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White Paper

Upstream Viral Safety: A Holistic Approach to Mitigating Contamination Risks

The safety of biologics from adventitious agents relies on a multilayered strategy that encompasses controlled manufacturing processes and the biomanufacturers' implementation of risk mitigation strategies to prevent contamination. This holistic approach has delivered safe products for human use for many decades and continues to meet today's expanding patient needs.

Viral Safety Upstream vs. Downstream

Viral safety relies on a framework that includes careful selection of raw materials, testing to detect virus in raw materials and process intermediates, and virus removal steps in downstream purification. For downstream processes, guidance documents outline expectations for viral safety, where the focus is on reducing levels of endogenous and adventitious virus. By contrast, there is no specific regulatory guidance recommending virus reduction steps upstream of the bioreactor. Despite the many controls, production of biopharmaceuticals or biologic therapies from cell-based processes is at risk of contamination with either bacteria or viruses. Although publicized viral contamination events are rare, their impact can be widespread, and they can have significant implications for the manufacturer. Such events affect organizations of all sizes and can result in costly investigations to identify the contaminant, facility shutdowns and overhauls of manufacturing processes. More importantly, these events may result in supply disruptions which can impact drug supply to patients. Combined, viral contamination can cause significant business disruption and often have far-reaching financial consequences for the companies involved (1). There is increasing awareness of the potential disruption caused by viral contamination and some biomanufacturers have been moved to implement dedicated virus reduction steps upstream of the bioreactor to mitigate these risks.

With newer modalities such as cell and and viral gene therapies, downstream purification may not have the opportunities for viral reduction as monoclonal antibody processes. In these cases, there may be higher risk of adventitious viral contamination and dedicated viral reduction steps upstream of the bioreactor may be an attractive option to reduce the risk of contamination.

This whitepaper describes how different elements of viral safety can be integrated to minimize the risk of bioreactor contamination and the resulting disruption to biomanufacturing operations.

Testing for the Presence of Virus

For biopharmaceutical production, viral safety in upstream cell culture processes relies heavily on comprehensive regulatory guidance describing characterization of the production cell line together with raw material controls and testing requirements, Figure 1. A baseline component of upstream safety is confirming the production cell line is free from adventitious microorganisms and includes testing for bacteria, fungus and mycoplasma.

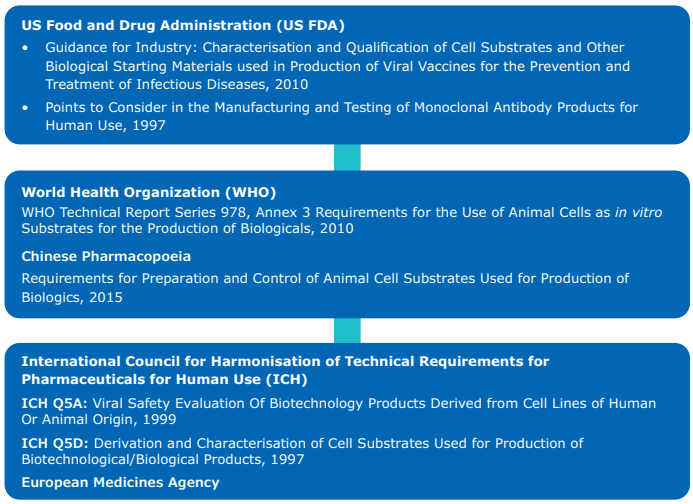

Figure 1. Regulatory guidance for raw materials and raw material testing

Typically, detection methods for adventitious virus include culturing with multiple permissive cell lines; these are long duration tests requiring 4-6 weeks. Molecular tests using polymerase chain reaction (PCR) methods offer manufacturers rapid testing results

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and can mitigate a potential contamination risk or reduce the potential for an upstream contamination progressing into downstream purification operations. Furthermore, molecular testing can quickly provide information on the source, identity or extent of contamination, allowing manufacturers to respond more rapidly to a contamination event and accelerate the resumption of normal operations. As molecular testing evolves, collaborative relationships between service providers and manufacturers become essential to fully leverage the benefits of different molecular methods in both normal business operations and in the unfortunate event of a contamination.

