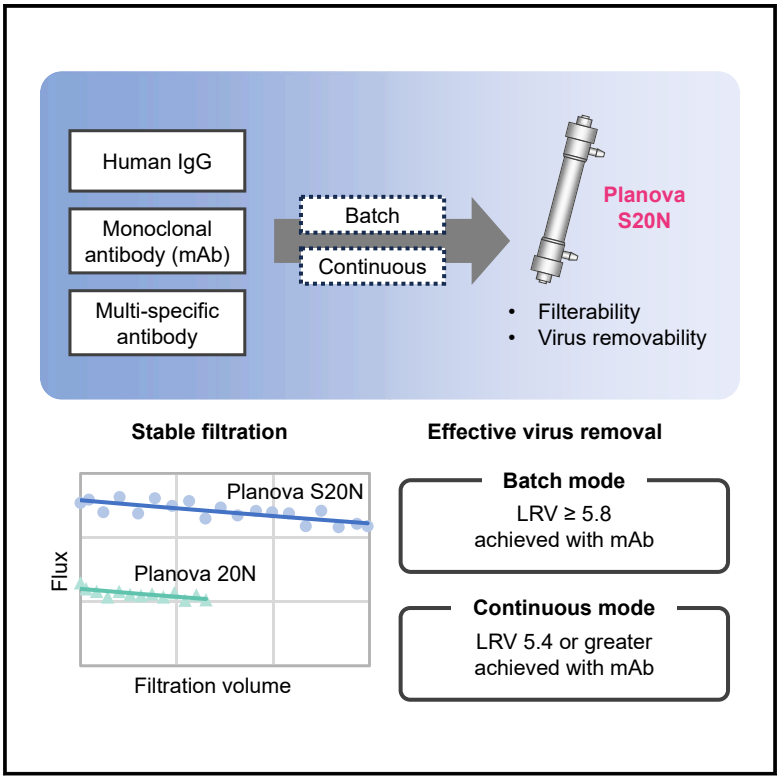
iScience

Article

Performance features of virus removal filters with novel regenerated cellulose hollow fiber membranes

Graphical abstract



Authors

Tomoko Hongo-Hirasaki, Hiroki Fukutomi

Correspondence

hongo.tb@om.asahi-kasei.co.jp

In brief

Microbial biotechnology; Biomedical materials; Biosafety

Highlights

- New Planova S20N filter achieves high virus removal capability for various viruses
- Good filterability and virus removal for human IgG, mAb, and multi-specific antibody
- Filtrations on new membrane reach flux twice that of conventional Planova 20N
- Planova S20N demonstrates good performance under continuous process conditions

Hongo-Hirasaki & Fukutomi, 2025, iScience *28*, 111701
February 21, 2025
https://doi.org/10.1016/j.isci.2024.111701

CellPress

iScience

CellPress
OPEN ACCESS

Article

Performance features of virus removal filters with novel regenerated cellulose hollow fiber membranes

Tomoko Hongo-Hirasaki[1,3,*] and Hiroki Fukutomi[2]
[1]Scientific Affairs Group, Bioprocess Division, Asahi Kasei Medical Co., Ltd., Nobeoka, Miyazaki 882-0031, Japan
[2]Scientific Affairs Group, Bioprocess Division, Asahi Kasei Medical Co., Ltd., Chiyoda-ku, Tokyo 100-0006, Japan
[3]Lead contact
*Correspondence: hongo.tb@om.asahi-kasei.co.jp
https://doi.org/10.1016/j.isci.2024.111701

SUMMARY

Virus removal filtration during the manufacturing process plays an essential role in ensuring the virus safety of biologics. Following the revision of International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q5A, the guidelines for the virus safety of biopharmaceuticals now state that an effective viral clearance process should achieve a viral reduction of 4 $\log_{10}$ or greater. Technological advances in the development of new manufacturing technologies for continuous production and the development of next-generation antibody drugs have increased the need for virus removal filters suited to a variety of applications. The newly available virus removal filter, Planova S20N, incorporates a newly developed regenerated cellulose hollow fiber membrane. In addition to stable filtration of a variety of antibody solutions, this filter demonstrates robust parvovirus removal under a variety of filtration operating conditions, including low flow rate filtration for continuous process.

INTRODUCTION

As indicated in the ICH Q5A guidelines,[1] viral clearance steps play an essential role in ensuring the virus safety of biopharmaceuticals and other biological products as one of the three basic principles along with testing and sourcing in the manufacturing process. Virus removal by filtration is widely implemented and recognized as a robust method.[2,3]

A virus logarithmic reduction value (LRV) greater than 4 is generally considered effective viral clearance.[4,5] The ICH Q5A Revision,[6] published as a step 4 document in November 2023, now includes a statement that virus LRV of 4 or greater is indicative of an effective viral clearance step targeting non-enveloped viruses. Using a virus filter designed to remove small viruses is also effective as a viral clearance step for smaller viruses, such as parvoviruses. Virus filtration is recognized as a robust and effective clearance step that is less susceptible to feed conditions owing to its size exclusion-based removal mechanism. Thus, achieving this degree of viral clearance is a factor in selecting a virus removal filter and selecting the process conditions, and several types of virus removal filters having parvovirus LRV of 4 or higher are available.

Research and development of antibody drugs in recent years has produced various antibody engineering technologies that harness the protein structure of antibodies, leading to more effective drug discovery and increased activity in the creation of next-generation antibodies.[7] However, these next-generation antibodies present challenges for virus removal filtration. Multi-specific antibodies (bispecific antibodies that bind to two antigens and multi-specific antibodies with multiple antigen-binding sites) allow a single antibody molecule to bind to multiple different targets but these molecules have a more challenging purification process as they are more prone to having impurities in the manufacturing process.[8] Fc-fusion proteins, which are composed of a crystallizable fragment (Fc) domain of immunoglobulin G (IgG) fused with a ligand, an active peptide or an extracellular domain (ECD) of a receptor,[9] have also been suggested to be unstable.[10]

Another recent development in pharmaceutical manufacturing technology is the shift from batch processing at each step to unique (or new) production methods, such as continuous production in which at least two or more unit operations are linked together for a reduction in space requirements and increased flexibility.[11] Continuous production approaches include end-to-end systems that link upstream and downstream processes and hybrid systems that combine continuous and batch production.[12,13] The virus filtration process in continuous production may be linked to the cell culture period in the upstream process for batch control purposes. While conditions of the immediately preceding chromatography process impact the virus filtration, cases of processing large volumes at low flow rates for long periods over several days have been reported.[14] For these processes, a virus removal filter that can filter stably under a variety of filtration operating conditions, including low flow rates, and that also demonstrates effective virus removal performance is required.

