avantor™

Enhancing Virus Clearance Process Capability using a Biodegradable Detergent

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To ensure the safety of biopharmaceuticals derived from mammalian cell culture, it is critical biomanufacturing processes can clear or inactivate potential viral contaminants.[1,2] Though rare, virus contaminations are a real threat to patients. Between 1985 and 2018, 45% of companies participating in a Consortium on Adventitious Agent Contamination in Biomanufacturing (CAACB) survey reported viral contamination events.[3]

The consequences of such events are dire for patients of course, and manufacturers could see catastrophic losses as well. Plant shutdown, loss of reputation and revenue, decrease in stock value and lawsuits are all real threats in a worst-case scenario. Purification process steps are the final shield to prevent viral contamination, and a viral inactivation solution can be an effective method to improve clearance and alleviate viral burden downstream.[4,5]

Global regulatory guidance requires adequate viral clearance, typically a minimum of 4 - 6 log higher than the total amount of potential virus in upstream feed stream, using different purification techniques including at least two orthogonal steps provide a minimum of 4 log reduction value (LRV) by each of the methods.[1,6]

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

- 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, product yield and purity in protein A purification
- efficient removal of detergent through subsequent purification steps
- readily biodegradable to meet EHS regulatory compliance standards

Purification: How viral inactivation fits in the process

Viruses can be introduced in the biomanufacturing process from many different routes, including — but not limited to — raw materials and contaminated cell lines. Retroviruses, in particular, are the major safety concern.

To mitigate this, a variety of viral safety measures and material testing have been implemented to ensure viruses, especially those derived from mammalian cells or plasma, are controlled to regulatory standards. The process flow diagrams (Figure 1) represent typical mAb purification processes, highlighting two commonly used viral inactivation strategies: detergent and low pH.

Enveloped viruses contain a lipid/protein coat which can be inactivated through disruption and denaturation of its structure by altering the environment. Inactivation using low pH typically consists of lowering the pH below 3.6 and incubating for at least 30 minutes with a temperature ≥15° C to achieve robust viral inactivation and kinetics consistency.[7] Detergent treatment disrupts the lipid bilayer of the enveloped viruses and leads to inactivation.

An efficient inactivation method, achieving greater than 6 LRV, can help in achieving overall viral clearance goals through the subsequent process steps (Figure 2).

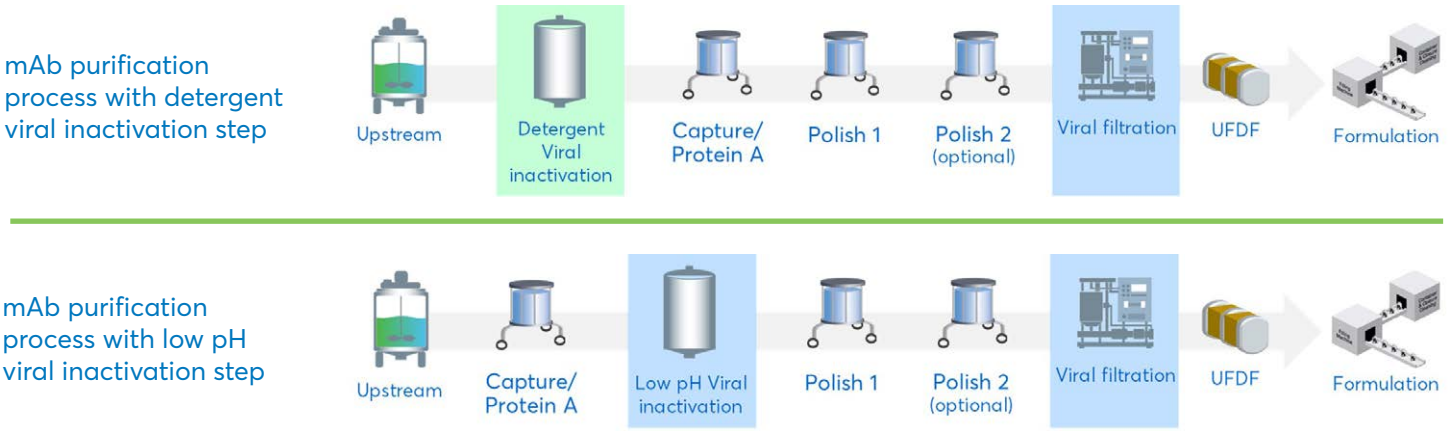


FIGURE 1: Typical viral clearance methods based on product/process requirements.

