Viral Clearance in mAb Downstream Processing

Considerations and Expectations for Unit Operations

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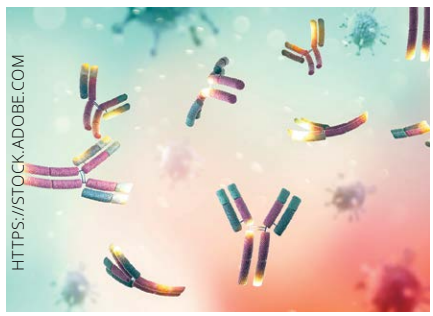
Since the initial discovery of viruses, viral contamination has been a human concern. Continuing throughout the development of biologic therapies in the 20th century, this concern came to a peak in the 1980s when human immunodeficiency virus (HIV) infections were caused by contaminated blood transfusions. Such events compounded by others from the 1960s and 1970s led to a need for globally harmonized regulatory requirements to ensure viral safety of efficacious biological treatments for patients. In April 1990, the first meeting to discuss harmonization efforts was attended by multiple government agencies and industry representatives from Europe, Japan, and the United States. That was the birth of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH).

Focusing on patient safety led to publication of the ICH Q5A guideline, which has been the global compass for safety in biotechnology products derived from cell lines of human or animal origin since its initial publication in 1995 (**1**). To prevent viral contamination in such products, biomanufacturers combine three complementary strategies: prevention, testing, and removal/inactivation. Thus, the viral clearance (VC) potential of a biomanufacturing process is a key aspect of the viral safety strategy. The main guiding principle to satisfy ICH guidelines is orthogonality using both dedicated and nondedicated processing steps for VC. Each step must be



independent from the others, with a different mechanism of action. Dedicated unit operations, such as chemical inactivation and virus filtration, maintain that orthogonality and establish physical barriers of safety. Nondedicated steps also might remove viruses physically, but usually to a lesser extent. The cumulative measurement of virus removal/inactivation by all steps within a manufacturing process provides a safety factor that should satisfy the viral safety requirements of most regulatory agencies.

General Considerations

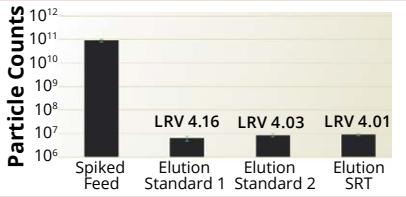
VC studies typically are performed before a company files its investigational new drug (IND) or biological licensing applications (BLAs) with the US Food and Drug Administration (FDA) — or equivalent regulators in other countries. Such studies should be performed after a downstream process has been "locked" because a change made to one step can have consequences on the unit operations that follow it. For example, applying a specialized intermediate wash after column loading in a protein A affinity capture step could result in a product pool with a different impurity profile than was obtained without the wash. As a result, that could change the impurity profile of all the subsequent downstream steps' product pools and in turn change the overall VC results.

Whether collaborating with an in-house or contracted virus-testing group, it is important to work closely together to establish an appropriate testing design for your process. Principles such as duplicate experiments, positive and negative controls, and statistical analysis of resulting data should be standard practice. An appropriate model-virus testing panel should be phase-appropriate and established by the testing group in consultation with strategic partners. For IND filing purposes with monoclonal antibody (mAb) processes based on Chinese hamster ovary (CHO) cells, for example, it is common to test only minute virus of mice (MVM) and xenotropic murine leukemia virus (XMuLV).

Virus clearance is expressed in terms of log-reduction value (LRV), the $\log_{10}$ of the ratio of input to output virus concentration in a step or process. An LRV ≥4 generally is considered to be robust, but anything between 1 and 3 log can contribute to VC of an overall downstream process. According to ICH guidelines, VC results <1 log are discarded (**1**). Summing the results from multiple steps will provide a cumulative LRV that can be used to calculate the overall process safety factor established by ICH.