Among commercially available virus removal filters, the Planova 20N virus removal filter made of regenerated cellulose

Table 1. HIC retention time for mAbs and h-IgG used in this study

Molecule	HIC retention time (min)
mAb A	6.97
mAb B	14.0
Human IgG	7.40

membrane in hollow fiber format is widely applicable to a wide variety of molecular and solution conditions.[15] However, as various molecules and processes have developed, there has been a need for virus filters that can be widely adapted to them. Against this background, a new virus removal filter called Planova S20N was developed to meet the technological developments and requirements of the industry while maintaining the advantages of the Planova 20N filter. The Planova S20N filter made of regenerated cellulose hollow fiber membranes with improved pressure resistance and capability for higher pressure filtration was achieved through improved hollow fiber membrane manufacturing technology.[16]

In this study, the performance features of Planova S20N filters were evaluated in comparison to the existing Planova 20N filters in terms of parvovirus removal under various filtration operating conditions and filtration performance with various antibody solutions, including human IgG (h-IgG), two types of monoclonal antibodies (mAbs) with different physicochemical properties, and a multi-specific antibody.

RESULTS

Summary of test materials

In this study, filtration characteristics and virus removal performance were examined for several molecules in various solutions representative of widely used biological products. For mAbs, two mAbs having different hydrophobicity were selected and evaluated to assess the impact of hydrophobicity, which is one of the characteristics associated with molecular stability and aggregation.[17] Additionally, a multi-specific antibody, which is representative of next-generation antibodies, was evaluated.

Planova S20N and Planova 20N filters show good filterability of mAbs with low and high hydrophobicity

Assessment of hydrophobicity by hydrophobic interaction chromatography (HIC) retention time showed mAb A to have low hydrophobicity (retention time, 6.97 min) and mAb B to have high hydrophobicity (retention time, 14.0 min), while the h-IgG used in this study had HIC retention time of 7.40 min (Table 1). Filtration of mAb A and mAb B in buffer mimicking the conditions commonly used in cation exchange chromatography (CEX) on Planova S20N and Planova 20N filters at the respective transmembrane pressure limits produced similar filtration profiles (Figure 1) and 3 h throughput values (Table 2) for both mAbs. The filtrations were stable with almost no flux decay and similar throughput in 3 h for both mAb A and mAb B irrespective of

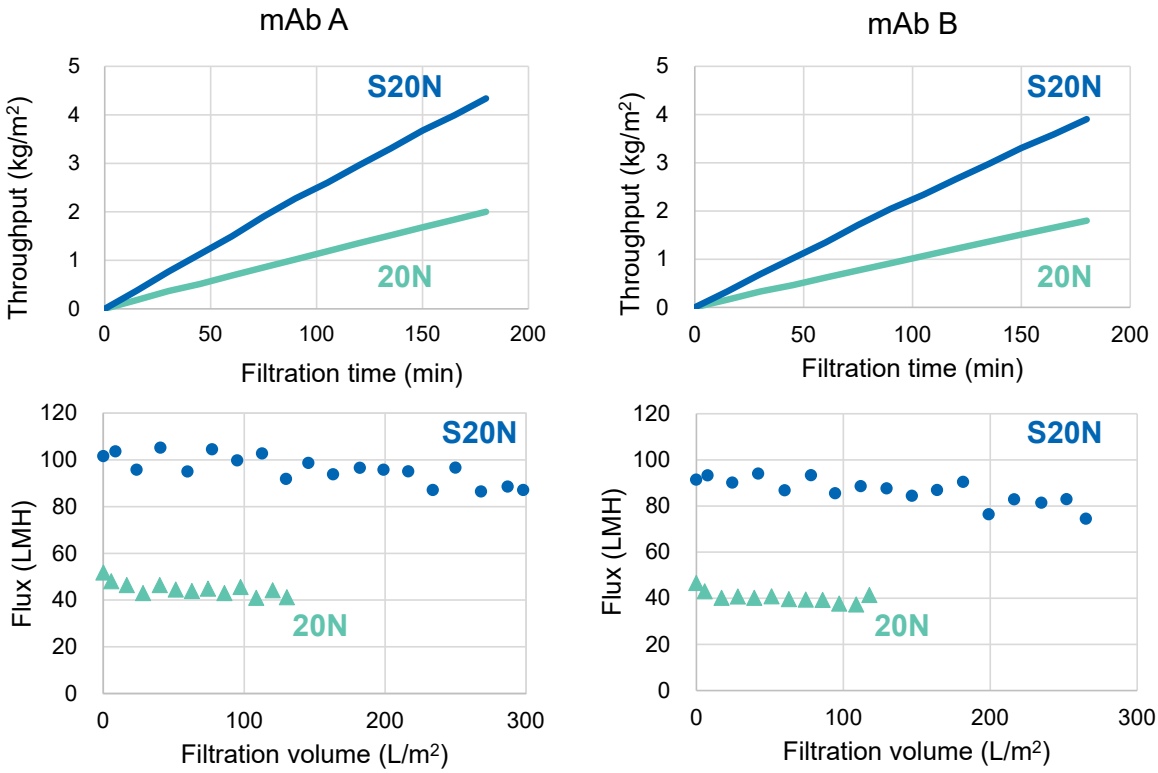


Figure 1. Throughput to 3 h and flux for two mAbs with low and high hydrophobicity on Planova S20N and Planova 20N filters

mAb A (left) with low hydrophobicity and mAb B (right) with high hydrophobicity were adjusted to 15 mg/mL in 100 mM acetate buffer, 200 mM NaCl (pH 5.5).