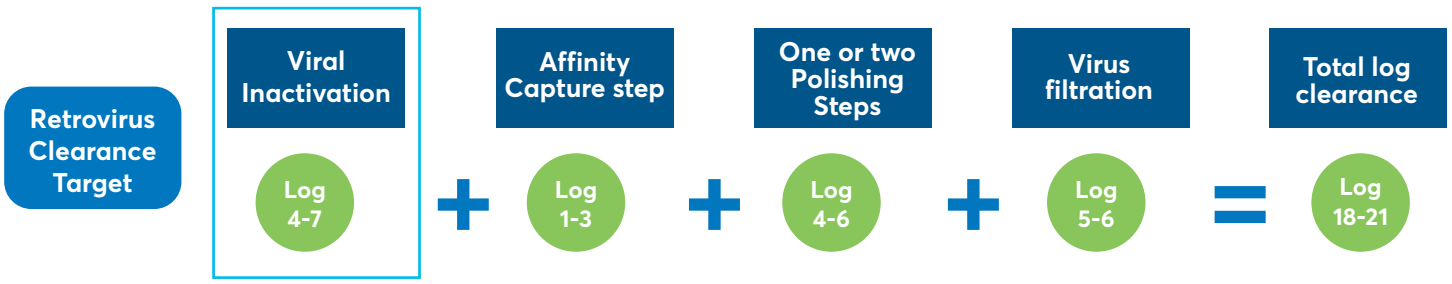


FIGURE 2: Viral clearance goal by process step.[6]

Viral Inactivation: Challenges and Opportunities

Viral inactivation steps such as low pH and detergent treatment boost the overall viral clearance capability in the downstream process — thereby meeting or exceeding regulatory requirements. Each inactivation step has a different efficiency based on virus type and a different effect on the protein of interest.

Although quite effective, low pH treatment presents several issues and risks to the process (Table 1).

Low pH viral inactivation	Detergent viral inactivation
Can cause aggregation	Usually do not cause aggregation
Difficult to reach tight titration range	No need of titration
Usually, no concern on titrant clearance	Need to demonstrate detergent clearance
Volume expansion of process intermediates due to titration	Minimizes volume expansion
Titration increases conductivity, may require dilution to adjust conductivity	Minimal change in conductivity of process intermediates
Doesn't fit well with continuous bioprocessing	Easy to fit with continuous bioprocessing

TABLE 1: Characterization of benefits and risks for each viral inactivation method

An additional challenge is the growing demand for structurally diverse monoclonal antibody (mAbs) and analogous proteins, namely Fc-fusion proteins, bispecific or multispecific antibodies. Low pH treatment may not be a workable approach for those complex proteins, as low pH has been shown to cause aggregation leading to decreased protein stability, potency and develop immunogenic response in patients.[8]

In that context, a detergent-mediated viral inactivation technique can be a better choice in preserving protein stability. Furthermore, this technique will be compatible with continuous column chromatography — once the harvest is treated with detergent, no process interruptions are needed for viral inactivation in between column operations.

If detergent inactivation is selected over low pH inactivation, there are environmental concerns restricting the use of certain detergents. Triton X-100 has been the industry standard for detergent-mediated viral inactivation; however, recent reports have raised concerns about Triton X-100's potential adverse effects, including acute oral toxicity, eye damage, skin irritation, and chronic aquatic toxicity.[9] Consequently, the detergent was added to the "substance of very high concern (SVHC) list" by the European Chemicals Agency under REACH regulations in December 2016.[10] In the EU, it requires authorization before use or an exemption applies.

Therefore, there is an increased need for a biodegradable detergent to meet regulatory requirements.

Detergent Viral Inactivation: Critical Parameters & Study Results

The virus inactivation (VI) capability of JTB VIS was investigated with three different model viruses, XMuLV, HSV-1 and BVDV. The most used model enveloped virus in 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.[11, 12]

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. Large volume testing method was applied for 120 min sample to maximize the assay sensitivity with low lower limit of detection (LOD).

