Ensuring Biopharmaceutical Safety with a Novel, High-Throughput Virus Removal Filter

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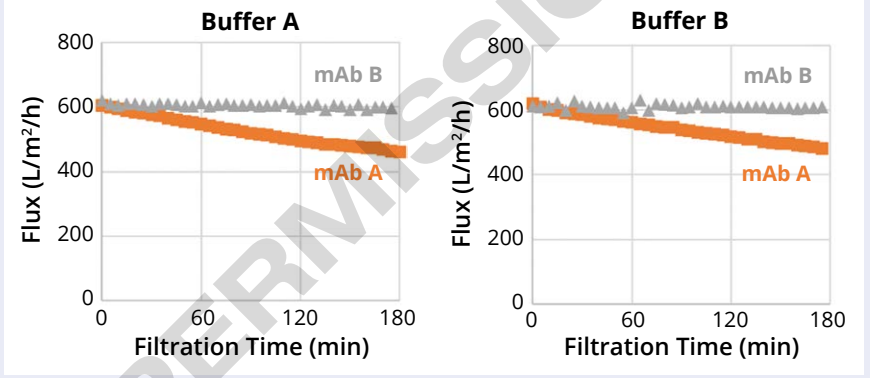
Biopharmaceuticals are increasing in application because of their high therapeutic efficacy and low incidence of side effects. The biopharmaceutical market has grown substantially and is expected to continue expanding, establishing monoclonal antibodies (mAbs) and related products as critical modalities in patient care. Many biologics are produced using mammalian cells, which have inherent risks of virus contamination. To date, no adverse events due to virally contaminated biopharmaceuticals have been reported. However, a contamination event could halt production and lead to supply shortages, an outcome with serious implications for both patients and healthcare providers.

To prevent viral contamination, biopharmaceutical manufacturing processes incorporate virus removal and/or inactivation steps. Membrane filtration often is applied for virus removal because it is effective for both enveloped and nonenveloped viruses, regardless of their physicochemical properties. The process is also capable of removing viruses across a broad size range, from parvoviruses (18–24 nm in diameter) to larger viruses, because the virus-removal mechanism is based on size exclusion.

Virus-removal capability is the primary performance requirement for such filters because it helps to ensure the safety of final drug products. According to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q5A guideline, an effective viral-clearance step generally gives reproducible reduction of virus load in the order of >4 $\log_{10}$ (**1**). The second performance requirement of a virus-removal filter is productivity, or the efficient processing of therapeutic proteins such as mAbs. An ideal virus-removal filter should combine high viral clearance with high flux per unit membrane area and minimal flux decay during filtration.

Recently, Asahi Kasei Life Science launched the Planova FG1 virus-removal filter. Presented in a hollow-fiber format, the membrane is made of hydrophilic-modified polyethersulfone (PES), offering pressure resistance, high scalability, and high flux. Here, we evaluate the filter's virus-removal capability using two different buffer solutions. We also assess filterability using two mAb types, three mAb concentrations, and two buffer conditions.

Figure 1: Flux for monoclonal antibody (mAb) solutions A and B under (LEFT) Buffer A and (RIGHT) Buffer B conditions



Materials and Methods

Samples: Two mAb solutions were prepared under conditions simulating the elution buffers used in cation-exchange (CEX, Buffer A: 50 mM acetate buffer, pH 5.0, conductivity = 15 mS/cm) and anion-exchange (AEX, Buffer B: 50 mM tris-HCl buffer, pH 7.0, conductivity = 5 mS/cm) chromatography.

Produced by Asahi Kasei Life Science, mAb A is an immunoglobulin G class 4 (IgG4) antibody with an isoelectric point (pI) of 8.0. It was collected as a process intermediate following protein A capture chromatography and subsequently purified through AEX and CEX chromatography steps. Purchased as a research reagent, mAb B is an IgG2 antibody with a pI of 6.5.

Virus Filtration: All filtrations were performed in dead-end mode with Planova FG1 filters at a constant pressure of 2 bar and at 25 °C.

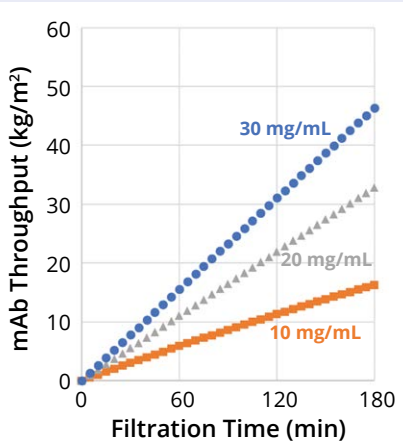
For the virus-removal capability study, mAb solutions of 10 mg/mL

This high-throughput filter with robust virus-removal capability is expected to **ENHANCE PRODUCTIVITY** and serve as a reliable solution in biopharmaceutical manufacturing.