Table 2. Filtration pressure and 3 h throughput for mAbs with low and high hydrophobicity on Planova S20N and Planova 20N filters

Filter	Pressure (kPa)	3 h throughput (kg/m^2)	
		mAb A	mAb B
Planova S20N	196	4.3	3.9
Planova 20N	98	2.0	1.8

degree of hydrophobicity. For these runs with filtration pressure twice higher for Planova S20N filters, flux and throughput were more than double for Planova S20N compared to Planova 20N filters.

Equivalent filterability of mAb A in four different buffer conditions

The 3 h throughput of 10 mg/mL mAb A in four different buffer conditions run on Planova S20N filters operated at 196 kPa for 3 h showed comparable filtration throughput of 3.6–3.9 kg/m^2 across the runs (Table 3). The different buffer conditions of composition, pH and conductivity used in this study did not affect the filtration performance of Planova S20N filters.

Effective viral clearance is achieved for various viruses

Filtration to 150 L/m^2 of 1 mg/mL h-IgG solution in 100 mM NaCl spiked with one of minute virus of mice (MVM), porcine parvovirus (PPV), pseudorabies virus (PRV), encephalomyocarditis virus (EMCV), bovine viral diarrhea virus (BVDV), or human immunodeficiency virus (HIV) on Planova S20N filters at 196 kPa followed by process pause for 30 min and additional 15 L/m^2 filtration produced total pool virus LRV greater than 4 for all viruses tested (Table 4). Planova S20N filters showed effective removal of a variety of viruses, DNA or RNA genomes, enveloped and non-enveloped, and a range of sizes.

Effective viral clearance is achieved for PPV from h-IgG with process pause over a range of filtration pressures

Filtration to 300 L/m^2 of 10 mg/mL h-IgG in 100 mM NaCl spiked with PPV was conducted on Planova S20N filters at three different filtration pressures of 49, 98, and 196 kPa, followed by process pause for 3 h and additional 30 L/m^2 filtration at the same filtration pressure conditions. The results showed high PPV LRV of 5.8 or higher at all filtration pressures tested (Figure 2). Further, the same level of virus removal was achieved after the 3 h process pause, with complete clearance at 49 and 196 kPa.

Table 3. Four different buffer conditions and 3 h throughput for mAb A on Planova S20N filters

Case	Buffer condition	3 h throughput (kg/m^2)
1	25 mM acetate buffer, pH 5.0, 14 mS/cm	3.8
2	25 mM histidine buffer, pH 6.0, 5 mS/cm	3.6
3	25 mM histidine buffer, pH 6.0, 14 mS/cm	3.6
4	25 mM Tris-HCl buffer, pH 7.2, 5 mS/cm	3.9

Table 4. Characteristics of viruses and removability results for h-IgG on Planova S20N filters

Virus	Abbreviation	Genome	Size (nm)	Virus LRV[a]
Minute virus of mice	MVM	DNA	18–24	≥5.3
Porcine parvovirus	PPV	DNA	18–24	≥5.8
Pseudorabies virus	PRV	DNA	120–200	≥5.4[b]
Encephalomyocarditis virus	EMCV	RNA	25–30	≥5.9[b]
Bovine viral diarrhea virus	BVDV	RNA	50–70	≥5.9
Human immunodeficiency virus	HIV	RNA	80–120	≥4.1[b]

[a]Total pool virus LRV after 30 min process pause and LRV was the average of the duplicate runs.
[b]Contract Research Organization (CRO) was contracted to perform the test. Large volume assay was used to expand the dynamic range of virus testing.

Effective viral clearance is achieved for MVM from mAb A in two buffer conditions

As virus removal filtration may immediately follow a chromatography step, we tested two buffers mimicking conditions commonly used in CEX and anion exchange chromatography (AEX). Filtration of 10 mg/mL mAb A spiked with MVM at 196 kPa for 3 h, followed by 30 or 120 min process pause and 10 L/m^2 recovery flush with the same buffer showed equivalent pooled MVM LRV of 6.0 for CEX buffer condition and 5.8 for

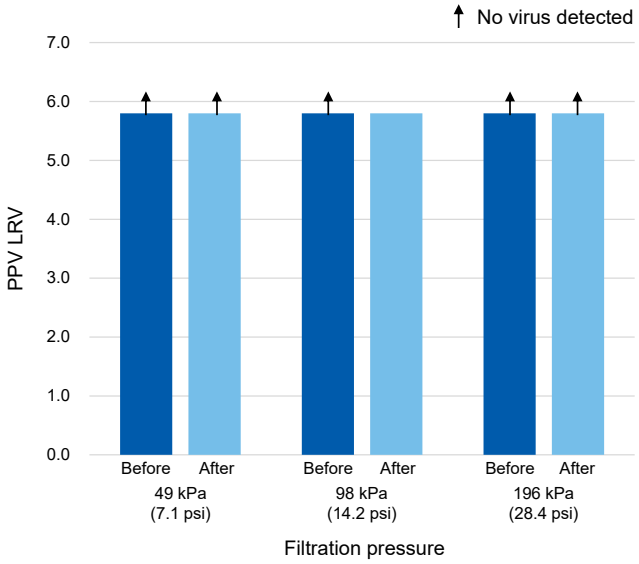


Figure 2. PPV removability for human IgG (h-IgG) on Planova S20N filters at different filtration pressures

For all runs, 10 mg/mL h-IgG in 100 mM NaCl spiked with PPV was filtered to 300 L/m^2 (before 3 h process pause, dark blue bars) and for an additional 30 L/m^2 under the same conditions (after pause, light blue bars). Up arrow indicates filtrate titer below the detection limit.

