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Add the following:

▲⟨1229.18⟩ VIRAL CLEARANCE METHODS

Viruses are infectious agents that cannot grow or reproduce apart from a living cell. They are substantially smaller than bacteria and cannot be removed by the typical sterilizing grade filter (see *Sterilizing Filtration of Liquids* ⟨1229.4⟩). Viruses are composed of genetic material, either DNA or RNA, and are surrounded by a protein coat and, in some viruses, by a membranous envelope. Viruses can introduce their DNA/RNA into living cells where they can replicate to spread the infection. Viruses cause a variety of diseases, e.g., common cold, influenza, warts, AIDS, Ebola, and smallpox. To minimize the risk to patients, sterile materials must be produced in a manner that minimizes the potential for viral contamination. Detailed treatment of this subject can be found in *Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin* ⟨1050⟩ and *Design, Evaluation, and Characterization of Viral Clearance Procedures* ⟨1050.1⟩.

Viruses are extremely diverse, vary widely in complexity, and can evolve rapidly, making their removal and destruction problematic. The greatest concern for viral removal is with the use of materials of an animal origin, such as biological processes where mammalian cells are used for the production of therapeutic proteins. Virus removal is of concern due to susceptibility of mammalian cells to viral contamination, difficulty in virus removal from mammalian cell culture processes specifically, and potential destruction of the biological product by removal/inactivation techniques. There are three major components to the prevention of viral contamination in biological processing:

1. Avoidance: Using cell lines and biological materials free of viral contamination to avoid initial ingress
2. Removal/Inactivation: Inclusion of viral clearance steps in the downstream processing of biologics to eliminate those present in the process and ancillary materials
3. Confirmation: Testing of in-process and finished materials to confirm absence of viral contaminants

Consideration for viral safety, the means for viral analysis, and the subsequent virus group selection for use in viral clearance studies can be found in ⟨1050⟩. The focus of this chapter is viral clearance methods:

"The objective of viral clearance studies is to assess process step(s) that can be considered to be effective in inactivating/removing viruses and to estimate quantitatively the overall level of virus reduction obtained by the process. This should be achieved by the deliberate addition ("spiking") of significant amounts of a virus to the crude material and/or to different fractions obtained during the various process steps and demonstrating its removal or inactivation during the subsequent steps" (*1*).

In the execution of viral clearance studies, the selection of appropriate viral challenges must consider the expected viral contamination (as determined from the material origin) and non-specific model viruses representing other potential viral contaminants. Biologic manufacturing processes typically include multiple viral clearance steps relying on different clearance modalities to ensure effective removal of different types of viruses (*2*). The clearance performance of each method is demonstrated independently in a scale-down model of the manufacturing process. Regardless of the viral clearance methods utilized, testing against a panel of potential virus contaminants is expected. The viruses used in the challenge studies should include both endogenous and non-endogenous viruses as appropriate to assess potential specific and nonspecific viral contamination (*1*,*3*). Viral clearance capability of the various methods varies greatly and is commonly reported as a log reduction value (LRV).

The expectations for virus clearance vary with the specifics of the product and process. The expected overall LRV must consider the initial viral population and an additional margin of safety. Considerations in establishing the minimum LRV should include:

- The appropriateness of the test viruses used
- The design of the clearance studies
- The log reduction achieved
- The time dependence of inactivation
- The potential effects of variation in process parameters on virus inactivation and removal
- The limits of assay sensitivities
- The possible selectivity of inactivation and removal procedure(s) for certain classes of viruses (*1*)

There are varied means of virus removal and clearance. The most widely used methods are filtration, chromatography, thermal, chemical (solvent/detergent combinations), and radiation treatments.

FILTRATION AND CHROMATOGRAPHIC METHODS

Various forms of filtration including ultrafiltration and nanofiltration can be used to capture larger viruses while allowing smaller materials to pass through the filter. The retention of viruses by filtration relies on principles similar to those for sterilizing filters as described in ⟨1229.4⟩. The primary mechanism for virus filtration relies on a sieving mechanism using filters with pore sizes smaller than the expected viral contamination as they may not be removed by the typical sterilizing grade filter. The filters used for viral removal may be rated by their

approximate molecular weight cutoff (MWCO) or their average pore size and typically provide multiple log reductions in virus concentration. The filters used for viral removal must be evaluated to confirm their compatibility with the fluid being processed, which typically varies with its position in the manufacturing process.

