WHITE PAPER

Moving Beyond Triton X-100: Selecting a Viral Inactivation Strategy for Today's Bioprocessing Workflows

Viral safety is a fundamental pillar of biopharmaceutical manufacturing. Whether producing monoclonal antibodies (mAbs), recombinant proteins, plasma-derived therapeutics, vaccines, or emerging cell and gene therapies, manufacturers must demonstrate robust viral clearance throughout the manufacturing process to ensure patient safety and regulatory compliance. For decades, detergent-mediated viral inactivation has been a cornerstone of that strategy because it effectively inactivates enveloped viruses while integrating well into downstream purification workflows.

Historically, Triton™ X-100 has been the industry standard for detergent-based viral inactivation. However, environmental regulations restricting octylphenol ethoxylates, evolving sustainability objectives, and recent updates to ICH Q5A(R2)[1] have accelerated the search for alternative chemistries that maintain viral safety while addressing changing regulatory and environmental expectations.

At the same time, biologics manufacturing itself has become more complex. Product pipelines now extend well beyond conventional mAbs to include bispecific antibodies, Fc-fusion proteins, recombinant proteins, viral vectors, and other advanced modalities, while manufacturing platforms have become increasingly flexible and intensified. In this environment, viral inactivation can no longer be viewed as a stand-alone unit operation. The selected technology must support product quality, downstream purification, analytical characterization, operational efficiency and sustainability alongside robust viral clearance.



This whitepaper explores how the criteria for selecting detergent-based viral inactivation technologies are evolving and uses J.T.Baker® Viral Inactivation Solution as a case study to examine the technical, operational, and environmental considerations that increasingly influence process development decisions.

SELECTING THE RIGHT VIRAL INACTIVATION STRATEGY

The challenge facing manufacturers today is not simply replacing Triton X-100; rather, it is selecting a viral inactivation strategy that fits the broader manufacturing process.

While robust viral clearance remains the primary requirement, it is no longer the only consideration. Process developers must also evaluate how viral inactivation technology affects molecule stability, downstream purification, analytical testing, implementation within existing manufacturing platforms and long-term regulatory and sustainability objectives. For many next-generation biologics, these factors can be just as important as viral reduction performance itself.

This broader decision-making process is reflected across the industry. According to BioPhorum, manufacturers continue to evaluate multiple Triton X-100 alternatives, with no single detergent yet emerging as the preferred replacement (Figure 1).[2] Instead, organizations are assessing candidate technologies based on a combination of viral efficacy, process compatibility, downstream removal, analytical support, regulatory acceptance, supply chain considerations and ease of implementation.

This shift reflects a broader change in process development philosophy. Rather than asking which detergent most closely resembles Triton X-100, manufacturers are increasingly evaluating which viral inactivation strategy best supports the overall performance, robustness and sustainability of the manufacturing process.

58%
of BioPhorum respondents were still using Triton X-100

63%
of companies that had switched were still in the research phase

84%
ranked regulatory approval among the most important selection criteria

Figure 1. Replacement of Triton™ X-100 remains an active area of technical evaluation across the biopharma industry, with BioPhorum Operations Group reporting that multiple alternatives are still under assessment and that no single detergent has yet emerged as a broadly accepted standard.

HOW DETERGENT-MEDIATED VIRAL INACTIVATION WORKS

Detergent-mediated viral inactivation has remained a preferred viral clearance strategy because it targets the lipid membrane, one of the most vulnerable structural components of enveloped viruses. Enveloped viruses rely on this outer membrane to infect host cells; detergents disrupt the lipid bilayer, causing the viral envelope to disintegrate and rendering the virus incapable of attaching to or entering target cells. Once the envelope is disrupted, the virus can no longer replicate or initiate infection.

