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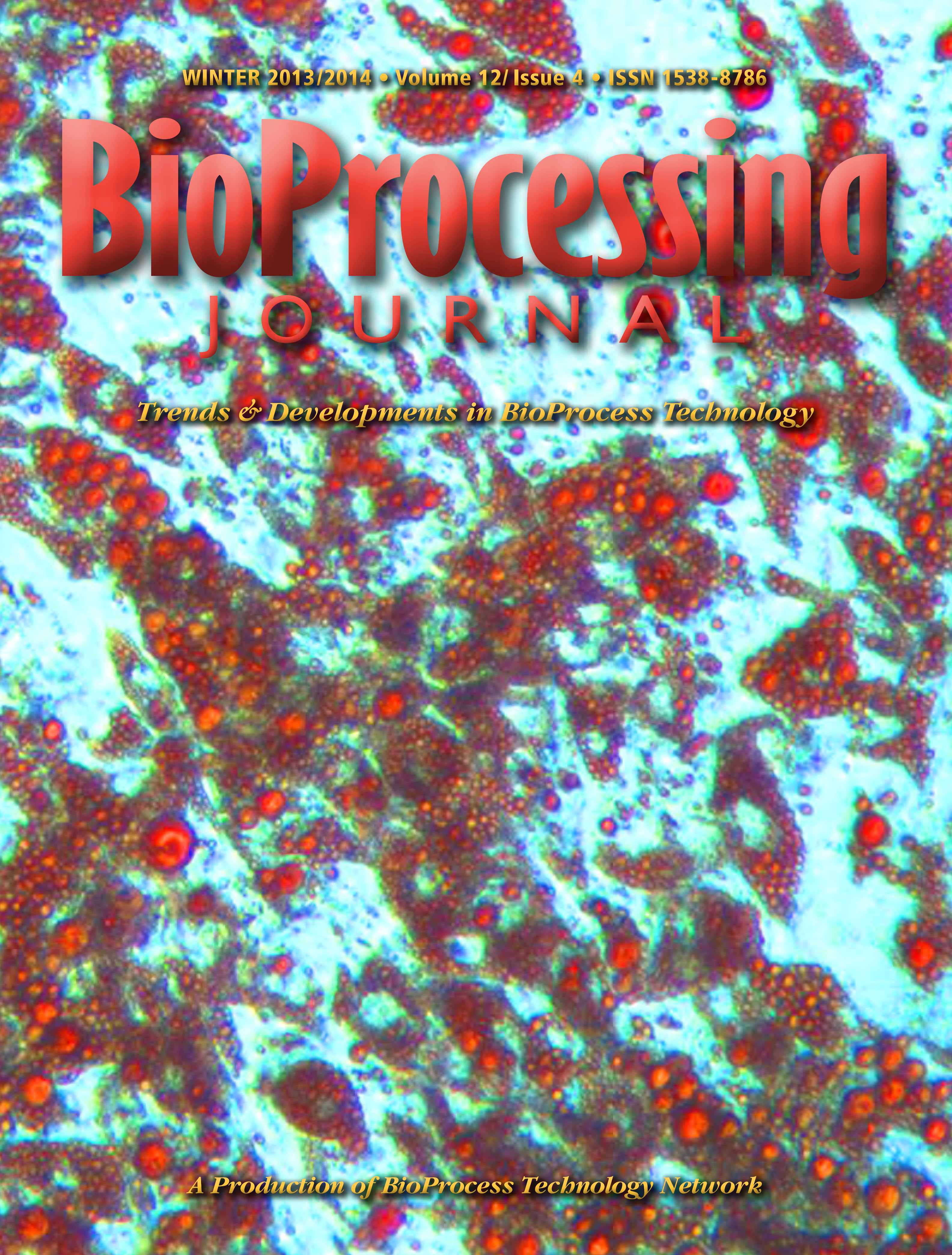
Trends & Developments in BioProcess Technology



A Production of BioProcess Technology Network

BioProcessing JOURNAL
TRENDS & DEVELOPMENTS IN BIOPROCESS TECHNOLOGY
SUPPLIER SIDE

Virus Clearance in Your Process from Start to Finish

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Introduction

The safety of biological products (biologics) derived from *in vitro* cultured cell lines of animal origin is dependent both on clinical studies to evaluate efficacy, and a matrix of controls throughout the manufacturing process to assure consistency, quality, and safety of the marketed product for human use. One major area of concern is virus safety. Through the combination of: (A) careful selection of raw materials; (B) testing of process intermediates; and (C) virus clearance evaluations of the individual steps in the manufacturing process; biologics manufacturers can demonstrate that their products are free from detectable adventitious and endogenous viruses.