Table 1: Results of viral-clearance study with porcine parvovirus (PPV) and minute virus of mice (MVM) spikes under Buffer A and Buffer B conditions (630 L of permeate/m^2 of membrane, including permeate after process pause) (LRV = log reduction value)

Buffer	Virus	Run	LRV (Pooled Permeate)
A	PPV	1	≥5.5
		2	≥5.5
	MVM	1	≥4.8
		2	≥4.8
B	PPV	1	≥4.8
		2	≥4.8
	MVM	1	≥5.5
		2	≥5.5

Figure 2: Filter throughput at mAb concentrations of 10, 20, and 30 mg/mL



were spiked with porcine parvovirus (PPV, concentration of 8.8 $\log_{10}$ as determined by tissue-culture infectious dose 50% ($TCID_{50}$)/mL) at 0.35% and with minute virus of mice (MVM, 9.1 $\log_{10}$ ($TCID_{50}$/mL)) at 0.16%. Virus-spiked mAb solutions were prepared in Buffer A and Buffer B as described previously and underwent prefiltration with a 0.1-µm pore-size filter. Virus filtration was performed until reaching a filtration volume of 600 L/m^2. Subsequently, pressure was released and maintained at 0 bar for 120 minutes, then brought to 2 bar until an additional 30 L/m^2 of permeate was collected.

Pooled permeate samples were collected, and the virus log reduction value (LRV) was calculated using the following formula:

$$LRV = \log_{10} (\textit{PPV or MVM titer in feed solution} \times \textit{sample volume}) \div (\textit{PPV or MVM titer in permeate sample} \times \textit{sample volume}).$$

For the filterability study, unspiked mAb solutions were filtered for three hours. Two buffer conditions were evaluated to determine the effect of buffer type on filtration. mAb solutions of 10, 20, and 30 mg/mL were filtered to evaluate the effect of protein concentration under Buffer B conditions.

Results

Virus Removal: We evaluated the Planova FG1 filter's virus-removal capability using PPV and MVM under two buffer conditions with 630 L/m^2 of throughput and a process pause. Table 1 lists LRVs from the pooled permeates. Under all tested conditions, the permeates exhibited virus titers below the quantification limit, with LRVs of ≥4.8, even after 120 minutes of process pause.

Effect of Buffer Conditions on Filterability: We studied the throughput of two mAb solutions (10 mg/mL) under two buffer conditions. Figure 1 illustrates the relationship between filtration time and flux for both buffer types. The Planova FG1 filter maintained a flux of >75% of the initial value over three hours of filtration with mAb A, and no flux decay was observed during filtration with mAb B. Additionally, for both mAb solutions, we observed no significant differences in filterability between the two buffer types. In all cases, throughput exceeded 15 kg/m^2 within three hours.

Effect of Protein Concentration on Filterability: Figure 2 illustrates the relationship between filtration time and throughput for mAb A at three concentrations (10, 20, and 30 mg/mL) under Buffer B conditions. Despite the increase in protein concentration, flux reduction remained minimal, and throughput improved with higher concentrations. Our results suggest that, in some cases, the Planova FG1 filter can maintain stable flux even at elevated protein concentrations, thereby enhancing productivity when filtration conditions are optimized.

Conclusion

The Planova FG1 filter exhibited high virus-removal capability, with LRVs of ≥4.8 for both PPV and MVM under all tested conditions. The filter also demonstrated good filterability, maintaining stable flux and high throughput across different buffer types and protein concentrations. This high-throughput filter with robust virus-removal capability is expected to enhance productivity and serve as a reliable solution in biopharmaceutical manufacturing.

Reference

1 ICH Q5A(R2). *Viral Safety Evaluation of Biotechnology Products Derived From Cell Lines of Human or Animal Origin*. International Council on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Geneva, Switzerland, 2023; https://database.ich.org/sites/default/files/ICH_Q5A%28R2%29_Guideline_2023_1101.pdf.

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Learn more about the Planova FG1 filter at https://asahikaseibioprocess.planova.ak-bio.com/bpi_planovafg1.