Raw Material Supplier Selection

Perhaps one of the most critical aspects to minimizing risk with raw materials is identifying suppliers who understand the requirements for biopharma production in terms of information on sourcing, control of quality systems, and raw material testing expectations as outlined in regulatory guidance documents (2,3). General 'best practices' for selecting raw materials to minimize viral contamination risks use the following framework:

- Replace animal-derived media components with non-animal origin component materials if possible
- If animal-derived components must be used, source them from lower risk geographies and perform comprehensive testing for the presence of virus
- Adopt chemically defined media where possible
- Partner with suppliers who offer higher risk components that have been pretreated with UV or gamma irradiation or high temperature short time (HTST)

Partnerships with suppliers who offer transparency in their quality and supply chain operations can simplify risk assessment and product selection. Our M-Clarity™ program provides clear product categorization based on specific levels of quality and available documentation to help customers select the right product for the intended use. This supplier-based product categorization simplifies purchasing decisions for customers and helps biomanufacturers make informed decisions that should ensure regulatory compliance during both development and scale-up.

Risk Analysis of Raw Materials

Risk analysis is routinely used to understand how different aspects of a manufacturing operation can contribute to potential contamination. Such analysis can identify vulnerabilities and help prioritize mitigation actions with consideration to raw materials, supply chain, equipment, processes and operators. Understanding risks can broaden perception of contamination, increasing the awareness and motivation of stakeholders to respond. The actions or mitigation efforts that cascade from risk analysis are largely determined by a stakeholder's risk tolerance–does increased understanding drive a change in behavior? Figure 2 highlights this balance.

Risk analysis may also accelerate development of a roadmap that could be implemented in the event of contamination. This roadmap could outline immediate actions, and prepare answers to important questions such as:

Who are the decision makers? Which external partners will perform testing? What happens to manufacturing and supply chain? What happens to lots of bulk product? What are the risks to equipment? How extensive are the cleaning and mitigation efforts? How will the site resume manufacturing operations?

Most tools to assess contamination risk consider the likelihood of a viral contamination event together with the ease of detection. Raw materials are evaluated individually and scored based on different parameters, Figure 3A. These individual scores can then be used to develop a composite value for each raw material, Figure 3B.

Risk Perception

How does your company evaluate risk?

Risk Tolerance

Does information change your behavior?

Figure 2. Understanding the profile of biomanufacturers to viral contamination risk.

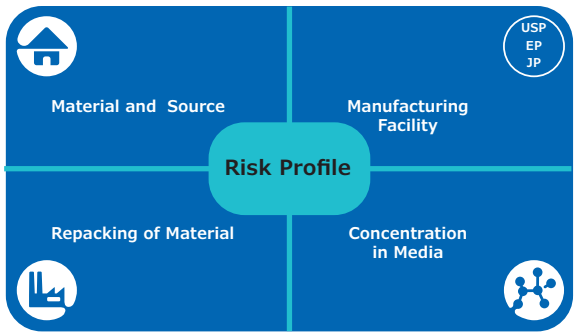


Figure 3A. Components of the risk profile used to assess the risk of raw materials.

	Low Risk Score	Higher Risk Score
Material and source	• Countries with robust and well-established Quality Systems • Synthetic/mined • Pharma grade • Low risk rodent exposure	• Countries with emerging Quality Systems • Animal derived • Industrial or <98% purity • High risk rodent exposure
Repacking	• No repacking • cGMP facility • Low risk rodent exposure	• Material repacked • No/unknown registration • High risk rodent exposure
Facility	• cGMP	• No/unknown registration
Concentration	<1 mg/L	>1000 mg/L

Figure 3B. Example of risk scoring for media raw materials used in cell culture. Materials with lower risk score are preferred for minimizing contamination risk.

This systematic analysis enables identification of higher risk components so they can be prioritized in mitigation efforts. In the example shown in Figure 4, sugar and hydrolysate were identified as high risk components: sugar is a rodent attractant, presenting issues for biomanufacturers looking to store bulk sugar and prepare solutions for cell culture media, while hydrolysates are plant-based and more likely to be susceptible to viral contamination from agricultural practices or pests.

