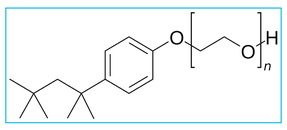
Safeguarding Biologics with a Revolutionary cGMP Compliant Viral Inactivation Solution: Pioneering a Sustainable Alternative to Triton X-100

Authors:
Tuhidul Islam, Willy Hesselink, Jungmin Oh, Nandkumar Deorkar, & Beth Fahnestock | Avantor, Bridgewater, NJ

Challenges and opportunities

To ensure the safety of biopharmaceuticals derived from human or animal origin, including plasma proteins, monoclonal antibodies, recombinant proteins, vaccines and gene therapies, it is critical that biomanufacturing processes can clear or inactivate potential viral contaminants.[1,2]



Triton™ X-100

One of the techniques for viral inactivation is detergent treatment.[3] Triton™ X-100[4] has been widely used for this process, however, it has been subjected to increased scrutiny due to potential environmental and health impacts.

In response, substance group 2-[4-(2,4,4-trimethylpentan-2-yl) phenoxy]ethanol, which includes Triton™ X-100, has been designated a "substance of very high concern" by the European Chemicals Agency's Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) regulations.[5]

Avantor has developed a novel viral inactivation solution, formulated with non-ionic detergent, that can improve the efficiency in virus inactivation. A recent study completed in conjunction with a CRO highlights the key benefits of J.T.Baker® Viral Inactivation Solution (JTB VIS):

J.T.Baker® Viral Inactivation Solution

- free of alkylphenols – similar structure to Triton X-100 without benzene ring
- achieves > 6.5 LRV prior to protein A capture step
- preserves integrity of protein of interest for product stability
- does not impact dynamic binding capacity, yield or purity in protein A step
- efficient removal of detergent through subsequent purification steps
- readily biodegradable to meet regulatory compliance standards and sustainability initiatives

Viral inactivation efficacy

The virus inactivation (VI) capability of JTB VIS was investigated with three different model viruses: XMuLV, HSV-1 and BVDV. Table 1 summarizes the characteristics of the virus panel. The most used model enveloped virus in monoclonal antibody (mAb), manufacturing is XMuLV which represents the relevant retrovirus-like particles that are endogenous to CHO cells. HSV-1 represents model for herpesviruses, while BVDV represents model for Hepatitis C virus which is a potential contaminant of human plasma-derived products.[6,7]

The VI study was performed at 15±0.5 °C and 4±0.5 °C temperature, and the timepoint samples, <1, 30, and 60 min were analyzed by standard testing method to observe the trend in virus killing ability of VIS. Results shown in Figure 1 and 2.

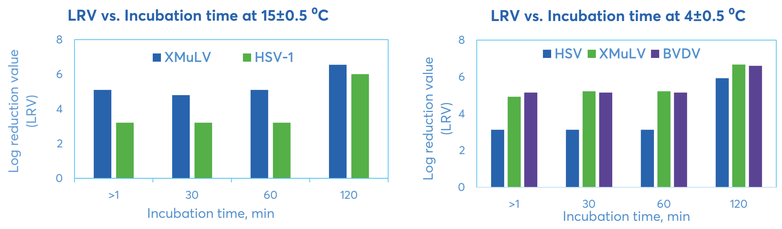


Figure 1 & 2: Log reduction value (LRV) by JTB VIS at 15±0.5 °C and 4±0.5 °C. Large volume testing method was applied for 120 min sample to maximize the assay sensitivity with low lower limit of detection (LOD).

Virus	Bovine viral diarrhea virus (BVDV)	Xenotropic murine leukemia virus (XMuLV)	Human herpes simplex virus type 1 (HSV-1)
Family	Flaviviridae	Retroviridae	Herpesviridae
Strain	NADL	NFS TH-1	HF
Genome	RNA	RNA	DNA
Enveloped	Yes	Yes	Yes
Size (nm)	40-60	80-110	150-240
Physiochemical Resistance	Low	Low	Medium

Table 1: Virus selection panel and virus characteristics.

Product integrity and stability

To determine the effect of detergent on product purity, JTB VIS-treated clarified cell culture harvest of IgG1, Fab and bispecific-antibody (bsAb) were loaded onto PROchievA™ column, purified Fc-fusion protein was conditioned and treated with JTB VIS prior to processing over an AEX column. Table 2 represents the summary of product purity data.

Process step	Protein molecule	VIS incubation time (minutes)	Purity (%)
Protein A (PROchievA™)	IgG_1	120	97
		Non-treated	97
	Fab	120	89
		Non-treated	89
	bsAb	120	89
		Non-treated	89
AEX (PolyQUAT™)	Fc-fusion protein	120	91
		Non-treated	91

Table 2: Impact of VIS treatment on product purity for protein A and AEX columns.

Structurally diverse monoclonal antibodies and analogous proteins, namely Fc-fusion proteins, bispecific or multispecific antibodies 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.[8] (Figure 3)

Bispecific antibody

To determine the effect of detergent on product purity, JTB VIS-treated clarified cell culture harvest of IgG_1, Fab and bispecific-antibody (bsAb) were loaded onto PROchievA™ column, purified Fc-fusion protein was conditioned and treated with JTB VIS prior to processing over an AEX column. Table 2 represents the summary of product purity data.