This mechanism offers several important advantages. Unlike some physical removal methods, detergent-mediated viral inactivation does not depend on virus size or filtration efficiency. Instead, it directly eliminates viral infectivity while maintaining compatibility with a broad range of biologic manufacturing processes. As a result, detergent treatment has become one of the most widely used orthogonal viral clearance steps.

Regulatory guidance recommends demonstrating viral clearance using representative model viruses that encompass a range of viral characteristics and resistance profiles.[3] For detergent-based viral inactivation, studies commonly employ xenotropic murine leukemia virus (XMuLV), human herpes simplex virus type 1 (HSV-1)/pseudorabies virus (PRV), and bovine viral diarrhea virus (BVDV). Together, these viruses represent retroviruses, herpesviruses and flaviviruses, providing confidence that viral inactivation strategies remain effective across structurally diverse enveloped viruses.

While the underlying mechanism is straightforward, successful implementation requires considerably more than effective viral disruption. Viral inactivation technology must perform consistently under representative manufacturing conditions, preserve product quality, integrate into downstream purification, and support analytical characterization throughout process development and commercial manufacturing. These broader requirements increasingly distinguish modern viral inactivation technologies from simple detergent replacements.

WHAT DEFINES A MODERN VIRAL INACTIVATION STRATEGY?

An effective viral inactivation strategy must first provide robust and reproducible viral clearance. Manufacturers must demonstrate sufficient log reduction values across representative model viruses while maintaining performance under worst-case operating conditions. Consistency across temperatures, process times and manufacturing scales is equally important because viral inactivation represents a validated critical process step.

Equally important is preservation of product quality. Many next-generation biologics exhibit greater structural complexity than traditional mAbs. Bispecific antibodies, recombinant proteins, antibody fragments, and Fc-fusion proteins may be more susceptible to aggregation, conformational changes, or reduced stability when exposed to certain processing conditions such as low pH viral inactivation. Consequently, process developers increasingly evaluate viral inactivation technologies not only for viral efficacy but also for their impact on critical quality attributes.

Downstream compatibility has also become a defining consideration. Viral inactivation should integrate seamlessly with purification operations rather than requiring extensive process redesign. Developers must understand how detergent treatment affects Protein A chromatography, polishing operations, resin performance, residual detergent clearance, analytical monitoring, and overall manufacturing efficiency.

Operational considerations have likewise become increasingly important. Ready-to-use formulations, simplified handling, reduced foaming, compatibility with continuous manufacturing, and ease of implementation all contribute to manufacturing robustness. As facilities seek greater flexibility and higher productivity, viral inactivation technologies that simplify operations while maintaining performance offer meaningful advantages.

Sustainability is another critical process attribute. Manufacturers increasingly seek chemistries that reduce environmental impact while supporting long-term regulatory compliance. This extends beyond replacing restricted chemicals; it includes evaluating biodegradability, waste reduction, worker safety, supply chain resilience and alignment with broader corporate sustainability objectives.

Taken together, these considerations illustrate why viral inactivation has evolved from a single unit operation into a multidisciplinary process development decision. Manufacturers must balance virology, protein chemistry, purification science, process engineering, regulatory expectations and environmental stewardship when selecting the most appropriate viral inactivation strategy.

The following sections examine how these considerations can be applied in practice through the evaluation of J.T.Baker® Viral Inactivation Solution, focusing not only on viral clearance performance but also on the broader process characteristics that increasingly define successful implementation in modern bioprocessing.

J.T.BAKER® VIRAL INACTIVATION SOLUTION

The evolution of viral inactivation technologies reflects the industry's broader shift toward designing processes that simultaneously improve product quality, operational efficiency, and environmental sustainability. One example of this approach is J.T.Baker® Viral Inactivation Solution, a detergent-based viral inactivation technology developed specifically to address both the regulatory and environmental pressures related to Triton X-100 and the changing technical requirements of next-generation biologics manufacturing.