Control experiments need to be accounted for in VC studies. For

Figure 1: MockV particle clearance with MabCapture C resin — MockV particles were spiked into in harvested cell culture fluid (HCCF) from Chinese hamster ovary (CHO) cells to a concentration of 1×10^{11} particles; SRT = short residence time.



chromatography studies, a baseline experiment should be run to help qualify scale-down tests. This qualification run at bench-scale uses the same buffers, columns, and so on as what will be used for virus-spiked experiments, except without the spike. Comparing pH, conductivity, elution, and impurity profiles all without virus present is good practice to ensure that a scale-down model is representative of the full-scale process. Guidelines suggest VC testing under worst-case conditions for a given unit operation, which are determined through a risk analysis (**2**).

VC studies commonly are performed in specialized facilities and are associated with high costs. In recent years, the use of mock virus particles (MVPs), as in MockV assay kits from Cygnus Technologies, has been reported for different filtration and chromatography applications (**3–5**). As illustrated in Figure 1, such tools can be used in typical bioprocess laboratory settings and can help companies understand potential VC for actual viruses.

Unit Operations

Monoclonal antibodies make up a large percentage of approved biotherapeutics as well as those in clinical development. As such, VC in mAb manufacture is broadly understood, with multiple published compilations about such performance for typical unit operations in mAb processes (**6–9**).

Affinity chromatography leverages the high specificity of a ligand for a given target, which allows the majority of impurities to flow through a column. Elution typically is accomplished using low pH buffers, as in the case of protein A affinity chromatography for mAbs.

Table 1: Comparing overload and B/E modes for viral clearance with POROS XS media

	Overloaded	Bind–Elute
Load amount (mAb1/resin)	2000 g/L	85 g/L
Viral clearance (log-reduction value, LRV) with 95% confidence limit		
Murine leukemia virus (MLV)	6.09 ± 0.89 LRV	6.19 ± 0.47 LRV
Minute virus of mice (MVM)	2.62 ± 0.39 LRV	2.17 ± 0.51 LRV
Pseudorabies virus (PRV)	≤5.71 ± 0.38 LRV	6.43 ± 0.42 LRV
Reovirus 3	8.03 ± 0.93 LRV	6.03 ± 0.29 LRV

For PRV, ≤ indicates that the virus level in the product pool (≤2000 grams monoclonal antibody per liter of resin) was below the detection limit of the large-volume titration assay.

Acidic solutions can interfere with VC evaluation when immunoassays use live cells that can be harmed by those conditions. Low pH also can inactivate enveloped viruses. For those reasons, quantitative polymerase chain reaction (qPCR) analysis is performed to measure viruses in this type of mAb-process sample.

LRVs for protein A affinity chromatography range between 2.5 and 5.5 for retroviruses and ≤3 for parvoviruses (**6**). Investigations into the reasons for such a wide range of clearance have suggested that mAb-specific virus–protein interactions can take place (**10, 11**). The VC capability of the Thermo Scientific MabCaptureC protein A resin has been evaluated using MVM and MVP particles, for which an LRV of 4 was obtained with an immunoglobulin G1 (IgG1) at both 3-min and 6-min residence times (Figure 1).

Chemical inactivation of enveloped viruses typically is achieved with a low-pH holding step. ICH outlines that a kinetic curve must be established for this time-dependent step. A good starting place for determining the parameters to apply here is standard E2888-12 from the American Society of Testing and Materials (ASTM). A modular claim of 5 log may be made if process parameters are maintained and incubation is held to at least 60 minutes. According to VC results compilations in the public domain (**6, 7**), LRVs of >4 log are achieved commonly with pH 3.5–3.8 and incubation periods >60 minutes (**6**). For cases in which target proteins are not stable at low pH, virus inactivation often is achieved using detergents (**12, 13**) in what is also a robust unit operation for VC. Note that when detergent inactivation is included in a mAb process, it is more commonly applied during cell-culture harvest before affinity capture.

