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mAbs Viral Clearance Study

Are Continuous Processes as Effective as Batch?

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Abstract

Growing demands for increased yields, higher throughputs, and reduced costs are pushing biopharmaceutical manufacturers to improve the efficiency of their processes in order to remain competitive. However, productivity improvements must not be made at the expense of product quality and protection against known and unknown viruses.

This application note describes how LFB Biotechnologies used the Resolute® BioSC System to conduct viral clearance studies and demonstrate that continuous (or intensified) processes are as effective as conventional batch approaches in their contribution to mAb downstream viral clearance. The study showed that the viral clearance performance of Protein A chromatography was as efficient in an SMCC process as in an equivalent batch setup. It also showed that viral distribution remains similar over the purification columns and cycles.

Introduction

Viral clearance plays a major role in ensuring product safety and quality and has long been a compulsory and critical element in biopharmaceutical clinical trial applications and marketing authorization submissions.

To keep up with growing demand, particularly in monoclonal antibody (mAb) applications, biopharmaceutical manufacturers are looking for new ways to boost productivity and resolve downstream processing bottlenecks. Improvements might involve integrating continuous chromatography techniques to replace legacy batch processes, which have limitations in efficiency, cost-effectiveness, and flexibility.

However, it is important to ensure that efficiency improvements are not made at the expense of product quality and protection against known and unknown viruses. When introducing a new system or process into their production lines, producers must show that it is safe and robust, that all product quality attributes are preserved, and that they have put all legally required risk mitigation measures in place, especially virus inactivation and removal processes.

Although virus clearance is typically carried out by dedicated removal or inactivation steps, other processes can also play an important role. Although typically envisaged as an initial mAb capture step in downstream processes, Protein A chromatography can also contribute significantly to viral clearance (viruses flow through columns along with other impurities, while target mAbs are captured by ligands in the affinity resin).

LFB Biotechnologies conducted a small-scale virus-spiking experiment to investigate whether the mode (batch versus continuous) has any impact on its viral clearance efficiency. [1] To assess viral clearance performance in continuous mode, LFB used Resolute® BioSC, operating the sequential multi-column chromatography (SMCC) process, a continuous capture process enabling footprint, volume, and COGs reduction.

Materials and Methods

Overview

The experiment conducted by LFB Technologies aimed to investigate the impact of the way the Protein A chromatography step is carried out (i.e., batch or multi-column) on viral clearance efficiency. Overall, the experimental methodology was typical of a viral clearance study: a model virus is deliberately injected into a sample and run through a scaled-down model of the studied process. The ability of the modeled process to clear the virus is then analyzed and documented.

Materials

- The starting material was a filtered harvested cell culture fluid (HCCF) from a mAb process. This material was virus-spiked at a 1% v/v ratio. The resulting material was passed through a 0.1 µm filter to remove potential virus aggregates before being loaded into Protein A packed columns.
- The selected virus was the murine minute virus (MMV). This small, non-enveloped parvovirus was chosen "because it serves as a model of potential contaminants of rodent-derived biopharmaceuticals and due to its very high resistance to physical or chemical treatments." [1]

Columns were packed with Protein A resin.