Comprehensive regulatory guidance suggests approaches for virus testing of biologics at early and later stages of clinical development and, although some countries have specific requirements, many of these approaches are harmonized worldwide.[1-5] In general, regulatory authorities expect purification processes to include multiple steps with complementary, or orthogonal, methods for virus reduction including inactivation and separation or removal. Each step in the process may be more or less effective for reducing levels of different test viruses, and therefore selection of the appropriate panel of test viruses for the specific product is critical for viral clearance studies.

The purification steps in each manufacturing process and the operation conditions of these steps are dependent on both the molecule and the preferences of each individual manufacturer. The capabilities of a few of these chromatography and filtration products are illustrated with monoclonal antibody (MAb) material and a generalized purification process with two viruses commonly used to evaluate MAb clearance: minute virus of mice (MVM) and murine leukemia virus (MuLV), Table 1.

Protein A chromatography is often the primary unit of a process designed specifically to capture the MAb product and separate it from impurities such as host cell proteins, nucleic acids, and cell culture components, which do not bind to the media. Virus reduction across protein A media was evaluated using a cell harvest that had been clarified using depth filters and spiked with purified MAb 05 to a final concentration of 1.0 g/L. This study evaluated two resin-based affinity media, ProSep® Ultra Plus ([PUP] EMD Millipore) which has a high dynamic binding capability, and Eshmuno® A (EMD Millipore), a high-capacity, acid and alkaline-resistant media. Each was tested in duplicate with MVM and MuLV. Columns containing the protein A media were approximately 1 cm in diameter with 5 cm bed height, and were sanitized and regenerated between runs. Following loading of the spiked clarified cell harvest, the columns were washed and the bound product was eluted with

TABLE 1. Characteristics of MVM and MuLV.

	MVM	MuLV
Family	*Parvoviridae*	*Retroviridae*
Size	~18–24 nm	~80–110 nm
Physico-Chemical Resistance	High	Low
Lipid Envelope	Non-enveloped	Enveloped
Category	Relevant virus	Specific model

a 0.1 M citric acid, 20 mM NaCl, pH 3.5 buffer, and then protein-containing fractions were assayed for the presence of virus by infectivity assays. The low pH conditions of the elution can cause inactivation of MuLV, so quantitative real-time polymerase chain reaction (qPCR) was used to measure virus removal. Overall loading of 22 g/L was achieved, which resulted in approximately 90% protein yield. Virus reduction results are presented in Table 2.

Both Eshmuno A and PUP were moderately effective in reducing levels of both parvovirus and retrovirus: MVM clearance was approximately 3 logs and MuLV removal was approximately 3.5 logs.

Low pH is commonly included after protein A affinity chromatography for inactivation of enveloped viruses. It would not be expected to inactivate parvoviruses such as MVM. Individual processes differ in the pH, buffer composition, temperature, and time of this step and, as with other inactivation methods, careful analysis of the kinetics of inactivation under the selected process conditions should be performed for each virus being tested. Table 3 shows low pH inactivation of MuLV in 3 g/L MAb G post-cation exchange pool at pH 3.8 over a 30-minute period.

Under these conditions, effective retrovirus inactivation was observed in MAb G within two minutes of pH reduction. For many processes, inactivation makes an important contribution to process clearance targets. However, the levels of virus inactivation under any one condition will be dependent on conditions and the virus being tested. If virus inactivation by low pH is evaluated as an individual process step, care should also be taken to differentiate reduction due to inactivation at other process steps which might use similar conditions.

TABLE 2. Log reduction values (LRV) achieved with protein A chromatography media.

Media	MVM LRV (Infectivity)	MuLV LRV (qPCR)
Eshmuno A	2.9	3.7
	3.4	3.8
Prosep Ultra Plus	3.2	3.4
	3.2	3.4

TABLE 3. LRV achieved with low pH inactivation of MuLV (1% v/v spike).