Polishing steps in downstream purification typically involve ion-exchange chromatography (IEC) and/or hydrophobic-interaction chromatography (HIC) for removal of product- and process-related impurities. For molecules with neutral or alkaline isoelectric points, anion exchange (AEX) provides a robust method for reduction of residual host-cell proteins and DNA in a flow-through operation. Average LRVs in the range of 5–6 for retro- and parvoviruses have been observed commonly with both flow-through and bind–elute separation modes (**6**). Such high LRV values generally are obtained with neutral or slightly alkaline pH and relatively low conductivity levels.

Thermo Scientific's POROS AEX resins have provided such LRV results in many VC studies. For example, researchers from Bristol Myers Squibb and Thermo Fisher Scientific investigated the VC of POROS 50 HQ resin for multiple mAbs. For a couple of those mAbs, the LRV was >5 log even with column loadings in the range of 200–500 g/L (**14**).

Other methods of polishing chromatography — e.g., HIC and cation exchange (CEX) — are used to remove aggregates and other impurities in some mAb downstream processes. In VC studies, however, such steps have accounted for just 13% of overall LRV contributions (**6**), with typical LRV values often <3 (**6, 7, 9**) that can vary widely for different mAbs and their specific process conditions. Scientists from Daiichi Sankyo have reported robust virus clearance for multiple viruses using POROS XS resin in both bind–elute and overloaded modes (Table 1) (**15**).

For HIC, data are more limited, but VC is also variable and mAb/process-dependent. However, positive VC results were reported by scientists at

Table 2: Viral clearance results for POROS Benzyl Ultra hydrophobic-interaction chromatography (HIC) flow-through unit operation in a case-study monoclonal antibody (mAb) process

Run	Yield	LRV
Qualification	85%	—
Xenotropic murine leukemia virus (XMuLV)	84%	>5.97
Minute virus of mice (MVM)	85%	4.56

Ambrx (now part of Johnson & Johnson) (**16**). They operated POROS Benzyl Ultra resin in flow-through mode at low conductivity and pH 5.5, obtaining LRVs of >5 and 4.5 for XMuLV and MVM, respectively (Table 2).

Virus-retentive filters operate based on the principles of size exclusion. The filter used should have pores that are smaller than the virus of concern but large enough for target proteins to pass through. Because this principle condenses down to just one variable (size), a testing approach with a single small-virus model such as MMV can be used to determine the LRV for a given filter (**17, 18**). The current generation of virus filters can achieve a median LRV of 5.8 (**6**).

A Key Aspect of Viral Safety

As part of ensuring patient safety, VC is important to the overall viral safety strategy in biomanufacturing. In downstream processes, chromatography steps can contribute in significant ways to the VC section of a company's regulatory package. Industry prior knowledge can guide some process development to meet viral safety requirements, but ultimately each developer must perform its own VC studies demonstrating robustness of well-characterized processes. Thermo Fisher Scientific's chromatography products are used successfully in many such processes, and technical experts within our organization can help you address many technical challenges.

References

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

2 Shah DA, et al. Worst-Case Conditions for Viral Clearance. *BioProcess Int.* 20(11–12)i 2022: 7–14; https://www.bioprocessintl.com/viral-clearance/worst-case-conditions-for-viral-clearance.

3 Orchard JD, et al. Using a Noninfectious MVM Surrogate for Assessing Viral Clearance During Downstream Process Development. *Biotechnol. Prog.* 36(1) 2020: e2921; https://doi.org/10.1002/btpr.2921.

4 Cetlin D, et al. Use of a Noninfectious Surrogate To Predict Minute Virus of Mice Removal During Nanofiltration. *Biotechnol. Prog.* 34(5) 2018: 1213–1220; https://doi.org/10.1002/btpr.2694.