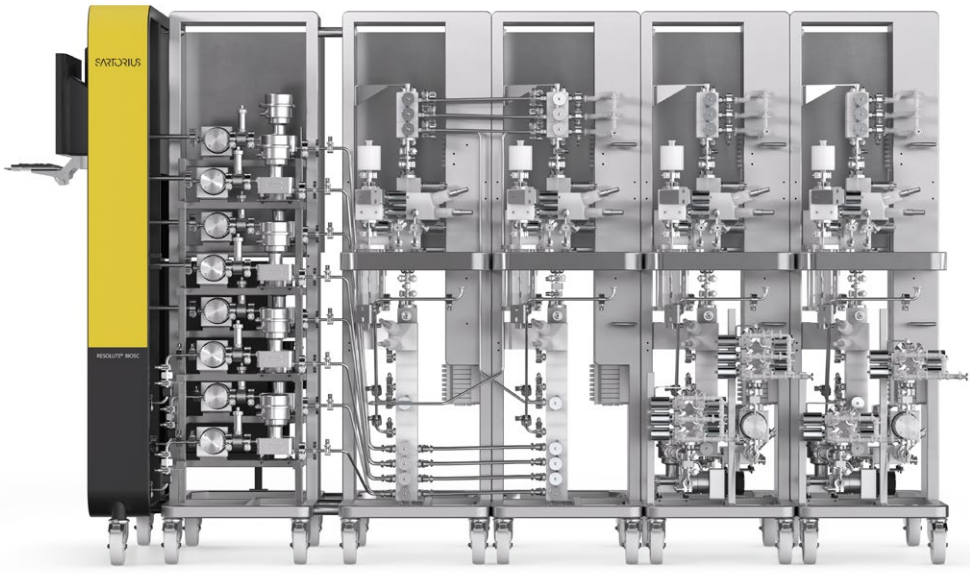

Figure 1: *Resolute® BioSC Pilot Enabling Multi-Column and Connected Processes*

Small-Scale Models

A small-scale model of the Protein A chromatography step was built for both continuous and batch designs. The Resolute® BioSC System was used to perform an SMCC process with a bind | elute capture step, using a 4-column setup running 7 purification cycles. The batch study used one small-scale column, running one purification cycle.

The "wash," which contains mAbs not fixed on the previously loaded column, is channeled towards the next column, thus resulting in minimal mAbs losses without requiring a dedicated column to recover this flux. Figure 2 describes the system designed as a small-scale model for the continuous SMCC process based around Resolute® BioSC.

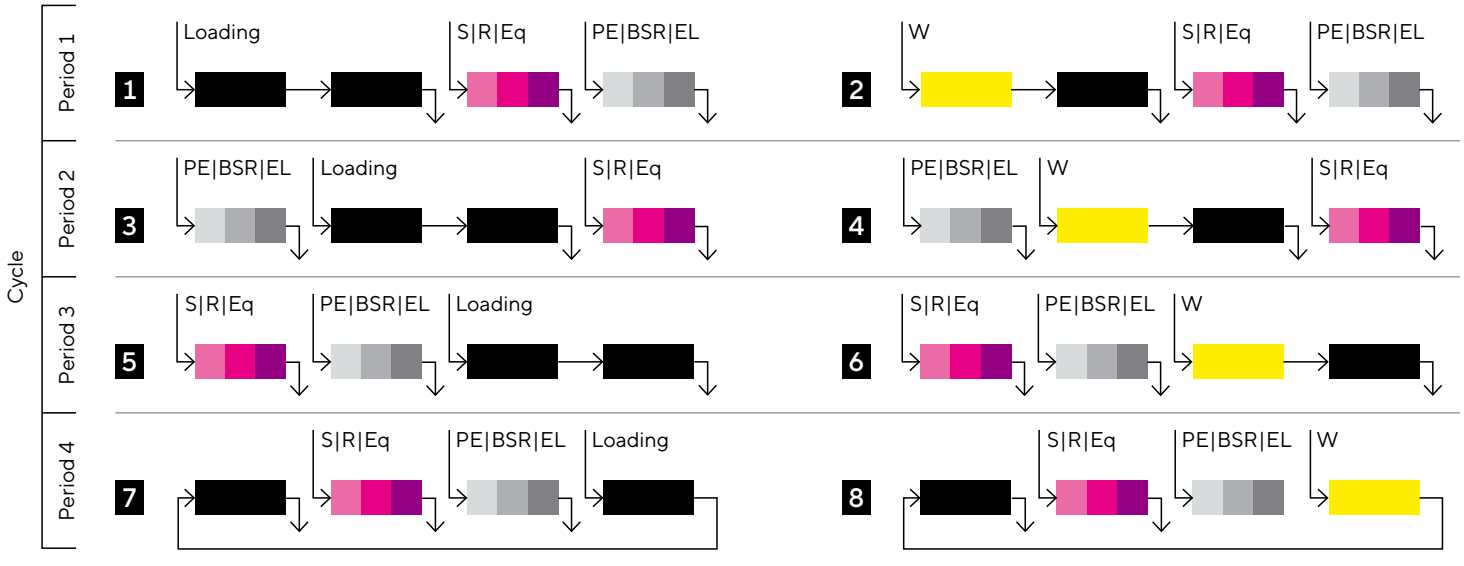

Figure 2: *Purification in SMCC-Mode With Four Columns*
(L = Loading, W = Wash, PE = Pre-Elution, BSR = Baseline Return, EL = Elution, S = Sanitisation, R = Regeneration, EQ = Equilibration)

Method

Both batch and continuous processes were first run without virus to asses how representative they are in terms of mAb yield and host cell protein (HCP) removal. For the SMCC process, loading was increased by about 30% compared with the batch process (39 g mAb/L of resin for SMCC versus 30 g mAb/L for batch).