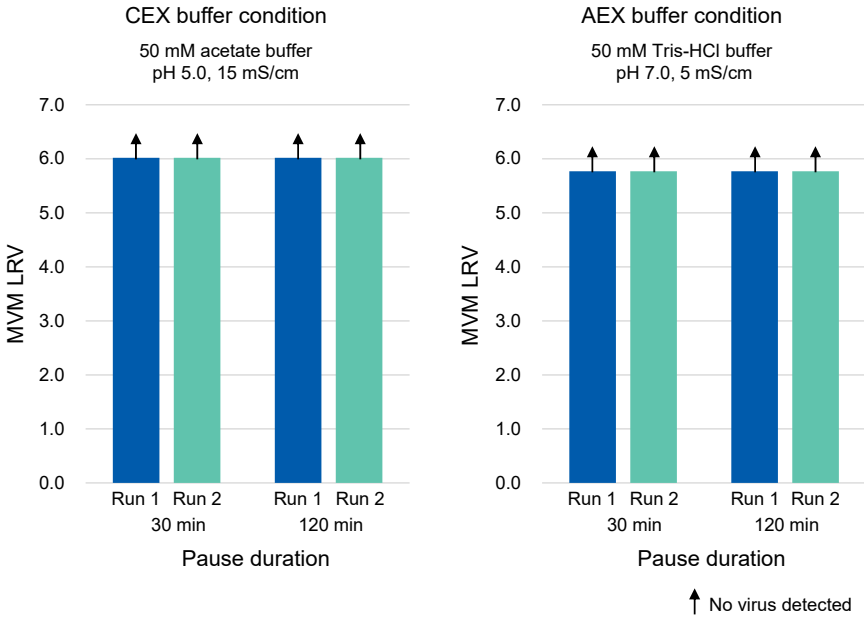


Figure 3. MVM removability for mAb A on Planova S20N filters with two buffer conditions mimicking elution solutions in CEX and AEX chromatography

For all runs performed in duplicate, 10 mg/mL mAb A spiked with MVM was filtered at 196 kPa for 3 h, followed by a process paused for 30 or 120 min and a recovery flush with the same buffer to 10 L/m^2. Up arrow indicates filtrate titer below the detection limit.

AEX buffer condition (Figure 3). There was no difference in pooled MVM LRV for both pause durations.

Planova S20N and Planova 20N filters show good filterability and MVM removal for a multi-specific antibody

For the filtration to 600 L/m^2 of multi-specific antibody spiked with MVM at 193 kPa on Planova S20N filters and at 83 kPa on Planova 20N filters, both filters showed stable filtration performance with little flux decay over time, and the flux on Planova S20N filters was roughly twice that on Planova 20N filters (Figure 4).

For the 600 L/m^2 filtration followed by a 1 h process pause and additional filtration of the buffer for 30 min, the pooled MVM LRV was 4.4 and 4.5 for Planova 20N filters, and complete clearance with MVM LRV of 4.7 was achieved for both runs on Planova S20N filters (Table 5). Thus, Planova S20N filters achieved better virus removability and had higher throughput for this multi-specific antibody.

Planova S20N filters show good PPV removal in low flux filtration conditions of continuous processing

In continuous processing, feed to the virus removal filter may be supplied a low but constant flow rate. Here, PPV spiked at 6.5 log $TCID_{50}$/mL into 10 mg/mL mAb A solution in a buffer condition commonly used in CEX and filtered on Planova S20N filters at a constant flow rate condition of 5 L/m^2/h (LMH) for about 100 h to reach a filtration throughput of 500 L/m^2 resulted in minimal pressure rise of less than 20.8 kPa (Figure 5), indicating stable filtration performance. PPV LRV shown in Table 6 was 5.4, 6.1, and >5.8 for the three runs.

DISCUSSION

Stable filtration for a variety of antibody solutions

The Planova S20N filter is made of highly hydrophilic regenerated cellulose, which makes it less prone to interactions with

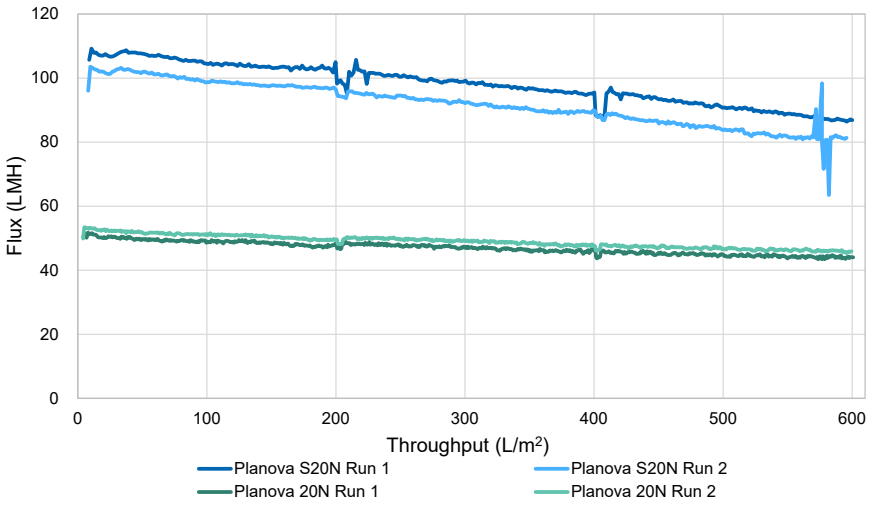


Figure 4. Filtration profiles for multi-specific antibody on Planova S20N and Planova 20N filters

Filtration of 6.0 mg/mL multi-specific antibody in 15 mM phosphate buffer, 400 mM NaCl (pH 7.5) spiked with MVM was conducted in duplicate to 600 L/m^2 at 193 kPa on Planova S20N filters (blue lines) and at 83 kPa on Planova 20N filters (green lines).

Table 5. MVM removability for multi-specific antibody with pause before recovery flush on Planova S20N and Planova 20N filters

Filter	Run	MVM LRV
Planova S20N	1	≥4.7
	2	≥4.7
Planova 20N	1	4.5
	2	4.4

Table 6. PPV removability for mAb A on Planova S20N filters with low flux, long duration filtration conditions mimicking a continuous process

Run	Filtrate PPV titer (log_{10} $TCID_{50}$/mL)	PPV LRV
1	1.00	5.4
2	0.75	6.1
3	≤0.73	≥5.8

the hydrophobic portions of proteins, affording it broad applicability to the virus removal filtration of antibody molecules.[18,19] The regenerated cellulose hollow fiber membrane has a gradient pore structure in which the pore size diameter gradually decreases from the inside of the hollow fiber to the outside.[20–23] For the filtration of solutions with a small amount of aggregate, the inner filter membrane layer with larger pores acts as a prefilter and in turn protects the next layer encountered in the filtration, which has smaller pores. The aggregates are captured in a stepwise manner by size,[24] which is thought to minimize the clogging effects and provide more stable filtration performance.