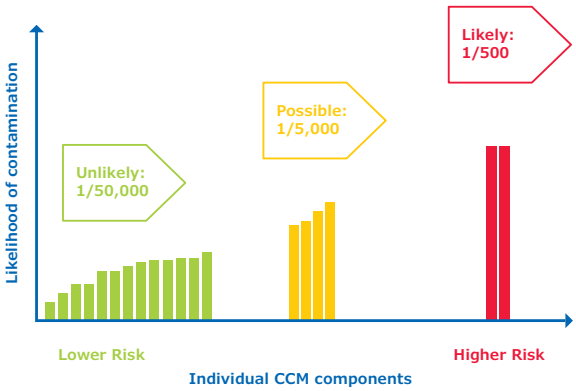


Figure 4. Example of risk profile assessment and likelihood of contamination event. This type of analysis helps prioritize risk mitigation actions.

Understanding the elevated viral contamination risks associated with sugar solutions led to the development of a supplier based HTST pasteurization process for glucose solutions that effectively inactivates many viruses including murine parvovirus, the main contaminant of concern in processes that use Chinese Hamster Ovary (CHO) cells. Although HTST treatment of glucose can be performed by biomanufacturers, the equipment is large, expensive and processing must be tightly controlled to minimize processing issues. The availability of HTST treated glucose offers a convenient way for biomanufacturers to mitigate viral contamination risk from a high-risk raw material before it enters the manufacturing plant.

Some suppliers offer cell culture media presterilized by gamma irradiation which offers a convenient solution for small-scale processes. However, not all cell culture media components are compatible with this treatment and most large-scale biomanufacturing operations depend on a point-of-use risk mitigation treatment upstream of the bioreactor.

Raw material selection occurs hand in hand with confirmation of cell culture performance: a 'lower risk' option is only useful if performance is acceptable. Assessing performance by characterizing cell growth, productivity, and the quality attributes of the protein being produced are important aspects of raw material selection. Of course, raw material risks should also be considered in the context of process design and flow as risk mitigation encompasses all aspects of the manufacturing environment.

Mitigation Technologies

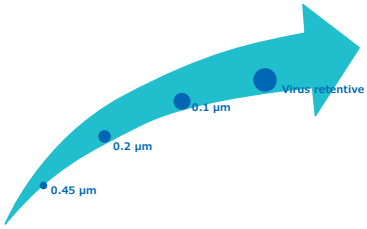
One approach to reduce the risk of viral contamination is to utilize gamma-sterilized, single-use components. Bioreactors, mixers, connectors and sampling systems are some options offered by suppliers for different steps in upstream processing. These pre-sterilized components offer the possibility of closed processing and provide flexibility, faster turnaround between campaigns, and minimize the impact to manufacturing timelines in the event of contamination.

Irrespective of whether they operate in single-use or more traditional stainless-steel operations, most manufacturers adopt some point-of-use treatment for processing cell culture media. In most cases, this may be limited to filtration through a sterilizing 0.2 µm membrane filter to prevent bacterial contamination. We previously explained how individual raw materials might be designated 'low risk' for viral contamination, however when considered in aggregate, the cumulative risk can be high and additional control may be needed before the media is used in a bioreactor. This is especially important as cells and rich media in the bioreactor offer an attractive environment for virus proliferation.

There are several treatment options for cell culture media to prevent viral contamination of the bioreactor. Two approaches that have been implemented include HTST treatment and filtration through virus retentive

membrane filters (1). While treating cell culture media with HTST is broadly effective, it requires a significant capital investment, involves large equipment and must be connected to a clean-in-place (CIP) system. Furthermore, some media components are known to be heat labile and cannot be processed using HTST.

Filtration has long been leveraged to mitigate the risk of microbial contamination in bioprocessing. Bioburden reduction, sterilizing and virus filtration are routine operations in downstream processing. Sterilizing-grade filters with 0.2 µm membrane are widely used for processing cell culture media. Although these filters meet the standards for sterilizing-grade performance, filters containing 0.1 µm pore size membrane are the preferred option for protection against contamination with mycoplasma or smaller organisms such as Spirochetes. However, 0.2 µm or 0.1 µm membrane filters will not prevent adventitious virus in cell culture media from entering the bioreactor. For this additional level of risk reduction, cell culture media should be processed through virus retentive filters of ~20 nm pore size, Figure 5.