Time (minutes)	LRV
2	5.3
5	5.6
10	≥ 5.3
30	≥ 5.3

Cation exchange chromatography (CEX) is often performed as a bind-elute step downstream of protein A and is implemented to reduce not only the levels of host cell protein and nucleic acid impurities, but also product aggregates. The position of CEX in a process is flexible as it can be either before or after anion exchange chromatography (AEX), and in some processes, the purification benefits are not needed and the step is omitted. Although not generally considered a process step that robustly delivers high levels of virus clearance, CEX can reduce virus levels, and can be a useful component in a process virus clearance plan.

Eshmuno HCX is a multi-mode CEX resin which works well at elevated conductivities. Virus reduction across HCX was evaluated in direct capture mode using a clarified cell harvest that had been spiked with purified MAb 05 to a final concentration of 1.5 g/L, 11.3 mS/cm, pH 6.0. Columns containing the HCX resin were approximately 0.8 cm in diameter with 10 cm bed height, and duplicate columns were evaluated with MVM and MuLV. Following loading of the spiked clarified cell harvest, the columns were washed and the bound product was eluted with a step gradient of 20 mM phosphate buffer containing 1 M NaCl, pH 7.0, and protein-containing fractions were assayed for the presence of virus by infectivity assays. Overall loading of 19 g/L was achieved and the virus reduction results are shown in Table 4. Eshmuno HCX resin removed less than 1 log MVM and should be considered an ineffective step. However, levels of MuLV reduction were consistently higher at approximately 2 logs.

Eshmuno CPX is a strong cation exchange resin designed to reduce levels of protein aggregates. Virus reduction across CPX was evaluated using a post-protein A pool of MAb 05 (11.5 g/L, 8.0 mS/cm, pH 4.7). Columns containing the CPX resin were approximately 0.8 cm in diameter with 10 cm bed height, and duplicate columns were evaluated with MVM and MuLV. Following loading of the spiked post-protein A pool, the columns were washed and the bound product was eluted with a step gradient of 50 mM sodium acetate buffer containing 1 M

TABLE 4. LRV achieved with Eshmuno HCX and CPX resin.

Resin	Process Pool	MVM LRV	MuLV LRV
Eshmuno HCX	MAb 05 clarified cell harvest	0.9	2.5
		0.8	2.0
Eshmuno CPX	MAb 05 post-protein A pool	0.4	≥ 4.1
		0.2	≥ 4.1

NaCl, pH 4.7. Protein-containing fractions were assayed for the presence of virus by infectivity assays. Using this procedure, loading of 40 g/L was achieved. Virus reduction results are presented in Table 4.

Eshmuno CPX, like the HCX, resulted in less than 1 log reduction in MVM and should be considered an ineffective step at reducing parvovirus levels. However, unlike Eshmuno CPX, no retrovirus was detected in the CPX elution pool indicating complete retrovirus clearance using this resin. Therefore, under these conditions with MAb 05 feed, Eshmuno CPX could be considered as an effective contributor to retrovirus clearance targets.

Anion exchange chromatography: Eshmuno Q (EMD Millipore) is a strong anion exchange resin which can be used with monoclonal antibody process streams in flow-through mode as a polishing step to remove trace impurities. Eshmuno Q resin demonstrates high dynamic binding capacity under a broad range of conditions. In binding mode, it can be used with high selectivity for separating blood factors in plasma processing. Unlike conventional anion exchange resins, Eshmuno Q resin is intrinsically stable against alkaline solutions used in column sanitization.

Virus reduction across Eshmuno Q resin was evaluated in flow-through mode using a MAb (5 g/L, 2.9 mS/cm, pH 7.5). Columns of ~8 mL (100*10 mm ID) were evaluated with MuLV and PPV†. Effluent fractions were assayed for the presence of virus by infectivity assays and the results are summarized in Table 5.

AEX operated in the flow-through mode resulted in at least 4 logs of virus removal and could be considered as an effective contributor to both parvovirus and retrovirus clearance. However, as with any chromatography operation, the run conditions for each molecule should be carefully assessed in order to understand the process parameters that impact product purity and virus removal.