Unlike traditional Triton X-100 formulations, J.T.Baker® Viral Inactivation Solution is based on a proprietary nonionic detergent that is structurally and functionally similar to Triton X-100 but does not contain alkylphenol ethoxylates. It is supplied as a ready-to-use 10% active aqueous solution manufactured under cGMP conditions, eliminating the need for on-site detergent preparation and reducing opportunities for operator error and process variability.

The formulation was designed with sustainability as a key consideration. The detergent is readily biodegradable, is not listed as a Substance of Very High Concern (SVHC) under REACH, and has been recognized through the U.S. Environmental Protection Agency's (EPA) Safer Choice program. OECD biodegradability testing demonstrated rapid degradation under aerobic conditions, reducing concerns regarding environmental persistence while providing manufacturers with an alternative aligned with evolving sustainability initiatives. Although these environmental characteristics are important, they represent only one aspect of successful detergent replacement. Ultimately, manufacturers require evidence that alternative technologies perform reliably within commercial manufacturing environments.

Demonstrating Robust Viral Inactivation

The primary objective of any detergent-based viral inactivation technology remains unchanged: rapid and reproducible inactivation of enveloped viruses under representative manufacturing conditions.

To evaluate viral clearance performance, J.T.Baker® Viral Inactivation Solution was tested using three model enveloped viruses recommended by ICH Q5A guidance: XMuLV, HSV-1, and BVDV. These viruses span a range of genome types, sizes, and resistance characteristics and provide confidence that the detergent performs consistently across representative viral families.

Viral inactivation studies were conducted using clarified harvest material at both 4°C and 15°C, temperatures representative of common bioprocessing conditions (Figure 2). Viral inactivation began immediately following detergent addition, and by the completion of the study, the solution achieved greater than 5–6 log_{10} reduction values (LRVs) across all virus models. Importantly, performance remained consistent across both temperatures, demonstrating that robust viral clearance can be maintained even under low-temperature process conditions.

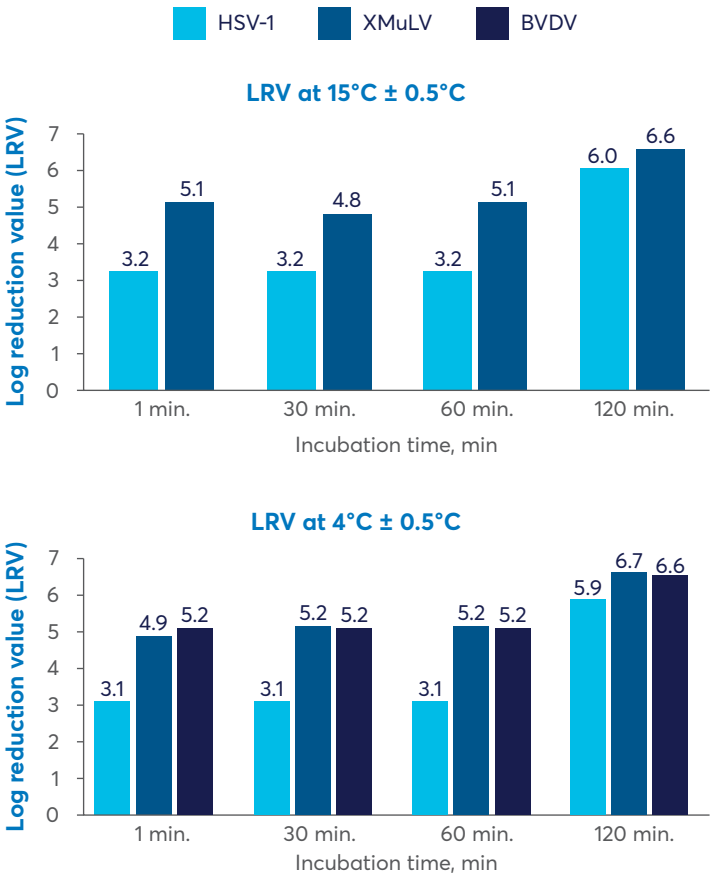


Figure 2. LRV by J.T.Baker® Viral Inactivation Solution at 15°C and 4°C using large volume method for the 120-minute sample to enhance assay sensitivity and achieve low limit of detection (LOD).