Two virus-spiking experiments were then conducted for each mode. Different column load values were used for the SMCC process: first 32 g mAb/L of resin, then 42 g mAb/L. For the batch process, 30 g mAb/L was used in both runs. Contact times used during the chromatography step were first defined for the batch process and then directly applied to the continuous design (taking into account specificities of the SMCC design, notably regarding pre-elution contact times).

After loading the filtered spiked material, the distribution of virus infectivity was evaluated in the various collected fractions. This was important to fully comprehend the mechanics ofviral clearance in both approaches. The following fractions were collected for virus titration:

- Batch Process:
  Unbound – Pre-Elution – Before Elution (before UV rise) – Elution – Regeneration 1 – Sanitisation – Regeneration 2
- Continuous (SMCC) Process:
  Unbound – Pre-Elution – Elution – Sanitisation | Regeneration

Table 1 provides a comprehensive overview of the operating conditions of this small-scale experiment.

Table 1: *Overview of the Operating Conditions in The Small-Scale Experiment*

			Virus-spiking experiments at small scale	
			SMCC	**Batch**
	Column volume (CV)	**(mL)**	**3.8**	**8.0**
Equilibration	Volume of equilibration buffer	(CV)	>5	>5
	Residence time	(min)	1.2	1.2
Column load	Column load	(g MAb/L of resin)	30–40	30
	Residence time	(min)	1.2	1.2
Wash	Volume of equilibration buffer	(CV)	5	N|A
Baseline return	Volume of equilibration buffer	(CV)	N|A	5
	Residence time	(min)	1.2	1.5
Pre-Elution	Volume of pre-elution buffer	(CV)	5	5
	Residence time	(min)	4.8	1.2
Baseline return	Volume of equilibration buffer	(CV)	5	5
	Residence time	(min)	4.8	4.8
Elution	Volume of elution buffer	(CV)	8	>3
	Residence time	(min)	4.8	4.8
Regeneration and sanitization	Volume of water	(CV)	ND	2
	Residence time	(min)	ND	4.8
	Volume of 2M NaCl solution	(CV)	ND	5
	Residence time	(min)	ND	4.8
	Volume of water	(CV)	ND	2
	Residence time	(min)	ND	3.9
	Volume of 0.5 M NaOH solution	(CV)	5	5
	Residence time	(min)	3.8	3.9
	Contact time (on hold)	(min)	ND	30
	Volume of water	(CV)	ND	5
	Residence time	(min)	ND	3.9
	Volume of 2 M NaCl solution	(CV)	5	5
	Residence time	(min)	3.8	3.9

(N|A = Not Applicable. ND = Not Done)

The distribution of MMV infectivity was measured for columns 1 and 3 and in the three SMCC phases: “Start of Production” (SoP), “Steady State,” and “End of Production” (EoP).

Reduction Factor (RF) was calculated as the ratio of virus quantity in the load sample over quantity in the fraction collected after the step (expressed in $\log_{10}$).

Table 2 contains a more detailed breakdown of the collected fractions for the SMCC cycles..

Table 2: *Collected Fractions During Virus-Spiking Experiments by Continuous Multi-Column Protein A Chromatography Step*

				Steady State																														
		SoP		Cycle 1				Cycle 2				Cycle 3				Cycle 4				Cycle 5				Cycle 6				Cycle 7				EoP		**SoP, Steady State & EoP**
Column		1*	2	3	4	1*	2	3	4	1*	2	3	4	1*	2	3	4	1*	2	3	4	1*	2	3	4	1*	2	3	4	1*	2	3	4	1 to 4
Collected fract	Unbound	✓		✓		✓		✓		✓										✓		✓				✓				✓		✓		✓(pool)
	Pre-Elution	✓		✓		✓		✓		✓										✓		✓				✓				✓		✓		✓(pool)
	Elution	✓	■	✓	■	✓	■	✓	■	✓	■	■	■	■	■	■	■	■	■	✓	■	✓	■	■	■	✓	■	■	■	✓	■	✓	✓	✓(pool)
	Sanitisation| Regeneration	✓		✓		✓		✓		✓										✓		✓				✓				✓		✓		✓(pool)

* denotes column association with UV dedector
✓ denotes collection and virus titration of the sample
■ denotes collection of the sample

