance followed by validation of performance using optimal clearance conditions. 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 Biocap™ 30 capsules containing approximately 30 cm² effective filtration area. The recommended flux for VR Series filters is 0.25 ml/cm²/min in order to allow sufficient residence time to obtain optimum adsorption. Based on the Biocap 30 filter area, the recommended flow rate for viral clearance studies is 7.5 ml/min. For process design purposes, the typical throughput for VR Series filters is >100 L/M². Scaling down to the 30 cm² Biocap 30 filter area, the throughput required to evaluate viral clearance to the endpoint of filtration would be approximately 350 ml.

Conclusion and Summary:

A number of field reports and experiments conducted at CUNO demonstrate that Cuno Zeta Plus VR Series filters offer an effective means to provide viral clearance. Virus retention has been observed with blood based solutions and with highly purified mammalian cell culture derived process fluids. These results support the use of Cuno Zeta Plus VR Series filters in upstream applications such as mammalian cell culture nutrient feed streams and in downstream purification streams following chromatography or prior to nanofiltration steps.

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 absorptive 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 nanofiltration membranes. The larger pore size of Zeta Plus VR Series filters ensures adequate process flow rates ($> 2.7 \text{ LPM/M}^2$) and minimizes product (protein) loss due to mechanical retention

VR Series filters are convenient to use and validate. Process scale 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 validate filter performance following installation. VR Series filters are available in a variety of configurations for viral clearance evaluation at bench scale. These small area 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 filters.

<i>Zeta Plus VR Series Reference Literature</i>	
Reference Description	Literature No.
Zeta Plus VR Filter Cartridges	LITZPVR
Zeta Plus VR Regulatory Support File	LITTDRSFVR
Zeta Plus BioCap Filter Capsules	LITZPBC
Zeta Plus ZPC & ZPB Sanitary Filter Housings	LITHS.ZPBC
Zeta Plus 8ZP1P and 12ZP1P Sanitary Filter Housings	LITZPH1P
<i>Other CUNO Reference Literature</i>	
LifeASSURE PB Filter Cartridges	LITCLAPB1
Mini Cartridge Housings	LITZRHMCH
Zetapor SP Sterilizing Grade Filter Cartridges and Capsules	LITCZR020SP
Zeta Plus Low Aluminum Series Media and Cartridges	LITZPLA2

Scientific Applications Support Services

The cornerstone of CUNO's philosophy is service to customers, not only in product quality and prompt service, but also in problem solving, application support and in the sharing of scientific information. CUNO's **Scientific Applications Support Services (SASS)** group is a market-oriented group of scientists and engineers who work closely with customers to solve difficult separation problems and aid in the selection of the most effective and economical filtration systems. CUNO offers specialized support to the pharmaceutical and biotechnology industry through our **Validation Support Services Program**. SASS routinely provides end-users with:



- Validation And Regulatory Support
- Extractable And Compatibility Analysis
- Filter System Optimization Studies
- CUNOCheck® 2 Integrity Tester Validation

For more information regarding CUNO's Validation Support Services, please contact CUNO Technical Services or your local CUNO Distributor.

APPENDIX



Zeta Plus® CA & SA & LA Series Filter Media development was prompted by the growing concern for metallic extractables, especially aluminum in parenteral solutions and infant food products. This premium grade media has been specifically designed for processes that require a high degree of filtration and low aluminum extractables. It is ideally suited for parenteral, blood fraction, dextrose, dialysis solutions and infant food/formula applications. The Zeta Plus CA & SA & LA Series are completely free of glass microfibers and asbestos, and are constructed from materials listed in CFR 21 "Food and Drugs." Stringent quality controls ensure maximum product performance for each production lot. For more information see CUNO literature LITZPLA2.