This temperature independence offers practical advantages for process developers because it reduces constraints on upstream and downstream operations. Rather than requiring narrowly defined operating conditions, manufacturers have greater flexibility to integrate viral inactivation into existing workflows while maintaining validated viral clearance.

Independent published work further supports these findings. In studies evaluating Triton X-100 substitutes across multiple clarified CHO harvest matrices, J.T.Baker® Viral Inactivation Solution demonstrated robust viral inactivation at relatively low detergent concentrations while maintaining performance across monoclonal antibody, bispecific antibody, and recombinant protein processes. These data suggest that effective viral clearance can be achieved while preserving flexibility during process development and optimization.

An increasingly important consideration in process development is understanding how detergent concentration influences both viral clearance and overall process performance.

Historically, detergent-based viral inactivation has often been viewed as a fixed operating parameter. However, greater flexibility in detergent concentration can expand process design space, allowing manufacturers to optimize viral safety while minimizing detergent burden during downstream purification.

Experimental studies demonstrated that J.T.Baker® Viral Inactivation Solution remained effective at concentrations substantially below traditional working levels. Viral clearance exceeding 4 log_{10} was achieved at concentrations as low as approximately 0.15% (v/v), while complete viral inactivation was maintained across higher concentration ranges. Independent studies similarly demonstrated robust viral reduction showing ≥5.6 log_{10} viral reduction for XMuLV and ≥6.17 log_{10} viral reduction for PRV, at low working concentrations following incubation at 18 °C for 120 minutes. (Figure 3).[4] For process development teams, this flexibility may simplify optimization activities. Lower detergent loading has the potential to reduce residual detergent burden, simplify downstream removal and provide greater freedom when designing manufacturing processes for different product modalities.

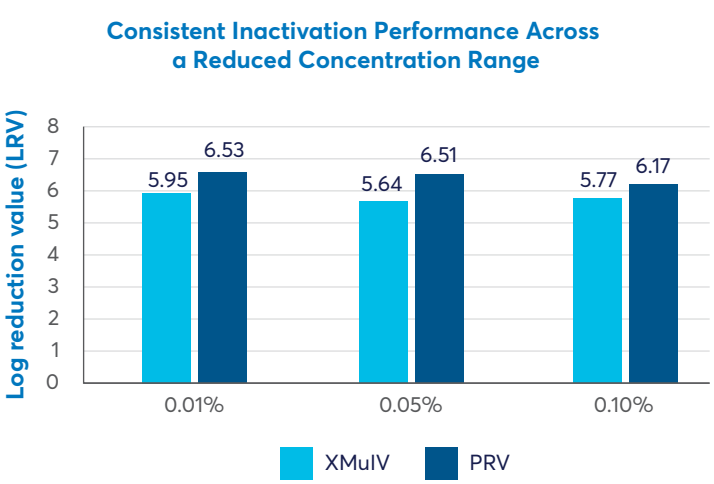


Figure 3. J.T.Baker® Viral Inactivation Solution achieves ≥5.6 LRV at 18 °C and 120 minutes, demonstrating robust efficacy at lower use concentrations.