Robust and high virus removability under a wide variety of operating conditions

The structure of the Planova S20N filter regenerated cellulose hollow fiber membranes is such that the hollow fiber walls have a larger hold-up volume than for Planova 20N filters.[25,26] The membrane pore structure is a three-dimensional network comprising multiple layers of connected voids and capillaries, and it is thought that by filtering antibody solutions with viruses through this network, viruses are caught as the solution crosses multiple layers. A large hold-up volume in the hollow fiber membrane likely indicates that the number of stages (number of layers) is large, and it follows that the virus capture capacity is large. In Planova 20N filters, parvoviruses are shown to be trapped in the center of the membrane wall.[27] As the Planova S20N filter has a larger hold-up volume in the hollow fiber walls than Planova 20N filters, there is additional capacity for virus capture. Consequently, Planova S20N filters are expected to exhibit robust and high virus removability for a wide variety of filtration operating conditions.

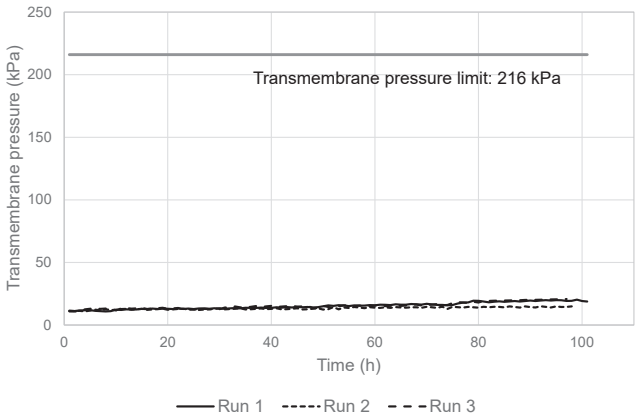


Figure 5. Transmembrane pressure profiles for low flux, long duration filtration of mAb A on Planova S20N filters mimicking a continuous process

Filtration of 10 mg/mL mAb A in a buffer that mimics the elution condition of CEX and spiked with PPV was conducted in triplicate to 500 L/m^2 on Planova S20N filters at a constant flow rate condition of 5 LMH for about 100 h.

Limitations of the study

In these studies, filterability and virus removability were evaluated for h-IgG solution, two mAbs, and one multi-specific antibody. Good filterability and virus removability were demonstrated for filtrations on Planova S20N and Planova 20N filters for these molecules. Filtration results can vary depending on molecule properties (e.g., when filtering new modified molecules) or for different solution additives and vastly different solution viscosity. To address these limitations, future studies should include a wider variety of molecule types and solution conditions. Along with including a wider variety of molecules and conditions, future studies should aim to clarify the filtration mechanisms, including clogging and virus removal, to support future process development.

RESOURCE AVAILABILITY

Lead contact

Further information or requests for reagents resources, and data should be addressed to the lead contact, Tomoko Hongo-Hirasaki (hongo.tb@om.asahi-kasei.co.jp).

Materials availability

This work did not generate new unique reagents and components.

Data and code availability

- This paper does not report the original code.
- All other data reported in this paper will be shared by the lead contact upon request.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

ACKNOWLEDGMENTS

We thank Ji Zheng and Jessica Waller at Bristol Myers Squibb for providing multi-specific antibody solution. We also thank our colleagues Christine Stouffer, Junji Hidaka, Keisuke Tsukamoto, Kumiko Michikawa, and Asami Inoue for providing essential data used in this study. We also thank our colleagues Naoto Watanabe and Linda Gudex for proofreading the manuscript. No funding was received for conducting this study.

AUTHOR CONTRIBUTIONS

Conceptualization, T.H.-H. and H.F.; methodology, investigation, and formal analysis, T.H.-H. and H.F.; supervision, T.H.-H.; writing – original draft, T.H.-H. and H.F.; writing – review and editing, T.H.-H. and H.F.

DECLARATION OF INTERESTS

The authors T.H.-H. and H.F. are employees of Asahi Kasei Medical Co., Ltd.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
  - Minute virus of mice (of minute virus of mice)
  - Porcine parvovirus
  - Bovine viral diarrhea virus
- METHOD DETAILS
  - Virus removal filter
  - Antibody solutions
  - Hydrophobicity analyses of mAb products by by hydrophobic interaction chromatography
  - Determination of the infectivity titer of virus
  - Calculation of virus log reduction value (logarithmic reduction value)
  - Evaluation of filterability of mAbs with high and low hydrophobicity
  - Evaluation of filterability of mAb A in four different buffers
  - Evaluation of the removability of viruses porcine parvovirus, of minute virus of mice, and bovine viral diarrhea virus
  - Evaluation of removability of EMC, pseudorabies virus, and HIV
  - Evaluation of porcine parvovirus removal under three filtration pressure conditions with process pause
  - Evaluation of of minute virus of mice removal using mAb A in two buffer conditions
  - Evaluation of of minute virus of mice removal using multi-specific antibody
  - Evaluation of virus removability of low flux filtration for continuous processes
- QUANTIFICATION AND STATISTICAL ANALYSIS

Received: August 9, 2024
Revised: November 17, 2024
Accepted: December 24, 2024
Published: January 3, 2025