SoP = Start of Production
EoP = End of Production

Results

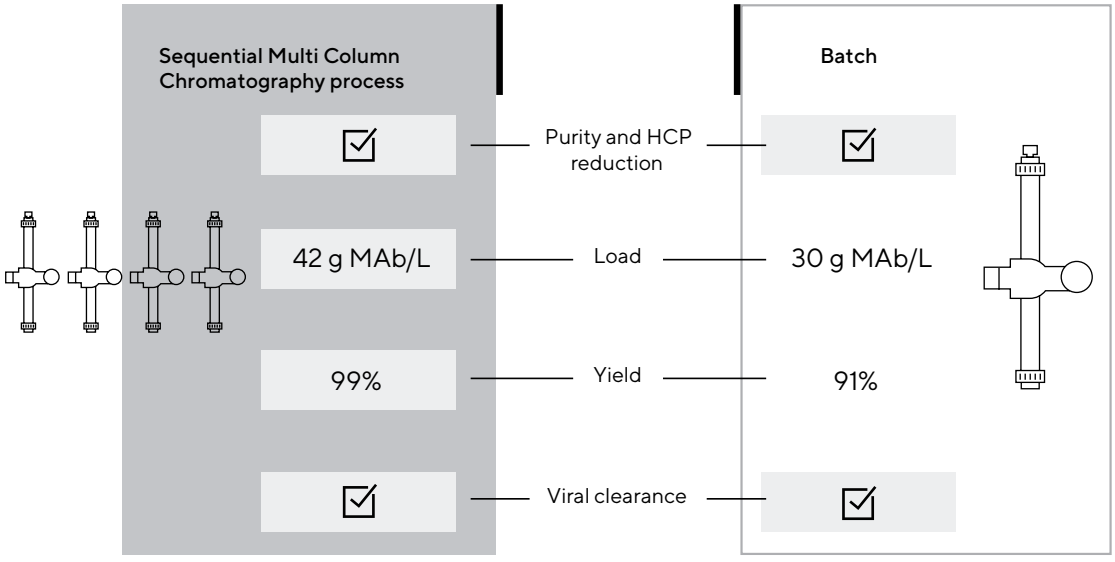

Figure 3: *Summary of the Main Findings of the Study.*

Both processes display the same efficiency in resolving viral clearance-related issues. However, SMCC offers a significantly higher yield; 99% for SMCC vs. 91% for batch (Table 3). SMCC is widely recognized for its significant productivity benefits -for more details on the subject, please visit the Sartorius website.

Small-Scale Model Qualification

Both small-scale models were first loaded without virus to confirm their representativeness. During this qualification phase, the Resolute® BioSC System | SMCC process achieved a global yield of 99%, which was better than the 91% obtained in batch mode despite a 30% increase in column load (39 g mAb/L of resin for SMCC, 30 g mAb/L for a batch), with an HCP reduction of 3.3 $\log_{10}$.

This finding clearly qualifies Resolute® BioSC as a highly effective tool for mAb purification studies.

Table 3: *Performance of Batch vs. Continuous Processes*

	SMCC study design (n = 1)(a)	Batch study design (mean ± SD (n = 3))
mAb yield (%)	99	91 ± 3
HCP reduction ($\log_{10}$)	3.3	4.4 ± 0.1

(a) Pool of all collected eluate fractions (i.e. from start to end of production and from the 4 columns).

Overall Viral Reduction and Partition

SMCC and batch processes produced similar results in terms of viral clearance and partition. Virus reduction factors (RFs) were close to 4 $\log_{10}$ in both cases, and the majority of viral content recovered in the unbound fractions.

Figure 4 shows RFs in the final pool of purification cycles for the SMCC setup and each collected fraction from the batch setup.