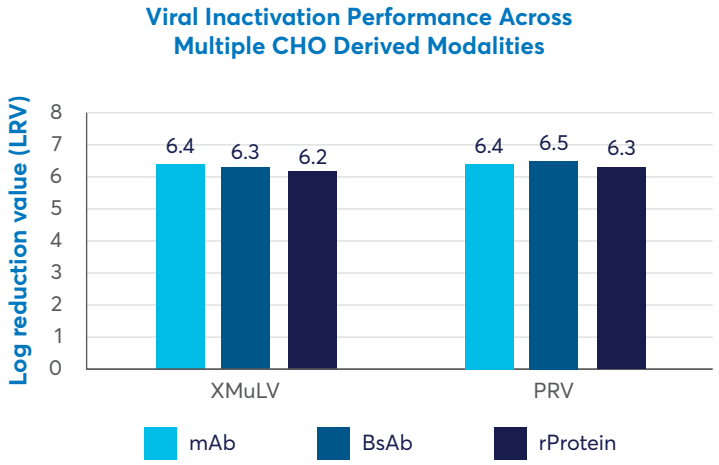


Figure 4. J.T. Baker® Viral Inactivation Solution performance across CHO-derived modalities treated with 0.05% final detergent concentration for 60 minutes at 18 °C.

Rather than viewing detergent concentration as a fixed specification, developers can evaluate concentration within a broader process design space that balances viral clearance, manufacturability, and downstream performance.

Protecting Product Quality Through Process Compatibility

Although viral reduction is essential, it is only one component of successful viral inactivation. A process that achieves excellent viral clearance, but compromises product quality or purification performance ultimately creates additional manufacturing challenges.

For many emerging biologics, product stability represents one of the most significant concerns. Bispecific antibodies, Fc-fusion proteins, recombinant proteins and other structurally complex molecules may be susceptible to aggregation or conformational changes during processing. Conventional low-pH viral inactivation, while highly effective for many products, is not universally appropriate and may require extensive molecule-specific optimization.

To evaluate process compatibility, J.T.Baker® Viral Inactivation Solution was assessed across multiple representative biologic modalities, including IgG1 monoclonal antibodies, Fab fragments, bispecific antibodies and Fc-fusion proteins. Following detergent treatment and downstream purification, product purity remained essentially unchanged relative to untreated controls across each modality evaluated (Figure 4).[4]

Structurally diverse mAbs and analogous proteins, such as Fc-fusion proteins or BsAbs, may not be suitable for low pH treatment. Low pH has been shown to cause aggregation leading to decreased protein stability and potency, and an immunogenic response in patients.[5] J.T. Baker® Viral Inactivation Solution preserved product quality far more effectively than low pH treatment, with 91% purity and 5% aggregates versus 46% purity and 49% aggregates after low pH condition (Figure 5).

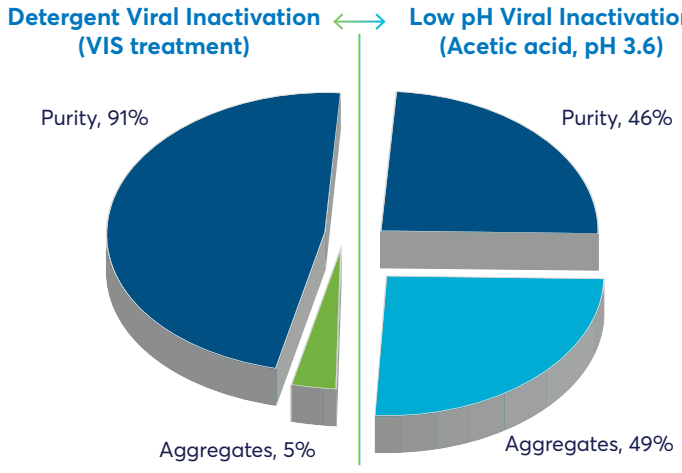


Figure 5. Enhancing product integrity through use of detergent-based viral inactivation.

To examine protein stability after extended detergent exposure, BsAb harvest was treated with 0.1% detergent for 24 hours, purified by protein A and analyzed using SEC-HPLC. No impact to protein stability occurred after extended exposure, as shown in comparative results in Figure 6.