DOES IT HAVE VIRUS INACTIVATION CAPABILITY OF > LOG 6.0?

The log reduction value (LRV) data are plotted in Figure 3. JTB VIS proved to have high efficacy through killing kinetics in less than one minute, and consistency for all the timepoint samples (Figure 3). It's interesting to note that temperature did not have an impact — even at low temperature where virus is more resistant.[13] These results are important for manufacturers who may need to shorten incubation time or operate at low temperatures as well as in clinical diagnostic applications.

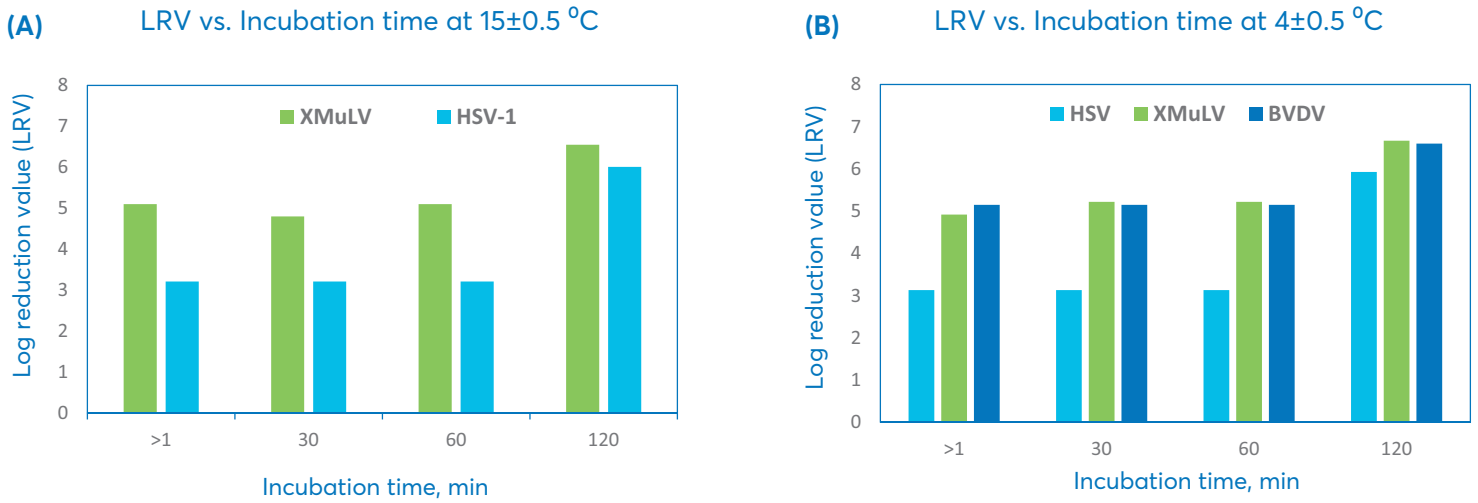
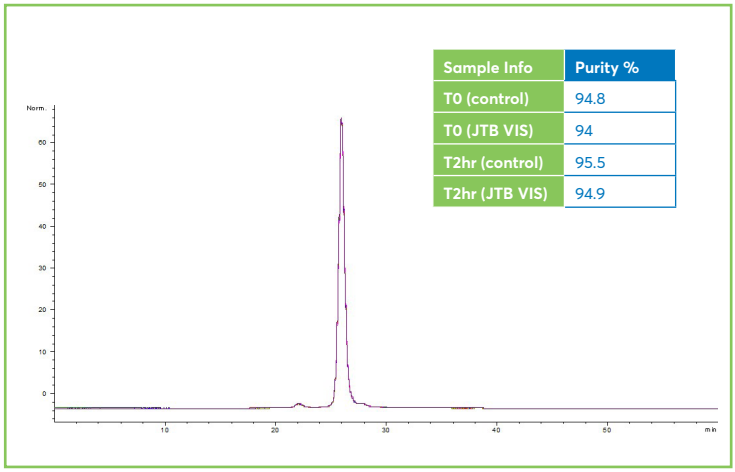


FIGURE 3: Log reduction value (LRV) by JTB VIS at (A) 15±0.5 °C and (B) 4±0.5 °C

DOES IT OFFER PRODUCT STABILITY AND CONTROLLED PROTEIN REACTIVE IMPURITIES?