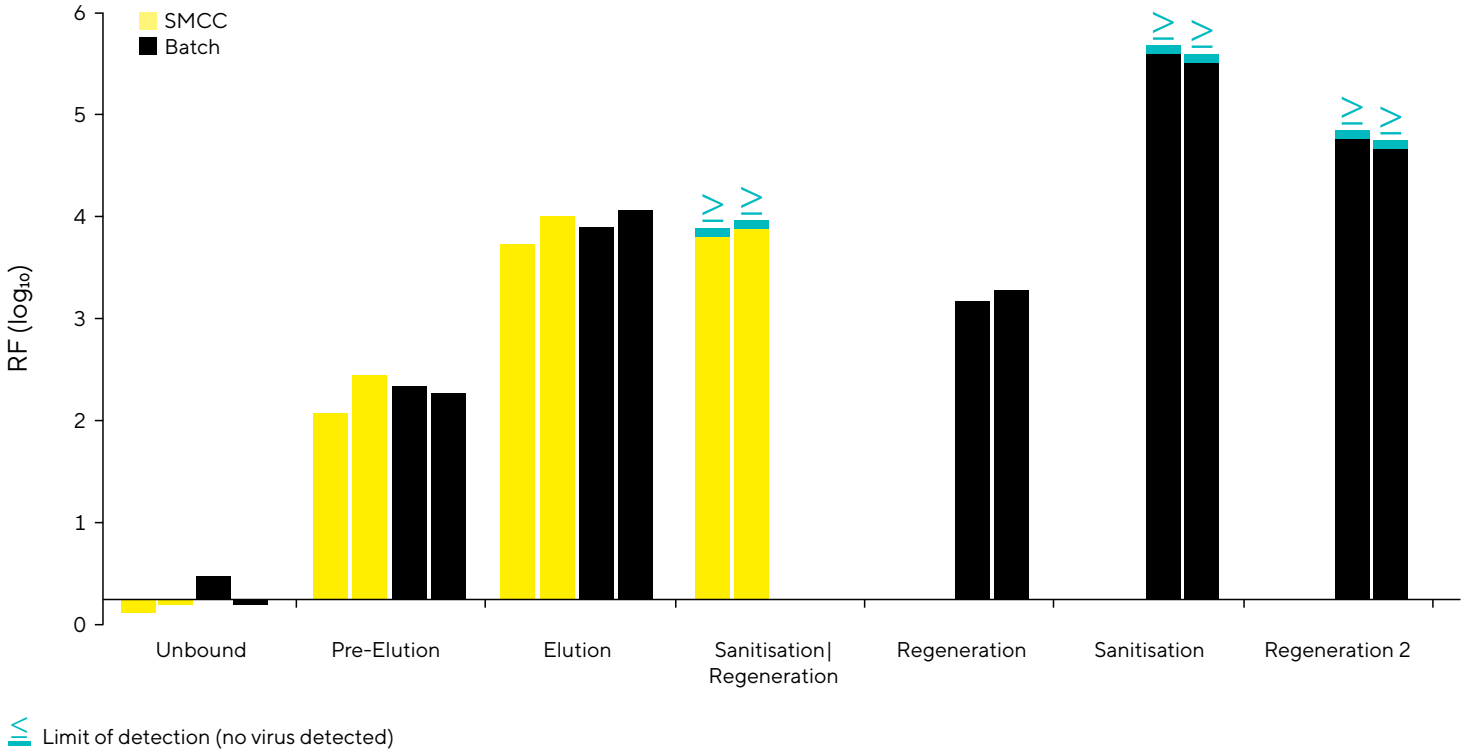

Figure 4: *Overall Viral Reduction Factors (RFs) of the Protein A Affinity Chromatography Step*

Note:
- RF < 1 $\log_{10}$: Considered negligible as per regulatory guidelines.
- RF >: The step may in fact reduce far greater quantities that can be quantified or claimed.

Figure 4 shows that very similar RFs are achieved at all comparable stages. RFs of 3.5 $\log_{10}$ and 3.8 $\log_{10}$ were obtained in the pool of eluate fractions for the SMCC process, compared to 3.7 $\log_{10}$ and 3.9 $\log_{10}$ in the batch experiments. These negligible differences indicate that the viral clearance capacity of the Protein A chromatography step is not impacted by using a continuous design instead of a conventional batch design.

Similar virus infectivity distribution was also observed in both SMCC and batch experimental designs, with the majority of the virus being recovered in the unbound fraction.

To quote the study authors concerning the SMCC process: "The distribution of MMV infectivity is demonstrated to be unchanged over the continuous bio-chromatography purification cycles and columns with the majority of virus in the unbound fraction, virus recovered in the Pre-Elution or Elution fractions, and no virus detected in the Regeneration | Sanitisation fraction".[1] This phenomenon is displayed in Figure 5.