REFERENCES

1. International Conference on Harmonisation Tripartite Guideline. Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin Q5A(R1). https://database.ich.org/sites/default/files/Q5AR1Guideline_0.pdf.
2. Roth, N.J., Dichtelmüller, H.O., Fabbrizzi, F., Flechsig, E., Gröner, A., Gustafson, M., Jorquera, J.I., Kreil, T.R., Misztela, D., Moretti, E., et al. (2020). Nanofiltration as a robust method contributing to viral safety of plasma-derived therapeutics: 20 years' experience of the plasma protein manufacturers. Transfusion *60*, 2661–2674. https://doi.org/10.1111/trf.16022.
3. Junter, G.-A., and Lebrun, L. (2017). Cellulose-based virus-retentive filters: a review. Rev. Environ. Sci. Biotechnol. *16*, 455–489. https://doi.org/10.1007/s11157-017-9434-1.
4. Europeans Medicines Agency. Note for guidance on virus validation studies: The design, contribution and interpretation of studies validating the inactivation and removal of viruses. CPMP/BWP/268/95. https://www.ema.europa.eu/en/documents/scientific-guideline/note-guidance-virus-validation-studies-design-contribution-and-interpretation-studies-validating-inactivation-and-removal-viruses_en.pdf.
5. Johnston, A., and Adcock, W. (2000). The Use of Chromatography to Manufacture Purer and Safer Plasma Products. Biotechnol. Genet. Eng. Rev. *17*, 37–70. https://doi.org/10.1080/02648725.2000.10647987.
6. International Conference on Harmonisation Tripartite Guideline. Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin Q5A(R2). https://database.ich.org/sites/default/files/ICH_Q5AR2_Guideline_2023_1101.pdf.
7. Elshiaty, M., Schindler, H., and Christopoulos, P. (2021). Principles and Current Clinical Landscape of Multispecific Antibodies against Cancer. Int. J. Mol. Sci. *22*, 5632. https://doi.org/10.3390/ijms22115632.
8. Sawant, M.S., Streu, C.N., Wu, L., and Tessier, P.M. (2020). Toward Drug-Like Multispecific Antibodies by Design. Int. J. Mol. Sci. *21*, 7496. https://doi.org/10.3390/ijms21207496.
9. Duivelshof, B.L., Murisier, A., Camperi, J., Fekete, S., Beck, A., Guillarme, D., and D'Atri, V. (2021). Therapeutic Fc-fusion proteins: Current analytical strategies. J. Separ. Sci. *44*, 35–62. https://doi.org/10.1002/jssc.202000765.
10. Fast, J.L., Cordes, A.A., Carpenter, J.F., and Randolph, T.W. (2009). Physical Instability of a Therapeutic Fc Fusion Protein: Domain Contributions to Conformational and Colloidal Stability. Biochemistry *48*, 11724–11736. https://doi.org/10.1021/bi900853v.
11. Godawat, R., Konstantinov, K., Rohani, M., and Warikoo, V. (2015). End-to-end integrated fully continuous production of recombinant monoclonal antibodies. J. Biotechnol. *213*, 13–19. https://doi.org/10.1016/j.jbiotec.2015.06.393.
12. Shirataki, H., Matsumoto, Y., Konoike, F., and Yamamoto, S. (2023). Viral clearance in end-to-end integrated continuous process for mAb purification: Total flow-through integrated polishing on two columns connected to virus filtration. Biotechnol. Bioeng. *120*, 2977–2988. https://doi.org/10.1002/bit.28464.
13. Botelho Ferreira, K., Benlegrimet, A., Diane, G., Pasquier, V., Guillot, R., De Poli, M., Chappuis, L., Vishwanathan, N., Souquet, J., Broly, H., and Bielser, J.M. (2022). Transfer of continuous manufacturing process principles for mAb production in a GMP environment: A step in the transition from batch to continuous. Biotechnol. Prog. *38*, e3259. https://doi.org/10.1002/btpr.3259.
14. Lute, S., Kozaili, J., Johnson, S., Kobayashi, K., and Strauss, D. (2020). Development of small-scale models to understand the impact of continuous downstream bioprocessing on integrated virus filtration. Biotechnol. Prog. *36*, e2962. https://doi.org/10.1002/btpr.2962.
15. Hongo-Hirasaki, T., Komuro, M., and Ide, S. (2010). Effect of antibody solution conditions on filter performance for virus removal filter Planova™ 20N. Biotechnol. Prog. *26*, 1080–1087. https://doi.org/10.1002/btpr.415.
16. Yoshida, M., Nakashima, S., Himeno, S., and Kajiyama, K. (2024). Porous hollow-fiber membrane and method for testing integrity. Patent application WO2022118943A1.
17. Le Basle, Y., Chennell, P., Tokhadze, N., Astier, A., and Sautou, V. (2020). Physicochemical Stability of Monoclonal Antibodies: A Review. J. Pharmacol. Sci. *109*, 169–190. https://doi.org/10.1016/j.xphs.2019.08.009.
18. Marques, B.F., Roush, D.J., and Göklen, K.E. (2009). Virus filtration of high-concentration monoclonal antibody solutions. Biotechnol. Prog. *25*, 483–491. https://doi.org/10.1002/btpr.177.
19. Hidaka, J., Taniguchi, H., and Futamura, A. (2022). Antibody purification methods. Technical Disclosure (journal of JIII), 500999. (in Japanese).
20. Tsurumi, T., Sato, T., Osawa, N., Hitaka, H., Hirasaki, T., Yamaguchi, K., Hamamoto, Y., Manabe, S.i., Yamashiki, T., and Yamamoto, N. (1990). Structure and Filtration Performances of Improved Cuprammonium Regenerated Cellulose Hollow Fiber (Improved BMM Hollow Fiber) for Virus Removal. Polym. J. *22*, 1085–1100. https://doi.org/10.1295/polymj.22.1085.

21. Tsurumi, T., Osawa, N., Hitaka, H., Hirasaki, T., Yamaguchi, K., Manabe, S.i., and Yamashiki, T. (1990). Structure of Cuprammonium Regenerated Cellulose Hollow Fiber (BMM Hollow Fiber) for Virus Removal. Polym. J. *22*, 751–758. https://doi.org/10.1295/polymj.22.751.

22. Hongo-Hirasaki, T., Yamaguchi, K., Yanagida, K., and Okuyama, K. (2006). Removal of small viruses (parvovirus) from IgG solution by virus removal filter Planova®20N. J. Membr. Sci. *278*, 3–9. https://doi.org/10.1016/j.memsci.2005.10.057.

23. Adan-Kubo, J., Tsujikawa, M., Takahashi, K., Hongo-Hirasaki, T., and Sakai, K. (2019). Microscopic visualization of virus removal by dedicated filters used in biopharmaceutical processing: Impact of membrane structure and localization of captured virus particles. Biotechnol. Prog. *35*, e2875. https://doi.org/10.1002/btpr.2875.