Chromatography steps common in biologic manufacturing processes are also able to remove viral contamination. Size exclusion and other chromatographic processes can result in multiple log reduction of viruses (*4–5*). The effectiveness of viral retention varies with the virus type and the specific chromatography process.

THERMAL METHODS

Some viruses are susceptible to destruction by thermal means. An in-line high-temperature short-time (HTST) process may be able to preserve the essential quality attributes of protein materials (*6*). HTST treatment is one of the proven techniques for the treatment of commercial-scale cell culture medium to mitigate the potential risk of contamination by many viruses. Medium precipitates may have an adverse effect on virus inactivation due to clogging of the HTST system and altering the HTST parameters, affecting medium composition and cell growth. Therefore, it is essential to validate the HTST parameters (*7–8*). Confirmation of efficacy must be demonstrated empirically as there are no defined thermophysical conditions for inactivation of viruses.

CHEMICAL METHODS

The common chemical means for viral clearance can include acidic or alkaline pH adjustments and solvent/detergent treatments. For example, low pH exposure (<4.0) is effective for the destruction of many enveloped viruses (*9*). Solvent/detergent processes can use incubation of materials in an organic solvent (e.g., tri-*N*-butyl phosphate) and detergent combination both of which must be removed afterward (*10*).

RADIATION METHODS

Animal-derived materials pose a risk of introducing viruses and *Mycoplasma* and *Acholeplasma* into biological harvest fluid during the upstream manufacturing process using mammalian cell substrates. Available data indicates that gamma radiation (25–40 kiloGrays) causes complete inactivation of target viruses and *Mycoplasma*. Although the gamma irradiation is highly effective, the efficacy and viral kill kinetics in serum appear to be dependent on the size of the target virus (*11*).

The diversity of viruses and the potential for adventitious contamination have led to biological processes that typically include a variety of different viral clearance steps to provide the greatest margin of safety. The demonstration is commonly performed empirically using a virus panel representative of both potential intrinsic and extrinsic contaminants as there is limited ability to predict the effectiveness of viral removal and destruction processes. As with any other processes included in the ⟨1229⟩ series, it should be demonstrated that the sum of the viral clearance efforts do not adversely affect the biological drug substance.

REFERENCES

1. Food and Drug Administration. Guidance for Industry: Q5A Viral Safety Evaluation of Biotechnology Products Derived From Cell Lines of Human or Animal Origin; Sept 1998.
2. Sofer G. Inactivation of Viruses: An Introduction to the Series. *BioPharm International*, June 2003 Supplement: S6–7.
3. European Medicines Agency. Note for Guidance on Virus Validation Studies: The Design, Contribution and Interpretation of Studies Validating the Inactivation and Removal of Viruses. EMEA, CPMP/BWP/268/95; 1996.
4. Aoyama, S. Adsorption study of egg-derived influenza virus with cellufine sulfate affinity chromatography media. BioProcess International, *Industry Yearbook*; 2015–2016:46.
5. Norling, L. Impact of multiple re-use of anion exchange chromatography media on virus removal. *J. Chromgr. A.* 2005;1069(1):79–89.
6. Hiatt, CW Kinetics of the Inactivation of Viruses. *Bacteriological Reviews*. June 1964;28(2):150–163.
7. Cao X, Stimphfl GM, et al. Identification of root cause analysis for cell culture media precipitates in viral deactivation treatment with high temperature/short-time method. *PDA J Pharm Sci Technol*. 2013;67(2):63–73.
8. Pohlscheidt M, Charaniya S, et al. Implementing high-temperature short-time media treatment in commercial-scale cell culture manufacturing processes. *Appl Microbiol Biotechnol*. 2014;98(77):2965–2971.
9. Mattila J, Clark M, Liu S, et al. Retrospective Evaluation of Low-pH Viral Inactivation and Viral Filtration Data from a Multiple Company Collaboration. *PDA J Pharm Sci Technol*. 2016;70:293–299.
10. Horowitz B, Bonomo R, et al. Solvent/Detergent-Treated Plasma: A Virus-Inactivated Substitute for Fresh Frozen Plasma. *Blood*. Feb 1992;79(3):826–831.
11. Nims RW, Gauvin G, Plavsic M. Gamma Irradiation of Animal Sera for Inactivation of Viruses and Mollicutes—a review. *Biologicals*. 2011;39(6):370–377.

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