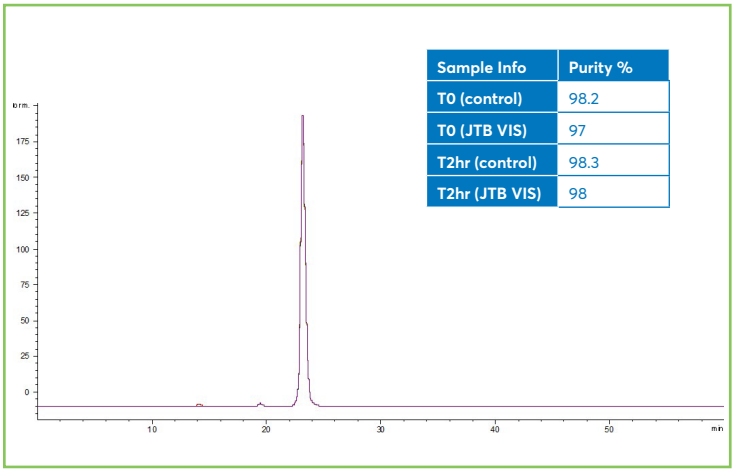
Maintaining protein integrity is crucial to the viral inactivation step for process yield and product quality. JTB VIS ensures that the integrity of protein of interest is not impacted when used for viral inactivation with mAb (IgG_1), Fab fragment (Figure 4A) and, more critically, with Fc-fusion protein and bispecific antibody (Figure 4B).

Impact Assessment on Purity of IgG_1 using J.T. Baker® Viral Inactivation Solution



Sample Info	Purity %
T0 (control)	94.8
T0 (JTB VIS)	94
T2hr (control)	95.5
T2hr (JTB VIS)	94.9

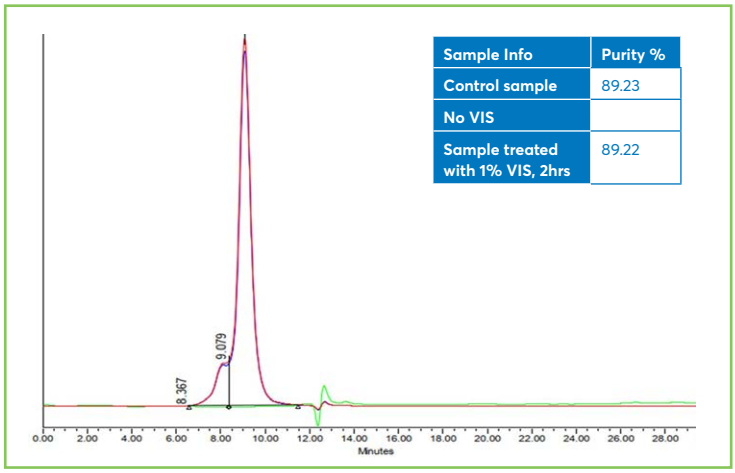
Impact Assessment on Purity of Fab using J.T. Baker® Viral Inactivation Solution



Sample Info	Purity %
T0 (control)	98.2
T0 (JTB VIS)	97
T2hr (control)	98.3
T2hr (JTB VIS)	98

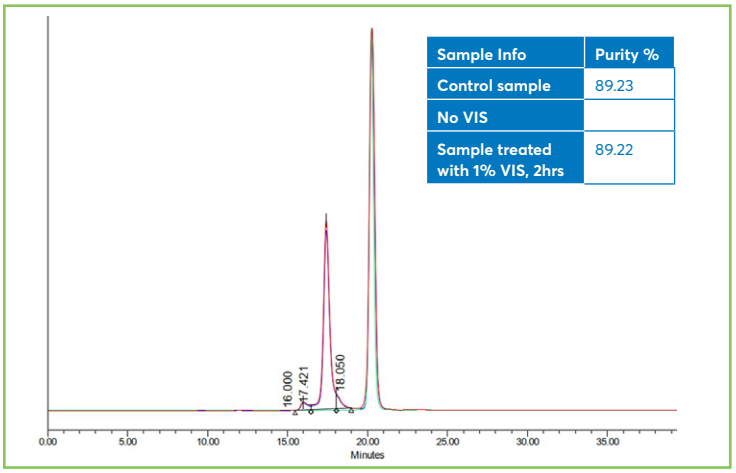
Figure 4A: Impact of VIS on protein stability tested with IgG_1 and Fab fragment.