Nanofiltration is included in most biologics manufacturing templates and is the only dedicated virus removal unit operation capable of removing both enveloped and non-enveloped viruses. Viresolve® Pro (EMD Millipore) devices are caustic, stable, small-virus filters that operate under a broad range of process conditions to remove virus in an operating time of four hours or less. Nanofiltration is considered one of the most robust process steps for virus removal and is generally evaluated as part of every process virus clearance study. Virus is removed based on size—the pores in the membrane allow passage of protein containing feeds but prevent passage of viruses larger than ~20 nm. Preparation for a virus clearance filtration study is important to assure the feed conditions are representative of the at-scale process. Seemingly small changes in feed, which are a consequence of shipping, storage, and handling can jeopardize the performance of the filtration device in the virus study resulting in high virus retention but sub-optimal volumetric throughput.

Virus reduction across Viresolve Pro micro devices was evaluated with purified MAb G at a concentration of 10 g/L, pH 5.0, under constant pressure at 30 psi. Titer assays of filtrate pools at a loading of 8 kg/m^2 revealed no detectable MVM or MuLV resulting in LRVs of at least 5 logs, Table 6.

Figure 1 shows that similar loading was achieved in the spiked and unspiked (baseline) runs, indicating that the spiking did not perturb the scale-down model, a critical component of any virus filtration study. Overall, these studies with MAb G illustrate that effective virus removal and high loading can be achieved using Viresolve Pro devices, confirming a high level of safety assurance to a process.

TABLE 5. LRV achieved with Eshmuno Q resin (1% v/v spike).

Resin	PPV LRV	MuLV LRV
Eshmuno Q	6.6	≥ 5.0
	≥ 5.7	≥ 4.7

TABLE 6. LRV across Viresolve Pro micro devices.

Devices	MVM LRV	MuLV LRV
Viresolve Pro Micro	≥ 5.0	≥ 5.3
	≥ 5.0	≥ 5.3

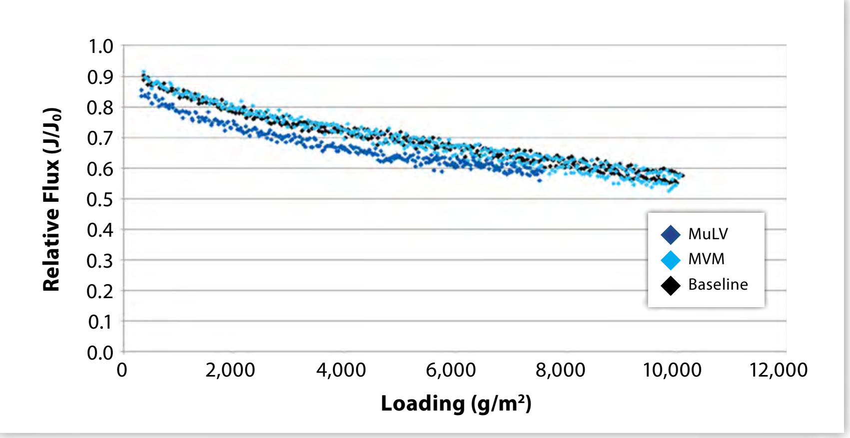

FIGURE 1. Volumetric throughput of spiked MAb G on Viresolve Pro micro devices. Purified MAb feed (10 g/L) with 10^6 TCID$_{50}$/mL MVM and 10^5 TCID$_{50}$/mL MuLV.

†PPV is similar to MVM and is approximately the same size and has similar physico-chemical characteristics.

Summary

EMD Millipore has over 20 years of experience in all aspects of the biologics manufacturing process and has developed a portfolio of products that can help purify an array of different molecules. Importantly, these products are offered at multiple sizes to facilitate scale-up from bench to large-scale manufacturing. Some products, such as the virus nanofilter, are dedicated to virus removal whereas others, such as the various chromatography media, purify the product but also have the potential to reduce virus levels. These purification tools separate the biologic from potential virus impurities using complementary methods based on specific ligands, charge, or size. Small-scale, well-designed, replicated studies with a panel of viruses encompassing a range of physicochemical properties are crucial to understanding the virus removal capabilities of each step in the manufacturing process. The overall process reduction is the sum of the individual step reduction factors and for retrovirus generally exceeds 15 $\log_{10}$. Our experience and array of product offerings can help your process development to reach both purification targets and the levels of virus reduction needed to assure regulatory approval.

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