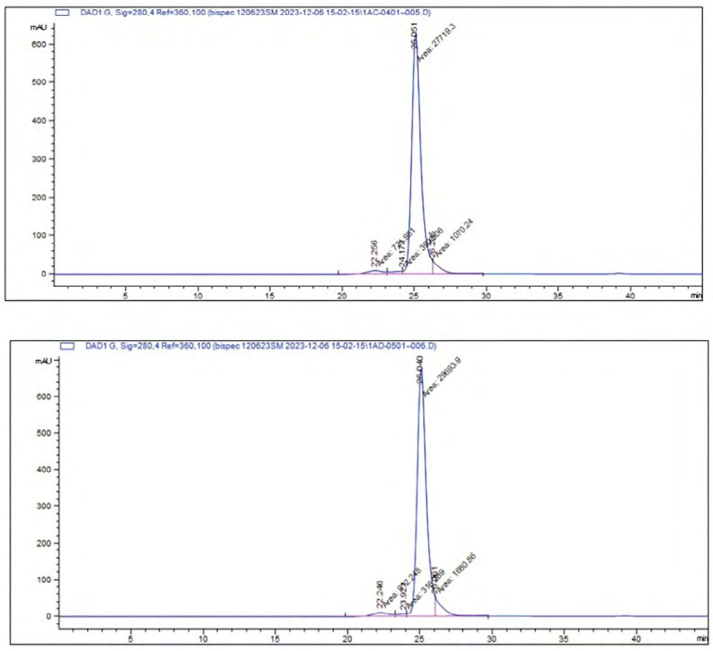


Figure 6. SEC profile of BsAb control (top) and after 24-hour detergent treatment (bottom).

Integrating with Downstream Purification

A successful viral inactivation technology must fit seamlessly into downstream purification operations. Protein A chromatography remains the workhorse capture step for mAb manufacturing, making compatibility with Protein A resins particularly important. Studies evaluating VIS demonstrated no measurable impact on Protein A dynamic binding capacity or product recovery following detergent treatment, indicating that viral inactivation can be incorporated without compromising one of the most critical purification operations.

Residual detergent must also be removed efficiently before final product manufacture. Clearance studies using J.T.Baker® Viral Inactivation Solution showed that detergent concentrations were reduced from approximately 1,000 ppm to below 2 ppm following Protein A chromatography and below the limit of quantitation following subsequent polishing operations using either anion exchange or hydrophobic interaction chromatography (Figure 7).

Impact of J.T.Baker® Viral Inactivation Solution treatment on binding capacity and recovery of Protein A resin

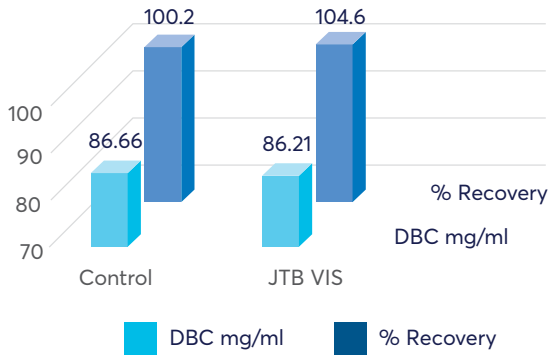


Figure 7. DBC and recovery after treatment with detergent.

Supporting analytical methods for residual detergent quantification, together with toxicological assessments establishing permitted daily exposure (PDE) values, provide manufacturers with additional tools for process validation and regulatory documentation. The results of a detergent clearance study shown in Figure 8 demonstrate that 1,000 ppm of detergent was reduced to < 2 ppm after Protein A and to <1 ppm/LOQ after single-step polishing by either anion exchange (AEX) or hydrophilic interaction chromatography (HIC).

Collectively, these data reinforce that viral inactivation cannot be evaluated independently of downstream purification. Instead, successful implementation depends on preserving the performance of the entire manufacturing platform.