Membrane Pore Size	Organism Retention
~20 nm Virus retentive	Parvovirus, mycoplasma, bacteria
0.1 µm	Mycoplasma, bacteria
0.2 µm	Bacteria
0.45 µm	Some bacteria

Figure 5. Filtration options for cell culture media.

Traditional virus filters designed for downstream processing typically do not perform well with cell culture media. Generally, complex cell culture media results in rapid fouling of these filters. In addition, the flux is too low for efficiently processing large volumes of media leading to high filter area requirements. Downstream virus filters may also retain beneficial components in the media which could impact cell growth or productivity. More recently, virus retentive filters designed specifically for processing chemically defined cell culture media have become available. These 'barrier' filters prevent microorganisms from entering the bioreactor by leveraging the virus retentive capabilities of downstream virus filters while offering superior processing for cell culture media, Figure 6.

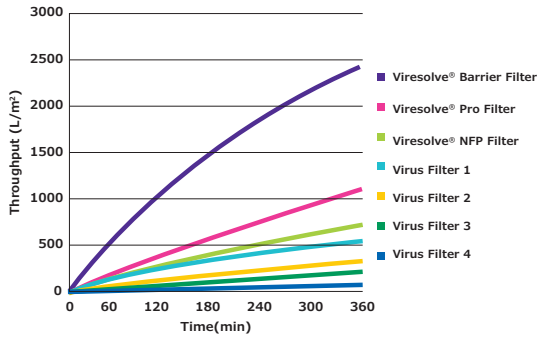


Figure 6. Comparison of traditional downstream virus filters (1-4), the Viresolve® NFP and Viresolve® Pro downstream virus filters and the Viresolve® Barrier filter designed specifically for processing cell culture media.

Because the membrane pore size of 'barrier' filters is designed to retain ~20 nm parvovirus, these filters typically provide more than four logs of parvovirus reduction while also offering protection against other microbial contaminants not completely retained by 0.2 µm or 0.1 µm filters.

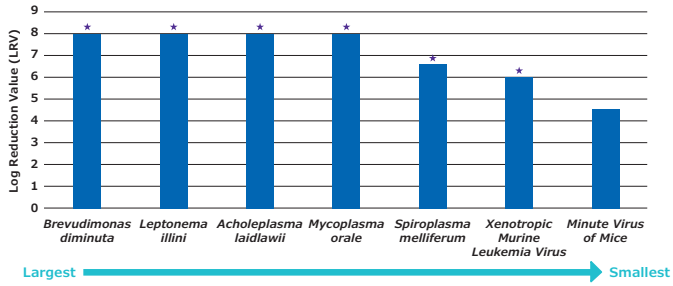


Figure 7. Retention of a panel of microorganisms on Viresolve® Barrier filters.

Importantly, as with raw material selection and irrespective of which filters are used for processing cell culture media, cell culture performance should be evaluated to identify any potential impact of the filter on cell growth, molecule productivity or protein quality characteristics.

Conclusions

By working within the parameters of this framework, biomanufacturers can effectively manage the risk of contamination in their upstream processes. Viral contamination can have devastating results, but through careful product selection, comprehensive testing plans and working with knowledgeable suppliers, biomanufacturers can minimize contamination risk. Figure 8 offers a general outline of different considerations: understanding the risks can prioritize mitigation actions to prevent viral contamination upstream and reduce the impact of disruption to operations. Ultimately, it is a manufacturer's tolerance for risk that will shape actions and mitigation plans.

Figure 8. Different options to mitigate upstream viral contamination.

References

1. Barone, P.W., Wiebe M.E., Leung, J.C et al (2020) Viral contamination in biologic manufacture and implications for emerging therapies. Nature Biotechnology https://doi.org/10.1038/s41587-020-0507-2
2. EMA/CHMP/BWP/814397/2011: *Guideline on the Use of Porcine Trypsin Used in the Manufacture of Human Biological Medicinal Products.* European Medicines Agency: London, UK, September 2014.
3. EMA/CHMP/BWP/457920/2012 rev 1. *Guideline on the Use of Bovine Serum in the Manufacture of Human Biological Medicinal Products.*London, UK, May 2013.

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