Impact Assessment on Purity of Fc-fusion protein using J.T. Baker® Viral Inactivation Solution



Sample Info	Purity %
Control sample	89.23
No VIS	
Sample treated with 1% VIS, 2hrs	89.22

Impact Assessment on Purity of Bispecific antibody using J.T. Baker® Viral Inactivation Solution



Sample Info	Purity %
Control sample	89.23
No VIS	
Sample treated with 1% VIS, 2hrs	89.22

Figure 4B: Impact of VIS on protein stability tested Fc-fusion protein and bispecific antibody.

DOES IT PROVIDE PROCESS COMPATIBILITY?

A viral inactivating reagent must be compatible with subsequent purification steps. As shown in Figure 5, JTB VIS does not impact dynamic binding capacity (DBC), product yield or purity.

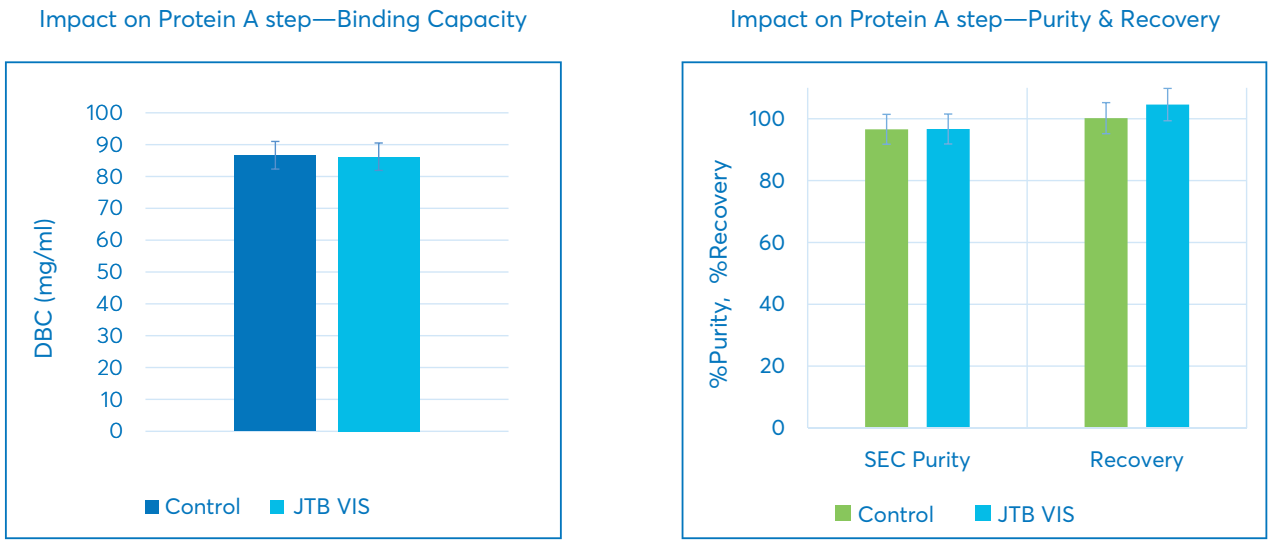


FIGURE 5: Impact of VIS on subsequent chromatography process performance

CAN IT SUCCESSFULLY BE CLEARED FROM THE FINAL PRODUCT?

One of the critical aspects of using detergent-mediated viral inactivation is that the subsequent purification process is able to remove the detergent from product. In this study (Table 2), protein A chromatography removed the JTB VIS detergent from the product stream to a level of <1 ppm (parts per million) (Figure 6).