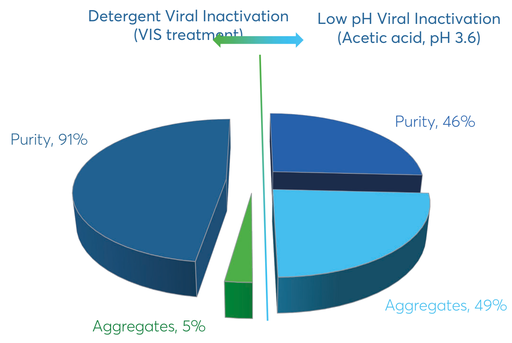


Figure 3: Enhancing product integrity though use of detergent-based viral inactivation.

To examine protein stability after extended detergent incubation times, the harvest of a complex bispecific antibody was incubated with 1% JTB VIS for 24 hours and compared to control sample without treatment. Samples were purified by protein A and analyzed using SEC-HPLC. No impact on protein stability occurred after extended exposure to JTB VIS as shown in comparative SEC-HPLC results shown in Figure 4.

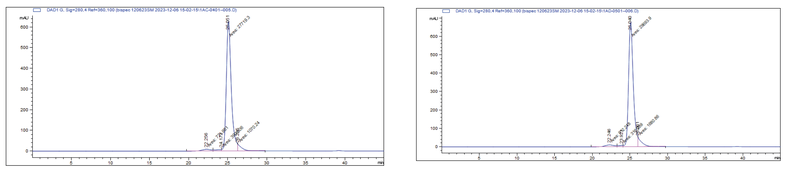


Figure 4: SEC profile of bsAb control (left) and after 24-hour treatment with JTB VIS (right).

Process compatibility

Compatibility with subsequent purification steps is pivotal for a detergent-mediated virus inactivation step. JTB VIS was evaluated with IgG_1 for compatibility with protein A purification process. There was no impact to binding capacity or recovery. (Figure 5)

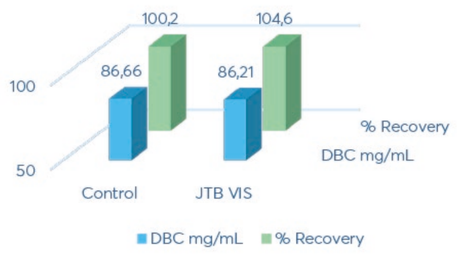


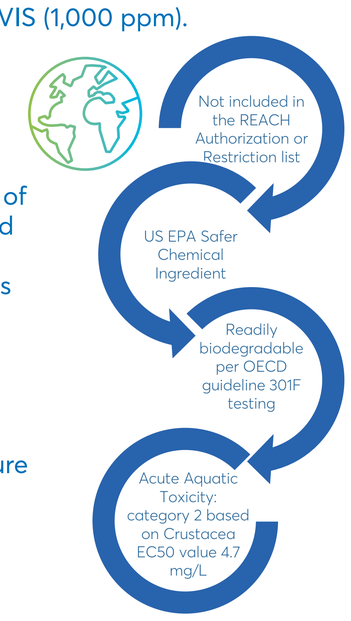
Figure 5: DBC and Recovery after treatment with JTB VIS.

Analytical data indicated excellent detergent clearance capability by protein A. The detergent amount in protein A eluate was below the limit of detection (<1 ppm) analyzed HPLC from a load concentration of 1% JTB VIS (1,000 ppm).

Environmental and health-based toxicity

For ensuring safety and compliance of applications using JTB VIS, permitted daily exposure (PDE), as defined in guidance by the European Medicines Agency[10] was determined by a qualified toxicologist.

Pharmacokinetic, clinical safety and nonclinical toxicology data were reviewed to identify point of departure (POD) for determining no-observed-adverse-effect level (NOAEL).



POD	Oral PDE value		Parenteral/ocular PDE value	
	(mg/day)	(mg/kg/day)*	(mg/day)	(mg/kg/day)*
NOAEL from oral 90-day toxicity study in rats	15	0.3	10	0.2

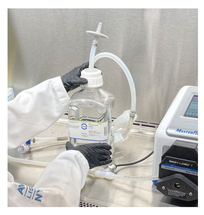
*Adjusted to mg/kg/day by dividing by a 50 kg body weight adult as per regulatory guidance

Table 3: PDE values for oral, parenteral and ocular routes of administration.

Sterile transfer

Optional pre-customized sterile assemblies are available to minimize operator intervention and contamination risk. Equipped with two 18" Wye Legs offering choice of fluid pathway for greater flexibility.

- Thermoplastic Elastomer (TPE) tubing as weldable option
- Silicon tubing with AseptiQuik® [9] G connector



Aseptic transfer assembly

Conclusion

Avantor introduces an advanced viral inactivation solution, manufactured under cGMP in a validated process, making it suitable for workflows that require increased regulatory control. It excels in efficiency, environmental safety and regulatory compliance. This ready-to-use biodegradable solution is free of alkylphenols and adheres to EHS standards, avoiding substance of very high concern (SVHC) designation. Its effectiveness in virus elimination remains high across temperatures without environmental toxicity. It maintains product stability and process performance while being easily detectable and removable. This innovation marks a substantial leap forward in viral inactivation technology.

References

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9. AseptiQuik® is a registered trademark of Colder Products Company. All other trademarks are the property of their respective owners
10. European Medicines Agency (2014) Guideline on Setting Health Based Exposure Limits for Use in Risk Identification in the Manufacture of Different Medicinal Products in Shared facilities. Published November 2014.