24. Tsukamoto, K., Hamamoto, R., Oguri, R., Miura, A., Iwasaki, T., and Sukegawa, T. (2024). Size-based analysis of virus removal filter fouling using fractionated protein aggregates. Biotechnol. Prog. *40*, e3391. https://doi.org/10.1002/btpr.3391.

25. Asahi Kasei Medical (2022). Validation Report for Planova 20N Filters (TAS34010-4.2) (Asahi Kasei Medical Co., Ltd).

26. Asahi Kasei Medical Co., Ltd (2024). Validation Report for Planova S20N Filters (TAS34156-1.3) (Asahi Kasei Medical Co., Ltd).

27. Leisi, R., Rostami, I., Laughhunn, A., Bieri, J., Roth, N.J., Widmer, E., and Ros, C. (2022). Visualizing protein fouling and its impact on parvovirus retention within distinct filter membrane morphologies. J. Membr. Sci. *659*, 120791. https://doi.org/10.1016/j.memsci.2022.120791.

28. Tattersall, P., and Bratton, J. (1983). Reciprocal productive and restrictive virus-cell interactions of immunosuppressive and prototype strains of minute virus of mice. J. Virol. *46*, 944–955. https://doi.org/10.1128/jvi.46.3.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
Minute virus of mice	ATCC	ATCC: VR-1346
Porcine parvovirus (PPV) NADL-2	ATCC	ATCC: VR-742
Bovine viral diarrhea virus 1 (BVDV) NADL	ATCC	ATCC: VR-1422
Chemicals, peptides, and recombinant proteins		
DMEM	Gibco	11965092
FBS	Serana Europe	S-FBS-NL-015
Horse serum	Gibco	16050122
Experimental models: Cell lines		
Nb324K	*Tattersall* et al.[28]	RRID: CVCL_U409
PK13	ATCC	ATCC: CRL-6489; RRID: CVCL_6433
MDBK	ATCC	ATCC: CCL-22; RRID: CVCL_0421
Software and algorithms		
LabSolutions LC/GC software	Shimadzu	https://www.shimadzu.com/an/products/software-informatics/labsolutions-series/labsolutions-lcgc
Excel	Microsoft	https://www.microsoft.com/en-us/microsoft-365/excel
Other		
Nexera lite inert high-performance liquid chromatography system	Shimadzu	https://www.shimadzu.com/an/products/liquid-chromatography/hplc-system/nexera-lite-inert/
Planova S20N	Asahi Kasei Medical	https://planova.ak-bio.com/products_services/planova-S20N/
Planova 20N	Asahi Kasei Medical	https://planova.ak-bio.com/products_services/planova-n/

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Minute virus of mice (of minute virus of mice)

Minute virus of mice (MVM) strain (VR-1346) was purchased from ATCC and host cell line Nb324K[28] was purchased from Yale University. Nb324K cells were infected with MVM at multiplicity of infection (MOI) 0.01 in Dulbecco's modified Eagle's medium (DMEM, Gibco) containing 3% Fetal bovine serum (FBS, Serana Europe) and cultured for 4 days at 37°C with 5% CO_2. The culture medium was exchanged with serum-free DMEM and cultured for an additional 3 days. MVM was concentrated by ultracentrifugation using a Type 45Ti rotor (Beckman Coulter Inc.) at 29,400 rpm for 2 h and applied to density gradient ultracentrifugation. Density step-gradient ultracentrifugation was conducted using 40%–60% sucrose/TNE buffer (50 mM Tris, 100 mM NaCl, 1 mM EDTA, pH 7.4) with an SW32 rotor (Beckman Coulter Inc.) at 24,000 rpm for 19 h, and purified MVM was recovered from between the 40% and 60% sucrose layers. Nb324K were authenticated and confirmed to be free of mycoplasma by the suppliers.

Porcine parvovirus

PPV NADL-2 strain (VR-742) and its host cell line PK13 (CRL-6489) were purchased from ATCC. PK13 cells were infected with PPV at MOI 0.01 in DMEM supplemented with 3% FBS and cultured for 2 days at 37°C with 5% CO_2. The culture medium was exchanged with serum-free DMEM and cultured for an additional 2–5 days. PPV stocks were recovered from the culture serum-free supernatant after removal of cell debris by low-speed centrifugation and 0.45 μm membrane filtration. PK13 were authenticated and confirmed to be free of mycoplasma by the suppliers.

Bovine viral diarrhea virus

BVDV 1 (BVDV) NADL strain (VR-1422) and its host cell line MDBK (CCL-22) were purchased from ATCC. MDBK cells were infected with BVDV at MOI 0.03 in DMEM supplemented with 10% horse serum (Gibco) and cultured for 2 days at 37°C with 5% CO_2. Crude

BVDV stocks were recovered from the culture supernatant after removing cell debris by low-speed centrifugation and 0.45 μm membrane filtration. MDBK were authenticated and confirmed to be free of mycoplasma by the suppliers.

METHOD DETAILS

Virus removal filter

Two virus removal filters with hydrophilic cuprammonium regenerated cellulose hollow fiber membranes were compared: Planova 20N (0. 001 m^2, Asahi Kasei Medical) with maximum operating pressure 98 kPa (1.00 kgf/cm^2) and Planova S20N (0.001 m^2, commercially available and 0.0003 m^2, specially manufactured for evaluations, Asahi Kasei Medical) with maximum operating pressure 216 kPa (2.20 kgf/cm^2). Detailed specifications of these two filter types are proprietary and not described further. Planova S20N has improved pressure resistance and requires only a leakage test for integrity testing.

Antibody solutions

H-IgG solution was prepared by diluting Venoglobulin IH 5% (Japan Blood Products Organization), a pharmaceutical product consisting of polyclonal antibody (molecular weight, 158,500 Da) separated from human plasma. Two different monoclonal antibody products (mAb A, IgG4, pI 8.0; and mAb B, IgG4, pI 7.5) collected as process intermediates after Protein A capture chromatography and further purified by AEX and CEX chromatography steps were produced by Asahi Kasei Medical for this study. Monoclonal antibody solutions were diluted for filtration based on concentration determined by UV absorbance at 280 nm using Nanodrop One (Thermo Fisher Scientific). A multi-specific antibody solution was kindly provided by Bristol Myers Squibb with concentration information.