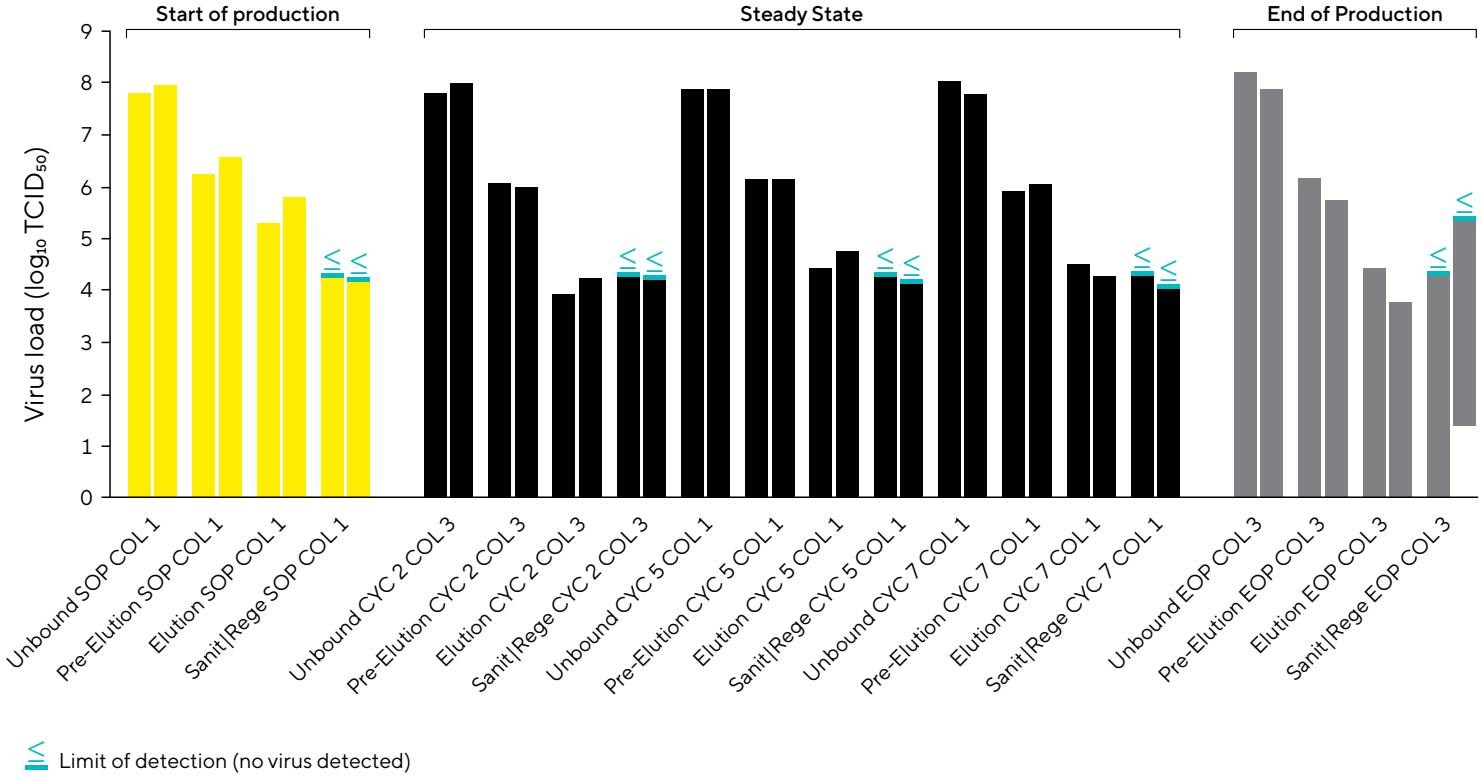

Figure 5: *MMV Infectivity in Collected Fractions by Protein A Chromatography Step in SMCC Study Design*

No Impact of Higher Column Loading on Viral Clearance Performance

Despite 30% higher loading (Table 1), substantial amounts of MMV were recovered in both designs and for both experimental rounds, again with similar infectivity distribution. This indicates that an increase in column load does not impact viral clearance efficiency in continuous mode in terms of overall viral reduction or viral distribution. This is important, as higher column loads are one of the defining features of the continuous approach in general, and of the SMCC technology in particular.

Efficiency of Regeneration | Sanitization Procedure in SMCC

Figure 5 also shows that no virus was detected after the SMCC regeneration | sanitization procedure. This indicates that any virus (or indeed any other impurity) still bound to the resin will be cleared after a purification cycle in a continuous multi-column setup.

Conclusion

This study undertaken by LFB Biotechnologies is an important milestone in demonstrating that the viral clearance performance of Protein A capture chromatography is maintained and remains efficient in all aspects with a continuous approach such as the SMCC technology.

The Resolute® BioSC System is a valuable asset for biopharmaceutical developers looking to integrate continuous manufacturing techniques.

References

[1] Goussen, C., Goldstein, L., Brèque, C., You, B., Boyer, S., Bataille, D., & Burlot, L. (2020). Viral clearance capacity by continuous Protein A chromatography step using Sequential MultiColumn Chromatography. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences, 1145, 122056. https://doi.org/10.1016/j.jchromb.2020.122056

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