Parameters	Description
Column/resin	PROchievA™ protein A resin
Column volume (CV)	5 mL (5.3 B.H. X 1.1 I.D.)
Resin load density	50 mg/mLr, IgG,
Residence time	1 min (flowrate: 5 ml/min)
Equilibration	PBS, pH 7.4, 10 CV
Loading	1% VIS (1:100 ratio) in harvest (detergent 0.1%, 1000 ppm)
Wash 1	PBS, pH 7.4, 10 CV
Wash 2	25 mM Tris, pH 8.0, 5 CV
Elution	100 mM Na-acetate, pH 3.6, 5 CV
Strip	100 mM Acetic acid, 5 CV
Sanitization	0.5 N NaOH

TABLE 2: Protein A chromatography process parameters

It can be postulated from the detergent's physicochemical properties that this detergent would simply be removed by any process chromatography step through bind-and-elute mode of operation.

DOES IT MEET REGULATORY COMPLIANCE STANDARDS?

Avantor has developed a readily biodegradable, free of alkylphenols, and environment, health and safety (EHS) compliant novel viral inactivation solution that shows improved efficiency compared to traditionally used detergents.[14] The biodegradability assessment, according to OECD guideline 301F, suggests that the key component is readily biodegradable under aerobic conditions in the environment. Furthermore, it is not listed on the Substances of very high concern (SVHC) list according to REACH regulations.[10]

SUMMARY

This ready-to-use, highly efficient, biodegradable reagent demonstrates high virus killing kinetics independent of operational temperatures (4 °C to RT). Other benefits of using this detergent include no environmental toxicity, no impact on product stability or process performance. This detergent can easily be removed from the product and be detected by a simple analytical assay.

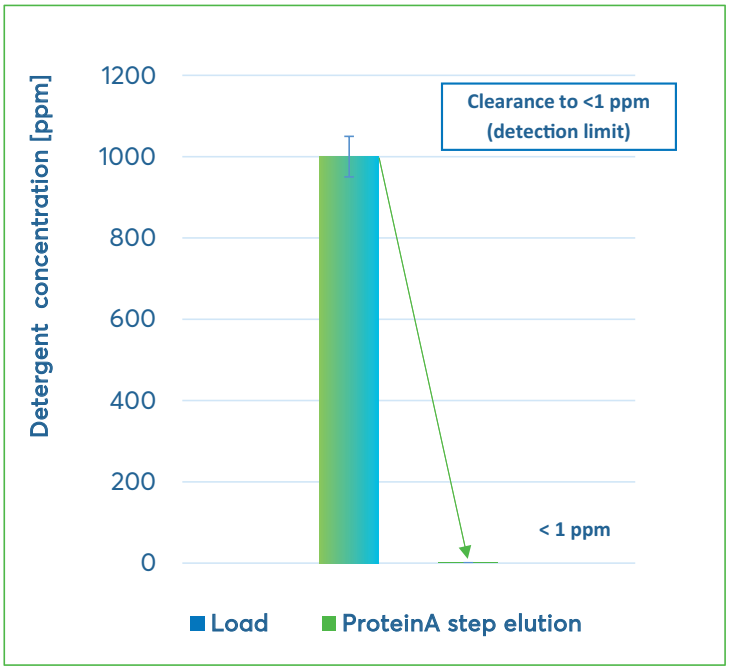


FIGURE 6: Log reduction value (LRV) by JTB VIS at (A) 15±0.5 °C and (B) 4±0.5 °C

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Tuhidul Islam is a Sr. Manager – Bioprocess Application at Avantor. He is focused on applications of new products and processes to overcome challenges in biologics manufacturing for different drug modalities. Tuhidul holds a PhD in Biochemical Engineering, with a particular focus on protein purification method development. He has 14+ years of experience in process development and cGMP manufacturing science for monoclonal antibodies, fusion proteins, Adeno-associated virus and Lentivirus.

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Nandu Deorkar is Senior Vice President, Biopharma Production Research & Development for Avantor. He is responsible for innovation strategy and planning, and execution of new products and technology development. During his more than 25-year career in research & development, Dr. Deorkar has been leading teams working on various aspects of upstream and downstream bioprocessing, single use systems, chemical/polymer R&D, drug development, formulation, drug delivery technologies, process development, and technology transfer. He has published more than 30 articles and holds more than 20 patents. Dr. Deorkar received Ph.D. from Indian Institute of Technology, Mumbai, India and MBA in Marketing from Fairleigh Dickinson University, Madison, NJ, USA.

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