Hydrophobicity analyses of mAb products by by hydrophobic interaction chromatography

The hydrophobicity of mAbs was compared based on HIC retention time. Experiments were performed on a Shimadzu Nexera lite inert high-performance liquid chromatography system equipped with an ultraviolet (UV) detector. A hydrophobic chromatography column (TSKgel Butyl-NPR; 4.6 mm I.D. × 10 cm; Tosoh Corporation) with a guard column was used for the separation. The HIC gradient was mobile phase "A" (20 mM of sodium phosphate, 1 M of ammonium sulfate, pH 7.0) with an increase from 0% to 100% of mobile phase "B" (20 mM of sodium phosphate, pH 7.0) in 25 min. Injection volume was 100 μL. Each mAb solution were adjusted to 0.3 mg/mL. Instrument control and data analysis were performed by Shimadzu LabSolutions LC/GC software.

Determination of the infectivity titer of virus

Viral infectivity was determined by $TCID_{50}$ assay after 10-fold serial dilution. Titer was calculated by the Spearman–Kärber Method.

Calculation of virus log reduction value (logarithmic reduction value)

The virus LRV for virus filtration trials is given by the following expression[4]:

$$Virus\ LRV = \log \frac{V1 \times T1}{V2 \times T2}$$

$V1 =$ *volume of starting material*, $T1 =$ *infectivity titer of starting material*, $V2 =$ *volume of material after the filtration*, *and* $T2 =$ *infectivity titer of material after the filtration*.

Evaluation of filterability of mAbs with high and low hydrophobicity

mAb A and mAb B were adjusted to 15 mg/mL in 100 mM acetate buffer, 200 mM NaCl (pH 5.5) and filtered in constant pressure, dead-end mode with compressed air on Planova S20N (0.0003 m^2) and Planova 20N filters for 3 h at 196 and 98 kPa, respectively. This evaluation was a single run.

Evaluation of filterability of mAb A in four different buffers

mAb A was adjusted to 10 mg/mL in the four buffer conditions shown in Table 3 and filtered in constant pressure, dead-end mode on Planova S20N filters (0.0003 m^2) for 3 h. This evaluation consisted of a single run for each condition.

Evaluation of the removability of viruses porcine parvovirus, of minute virus of mice, and bovine viral diarrhea virus

Feed of 1 mg/mL h-IgG in 100 mM NaCl was spiked with PPV, MVM, or BVDV and was filtered at 196 kPa in constant pressure, dead-end mode on Planova S20N filters (0. 001 m^2) to 150 L/m^2 followed by a process pause of 30 min and additional filtration of the same feed to 15 L/m^2. Filtrate samples were assayed for virus titer and pooled virus LRV was reported for each filtration. Evaluations were performed in duplicate. LRV was the average of the duplicate runs.

Evaluation of removability of EMC, pseudorabies virus, and HIV

Feed of 1 mg/mL h-IgG in 100 mM NaCl was with EMC, PRV, or HIV and was filtered at 196 kPa in constant pressure, dead-end mode on Planova S20N filters (0. 001 m^2) to 150 L/m^2 followed by a process pause of 30 min and additional filtration of the same feed to

15 L/m^2. The evaluations were performed in duplicate. LRV was the average of the duplicate runs. These runs, including virus preparation, were conducted at ViruSure Gmb under contract.

Evaluation of porcine parvovirus removal under three filtration pressure conditions with process pause

PPV was spiked to 10 mg/mL h-IgG in 100 mM NaCl and was filtered on Planova S20N filters (0. 001 m^2) to 300 L/m^2 followed by a process pause of 3 h and additional filtration of the same feed to 30 L/m^2 in constant pressure, dead-end mode at 49, 98, or 196 kPa. Filtrate samples were assayed for PPV titer, and PPV LRV before and after the process pause was determined. The evaluations were performed in duplicate.

Evaluation of of minute virus of mice removal using mAb A in two buffer conditions

Filtration of 10 mg/mL mAb A in spiked with MVM to 6.5 log $TCID_{50}$/mL in 50 mM acetate buffer, pH 5.0, 15 mS/cm (CEX buffer condition) or 50 mM Tris-HCl buffer, pH 7.0, 5 mS/cm (AEX buffer condition) on Planova S20N filters (0. 0003 m^2) was conducted at 196 kPa in constant pressure, dead-end mode for 3 h. Filtrations with each buffer condition were subjected to a process pause of 30 or 120 min and then repressurized to 196 kPa for additional filtration to 10 L/m^2. Filtrate samples were assayed for MVM to determine pooled MVM LRV for each filtration. The evaluations for each condition were performed in duplicate.

Evaluation of of minute virus of mice removal using multi-specific antibody

Multi-specific antibody adjusted to 6.0 mg/mL in 15 mM phosphate buffer, 400 mM NaCl, pH 7.5 and spiked with MVM at >5.8 log $TCID_{50}$/mL was filtered in duplicate at 193 kPa on Planova S20N filters (0.001 m^2) and at 83 kPa on Planova 20N filters in constant pressure, dead-end mode to 600 L/m^2, and after a process pause of 1 h and filtration of the same feed for 30 min, pooled MVM LRV was evaluated. The evaluations for each condition were performed in duplicate.

Evaluation of virus removability of low flux filtration for continuous processes

Filtration at constant, low flow rate of 5 LMH was conducted using a Masterflex L/S pump (Cole-Parmer with L/S Easy-Load II pump head. PPV spiked at 6.5 log $TCID_{50}$/mL into 10 mg/mL mAb A in 25 mM acetate buffer, 150 mM NaCl, pH 5.0 was supplied fresh every 24 h. Three filtrations were carried out in dead-end mode on Planova S20N (0.003 m^2). Filtrate collected and assayed for PPV titer to determine the pooled PPV LRV.

QUANTIFICATION AND STATISTICAL ANALYSIS

The number of filtration experimental repeats is indicated for each experimental methodology or corresponding figures and tables. Excel (Microsoft, WA, USA) was used for statistical analysis and data visualization.