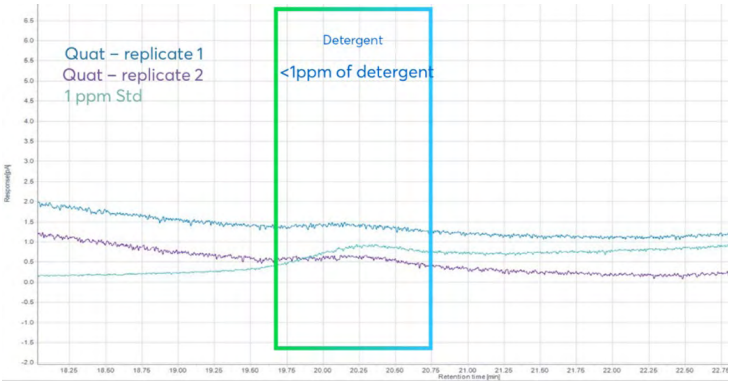


Figure 8. 2 mg/mL fusion protein, 0.1% detergent, AEX clearance.

OPERATIONAL CONSIDERATIONS BEYOND VIRAL CLEARANCE

While technical performance remains the primary selection criterion, practical manufacturing considerations increasingly influence technology adoption. Ready-to-use formulations eliminate on-site preparation and simplify material handling, reducing opportunities for process variability while supporting manufacturing efficiency. Lower viscosity and reduced foaming characteristics further improve operational performance by minimizing common processing issues, such as excessive foam generation, vent filter wetting, pressure fluctuations, and potential protein losses associated with foam formation.

J.T.Baker® Viral Inactivation Solution was also designed for compatibility with both traditional batch manufacturing and continuous bioprocessing workflows. As manufacturers continue adopting intensified processing strategies, technologies that integrate across multiple manufacturing platforms become increasingly valuable because they reduce the need for process-specific reformulation or redevelopment.

These operational attributes may appear secondary to viral clearance, yet they frequently determine how easily new technologies can be implemented at commercial scale.

LOOKING AHEAD

The replacement of Triton X-100 represents more than a response to changing regulations. It reflects a broader evolution in how manufacturers evaluate critical process technologies.

Modern viral inactivation strategies are expected to achieve far more than robust virus clearance alone. They must preserve product quality, integrate with downstream purification, support analytical characterization, simplify manufacturing operations, accommodate increasingly complex biologics and contribute to long-term sustainability objectives.

Viral inactivation thus becomes an integrated element of process development rather than an isolated unit operation.

J.T.Baker® Viral Inactivation Solution illustrates how detergent-based viral inactivation technologies are evolving to meet these expanding expectations. The data presented in this whitepaper demonstrate that effective viral clearance can be combined with process compatibility, downstream integration, analytical support, and environmental stewardship without requiring manufacturers to compromise product quality or manufacturing robustness.

As biologics continue to diversify and manufacturing platforms become increasingly flexible, the technologies that deliver the greatest value will be those that support the entire process, not just one critical step. Viral inactivation is no exception. The future of viral safety will be defined not only by how effectively viruses are eliminated, but by how well viral inactivation technologies contribute to resilient, efficient ,and sustainable biomanufacturing.

Endnotes

1 https://database.ich.org/sites/default/files/ICH_Q5A(R2)_Guideline_2023_1101.pdf
2 BioPhorum Operations Group Ltd. (2025, September), *Replacement of Triton X-100 in the biopharma industry.* DOI: 10.46220/2025DG001
3 https://www.fda.gov/media/163115/download
4 Du, Y. and Wu, S. (2025). Exploring the design space for Triton X-100 substitutes in viral inactivation applications . Biotechnology Progress, e70069 . https://doi .org/ 10.1002/btpr.70069
5 Lundahl MLE, Fogli S, Colavita PE, Scanlan EM. Aggregation of protein therapeutics enhances their immunogenicity: causes and mitigation strategies. RSC Chem Biol. 2021 May 4;2(4):1004-1020. doi: 10.1039/d1cb00067e.

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