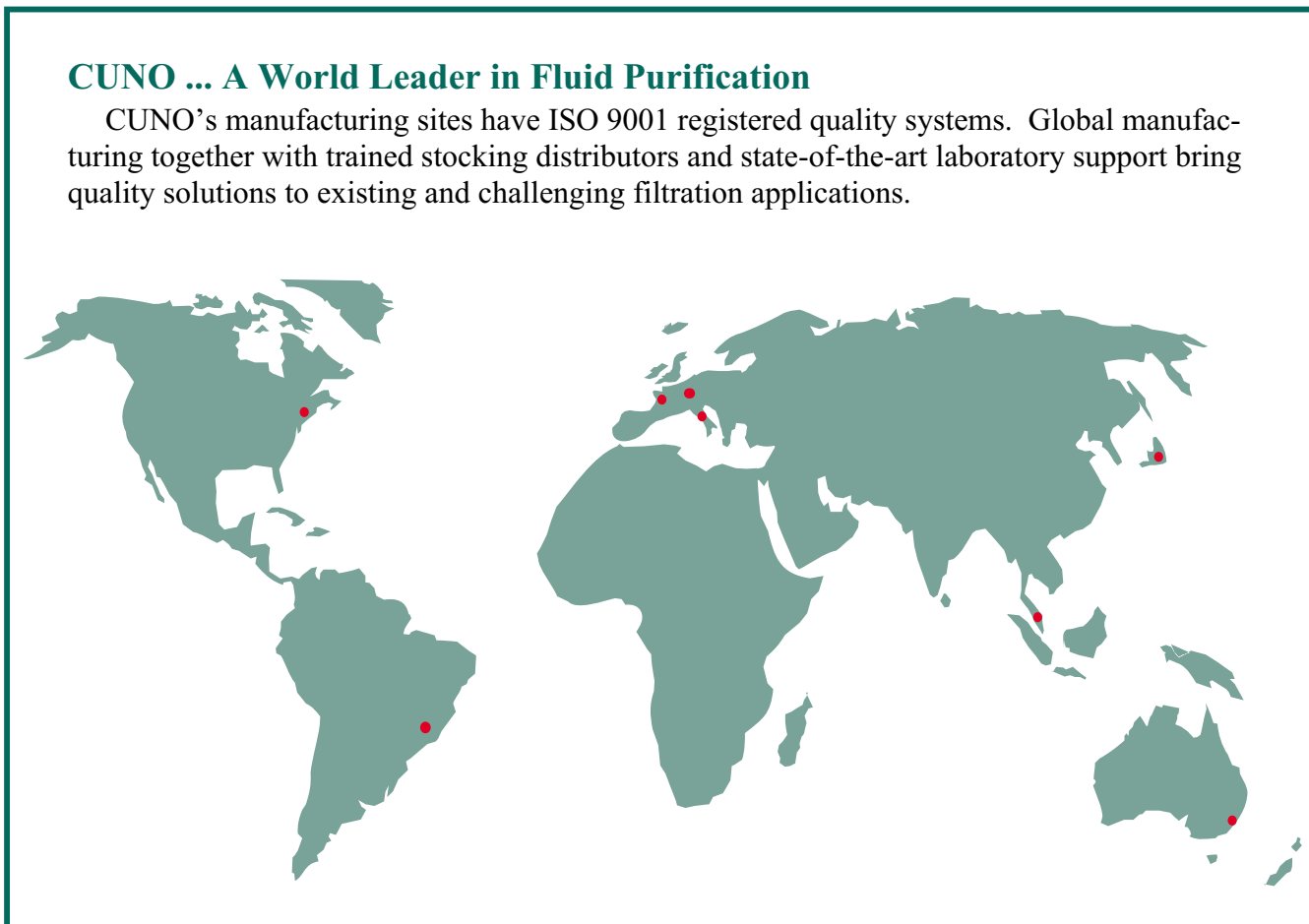
LifeASSURE® PB Cartridge and Capsule Filters are CUNO's latest advance in membrane filter technology. Encompassing two leading-edge processes, FlexN™ membrane manufacture and MaxMedia™ pleating construction, the LifeASSURE PB series of filters offers unmatched protection of final membrane filters, as well as exceptionally long service life. Designed with pleated Nylon 66 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

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