PALL Biotech

Technical Regulatory Topic

Risk Mitigation for Adventitious Agents in Biotechnology Products

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1 Introduction

Biotechnology products derived from cell lines are inherently at risk of contamination by adventitious agents. Adventitious agents that are of concern in most biological manufacturing bioprocesses are mainly viruses and bacteria (including mycoplasma). Adventitious agent contamination events can arise from a contaminated cell line source or during production, e.g., from cell substrates, raw materials, equipment, facilities and/or operators. Although contamination of bioreactors and biologic therapeutics is rare, several manufacturers have reported such incidents in biotechnology manufacturing processes in the past decade, and the consequences can be catastrophic. Therefore, regulatory agencies worldwide mandate testing for adventitious agents in each lot/batch of material in adherence with current Good Manufacturing Practices (cGMP). Bacteria and other contaminants can be easily removed by 0.1 µm or 0.2 µm sterilizing grade membranes, however small viruses are not removed by these filters. Manufacturers have to demonstrate the ability of the manufacturing process to produce a biopharmaceutical product that is safe for human use.

The ICH Q5A[1] guideline is the primary regulatory guideline for viral safety in biologic manufacturing processes based on animal and human cell cultures. The safety assurance is achieved through the application of a robust and effective virus testing program, which adopts a three-tiered approach:

1. Selecting and testing of cell lines and other raw materials
2. Assessing capacity for viral clearance and inactivation by a manufacturing process
3. In-process and/or final product virus testing.

2 Selecting and Testing of Cell Lines and Other Raw Materials

Cell culture in a bioreactor is a perfect environment for the proliferation of adventitious agents and the introduction of a very low-level contamination can quickly replicate into a major contamination. Hence, risk mitigation strategies include use of animal-origin free raw materials or pre-treatment of raw materials and screening of cell banks, which enter the bioreactor. Technologies considered for pre-treatment of raw materials may include:

1. Nanofiltration (size-exclusion based filtration using nominal 20 nm filters)
2. High temperature short time (HTST), for example, 102 ºC for 10 seconds)
3. UV-C (254 nm)
4. Gamma irradiation (30 to 50 kGy).

An important part of qualifying a cell line for use in the production of a biotechnology product is the appropriate testing for the presence of viruses. Numerous assays can be used for the detection of endogenous and adventitious viruses. ICH Q5A and USP <1050>[2] outlines examples of these assays which include:

- infectivity assays, *in vitro* and *in vivo*
- antibody production tests (e.g., mouse antibody production (MAP), rat antibody production (RAP) and hamster antibody production (HAP))
- electron microscopy (EM) studies
- reverse transcriptase polymerase chain reaction (RT-PCR)
- next generation sequencing (NGS), etc.

With scientific advancements, manufacturers are encouraged to discuss adoption of alternative novel techniques and/or rapid microbiological methods with the regulatory authorities. These novel techniques and methods may be acceptable when accompanied with adequate supporting data.

[1] ICH Q5A(R1) *Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin*, 1999. https://www.ich.org/page/quality-guidelines, accessed 05 January 2021.

[2] United States Pharmacopeia (USP) <1050> "*Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin*," 2013.

3 Assessing Capacity for Viral Clearance and Inactivation by a Manufacturing Process

The ICH Q5A regulatory guideline indicates that a manufacturer of biological products for human use should demonstrate the capability of the manufacturing process to remove or inactivate potential contaminants. The aim of a viral clearance study is to assess the effectiveness of individual steps in the manufacturing process at removing or inactivating viruses. Virus safety in biomanufacturing typically relies on scale down models in which manufacturers demonstrate the capability of process steps to reduce or remove viruses.

The first study is required before Phase I clinical trials, in which the process should be evaluated for inactivation or removal of an enveloped and a small nonenveloped virus, and at least two orthogonal steps should be used for achieving this. A second and more complex study is then conducted before manufacturing Phase III materials to provide evidence of the effective and adequate clearance of relevant and known viruses, as well as the removal of a range of non-specific viruses to show robustness of the process step.
A viral clearance study with at least four to five viruses for late stage is considered state-of-the-art, and log-reduction values (LRV) of four or higher are perceived as robust and effective safety measures. The robust and reliable capability to eliminate viruses must be demonstrated by a risk-based approach. The requirements demand a statistically independent combination of methods (orthogonal technologies) for removing enveloped and nonenveloped viruses. These methods are based on the different physical principles of removal and inactivation, and yet are complementary to each other.

Several methods can be used for virus clearance in bioprocessing. These include inactivation methods such as solvent and detergent (SD) or chemical treatments, low pH, chromatography, and removal by size exclusion filtration. Treatments with solvents and detergents or low pH have significant limitations in their ability to inactivate small nonenveloped viruses. SD treatments were commonly used for plasma proteins and were considered the gold standard for inactivating enveloped viruses. Low pH inactivation of retroviruses is reported to be highly dependent on time, temperature, pH, and relatively independent of the recombinant protein type or conductivity conditions.

Nanofiltration, membrane chromatography, and UV-C, together used as a technology platform for virus clearance by removal, adsorption, and inactivation, provide robust and efficient clearance capability for all viruses with major focus on small, nonenveloped viruses such as porcine parvovirus (PPV) or minute virus of mice (MVM). Driven by regulatory guidance, technologies with the capability to remove or inactivate small, nonenveloped viruses should be implemented from an early stage into the downstream process of a biopharmaceutical to fulfill virus-clearance expectations.

4 In-Process and Final Product Virus Testing

Virus testing is performed at different stages of the bioprocess, and includes unprocessed bulk, purified bulk, and final product. A representative sample of the unprocessed bulk, removed from the production bioreactor prior to further processing, represents one of the most suitable levels at which the possibility of adventitious virus contamination can be determined with a high probability of detection. Appropriate testing for viruses should be performed with the unprocessed bulk. The unprocessed bulk product then goes through multiple purification steps to ensure the final product meets the quality target product profile. With respect to the virus testing performed, traditional infectivity assays, *in vitro* and *in vivo,* and PCR methods are employed, though adoption of alternate novel technologies seems likely in the future.

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