5 Gulla KC, et al. High-Throughput Chromatography and Analytics Can Inform Viral Clearance Capabilities During Downstream Process Development for Biologics. *Biotechnol. J.* 16(9) 2021: 2000641; https://doi.org/10.1002/biot.202000641.

6 Ajayi OO, et al. An Updated Analysis of Viral Clearance Unit Operations for Biotechnology Manufacturing. *Curr. Res. Biotechnol.* 4, 2022: 190–202; https://doi.org/10.1016/j.crbiot.2022.03.002.

7 Cipriano D, Burnham M, Hughes JV. Effectiveness of Various Processing Steps for Viral Clearance of Therapeutic Proteins: Database Analyses of Commonly Used Steps. *Meth. Molec. Biol.* 899, 2012: 277–292; https://doi.org/10.1007/978-1-61779-921-1_18.

8 Stanley B, et al. Twenty Plus Years of Data Demonstrating Virus Filtration as an Effective and Robust Step for Large Virus Removal. *PDA J. Pharm. Sci. Tech.* 76(1) 2022: 1–8; https://doi.org/10.5731/pdajpst.2020.012591.

9 Remington KM. Fundamental Strategies for Viral Clearance — Part 1: Exploring the Regulatory Implications. *BioProcess Int.* 13(1) 2015: 10–15, 26; https://www.bioprocessintl.com/viral-clearance/fundamental-strategies-for-viral-clearance-part-1-exploring-the-regulatory-implications.

10 Bach J, Connell-Crowley L. Clearance of the Rodent Retrovirus, XMuLV, by Protein A Chromatography. *Biotechnol. Bioeng.* 112(4) 2015: 743–750; https://doi.org/10.1002/bit.25484.

11 Pan C, et al. Characterizing and Enhancing Virus Removal by Protein A Chromatography. *Biotechnol. Bioeng.* 116(4) 2019: 846–856; https://doi.org/10.1002/bit.26866.

12 Miesegaes G. Viral Clearance by Traditional Operations with Significant Knowledge Gaps (Session II): Cation Exchange Chromatography (CEX) and Detergent Inactivation. *PDA J. Pharm. Sci. Technol.* 68(1) 2014: 30–37; https://doi.org/10.5731/pdajpst.2014.00964.

13 Conley L, et al. Evaluation of Eco-Friendly Zwitterionic Detergents for Enveloped Virus Inactivation. *Biotechnol. Bioeng.* 114(4) 2017: 813–820; https://doi.org/10.1002/bit.26209.

14 Hung J, et al. Impact of Virus–Antibody Interactions on Viral Clearance in Anion Exchange Chromatography. *J. Chromatog. A* 1633, 2020: 461635; https://doi.org/10.1016/j.chroma.2020.461635.

15 Masuda Y, et al. Cation Exchange Chromatography Performed in Overloaded Mode Is Effective in Removing Viruses During the Manufacturing of Monoclonal Antibodies. *Biotechnol. Prog.* 35(5) 2019: e2858; https://doi.org/10.1002/btpr.2858.

16 Knudsen N, Menendez A, Appel J. POROS Benzyl Ultra Viral Clearance and Impurity Removal for a Novel Antibody Format Used in an Enhanced Antibody Drug Conjugate (ADC). Thermo Fisher Scientific, 2023; https://assets.thermofisher.com/TFS-Assets/BPD/posters/poros-benzyl-utlra-clearance-poster.pdf.

17 Chen D. Viral Clearance Using Traditional, Well-Understood Unit Operations (Session I): Virus-Retentive Filtration. *PDA J. Pharm. Sci. Technol.* 68(1) 2014: 38–50; https://doi.org/10.5731/pdajpst.2014.00965.

18 Gefroh E, et al. Use of MMV as a Single Worst-Case Model Virus in Viral Filter Validation Studies. *PDA J. Pharm. Sci. Technol.* 68(3) 2014: 297–311; https://doi.org/10.5731/pdajpst.2014.00978.

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