Application Note

Ensuring Compliance:

Regulatory guidance for virus clearance validation

Introduction

To assure virus safety of biological therapeutics, regulatory guidance advocates an approach in which control occurs at various stages of the drug manufacture. Specifically, manufacturers should:

1. Select and test their source materials for the absence of virus
2. Test the capacity of the production process to remove or inactivate viruses
3. Test the biologic at appropriate stages of production for freedom from detectable viruses

The concern over virus contamination in the manufacture of biological therapeutics is related to viruses endogenous to the cell line (e.g., murine retrovirus) and adventitious viruses, which can be introduced during manufacturing (e.g., parvovirus). To date, biological therapeutics derived from cell lines have not been implicated in the transmission of viruses to humans. Nevertheless, to assure product safety, steps that can be shown to remove or "clear" virus should be a part of the manufacturing process for all biologics.

For products generated from plasma, the threat of virus contamination is high because blood is known to carry a number of human pathogenic viruses (e.g., HAV, HBV, HIV and B19) and virus transmission to humans has occurred through administration of virus-contaminated products. However, recent improvements in blood screening together with manufacturing modifications to assure virus removal during purification has reduced the risk of virus contaminations in plasma-derived products.

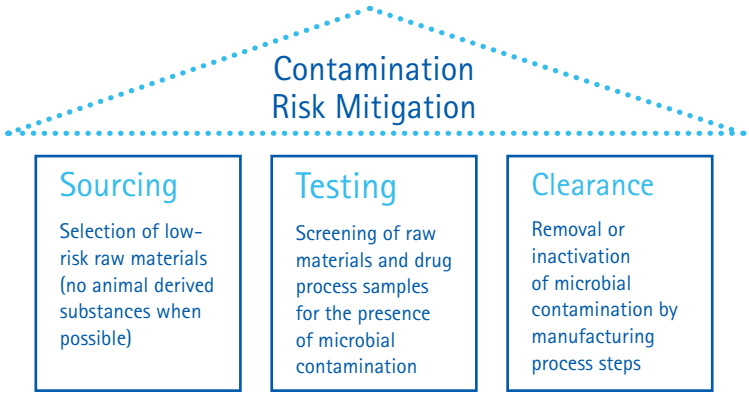
Biopharmaceutical manufacturers are required to test and validate the effectiveness of the manufacturing process to achieve an appropriate level of virus safety. The validation approach is generally modeled after the current regulatory guidance. This document summarizes the broad principles for assessing virus clearance of biological therapeutics.

A brief working summary of the virus clearance guidance

The regulatory guidance for virus safety promotes a comprehensive integrated risk-based approach in which raw materials are carefully selected and controlled, in -process material is screened for the presence of virus, and steps in the manufacturing process are evaluated for the ability to remove or inactivate virus.

Because cell lines used as protein expression systems commonly contain retrovirus-like particles, it is essential that manufacturers of biologics have a means for clearing these known contaminants. Before a biologic is administered to humans in Phase 1 trials, the production operations that are likely to contribute to viral clearance should be identified and assessed for clearance capabilities.

To meet this requirement, and determine the level of clearance contributed by the individual operations, manufacturers commonly perform studies in which model viruses, usually a retrovirus and a parvovirus,



are "spiked" into the biologic and a small-scale version of the purification operation is run. The level of virus in the biologic before and after the unit operation is measured to assess clearance. Clearance levels are generally referred to as reduction factors and are expressed in log values.

Subsequent clearance evaluations will typically be performed before the start of Phase III or commercial production, particularly if the manufacturing process has been modified since the earlier virus clearance assessment. These tests will usually involve an expanded panel of viruses with a broader range of physicochemical properties to assess the robustness of the process for removal of different types of potential virus contaminants.

Overall reduction factor targets for a process are usually dependent on levels of endogenous retrovirus particles in the cells. However, the history and type of production cell line, materials used for production, experience of the biologics manufacturer with the cell line and published data, all impact clearance targets. Dependent on the clearance targets, manufacturers may evaluate three or more operations in a purification scheme for clearance capabilities. Most importantly, the manufacturer will need to understand the mechanism of virus clearance at each step, in order to assure complementary or orthogonal processes of virus inactivation or removal. This requirement assures effectiveness of the overall process for clearance capabilities against a broad range of potential virus contaminants.

Although specific differences exist in different geographies, this general approach is advocated worldwide as a means of ensuring the virological safety of biologically-derived therapeutic products.

Virus clearance regulatory agencies

EMA

European Medicines Agency (EMA) is the scientific agency of the European Union responsible for protection and promotion of public and animal health. It coordinates evaluation and monitoring of centrally authorized products, develops technical guidance and provides scientific advice to sponsors in member states. EMA replaced EMEA (The European Agency for the Evaluation of Medicinal Products) in 2004.
www.ema.europa.eu/ema.

ICH

The ICH (The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use) is a project that brings together the regulatory authorities of Europe, Japan and the United States to discuss scientific and technical aspects of drug registration. The purpose is to make recommendations to achieve greater harmonisation in the interpretation and application of technical guidelines and requirements for registration in order to streamline testing during the research and development of new medicines and eliminate unnecessary delay in the global development.
www.ich.org

FDA

The FDA's Center for Biologics Evaluation and Research (CBER) is responsible for most, if not all, of the human drug products and therapeutic biologicals that would be considered at risk for virus contamination. They issue guidance for manufacturers on product development and the testing needed to assure that products for human and veterinary use meet stringent safety requirements. The FDA grants marketing approval of new medicinal products and enforces laws to assure product safety.
www.fda.gov/BiologicsBloodVaccines

Summary of regulatory guidance for virus clearance validation studies

Objective

A virus clearance study performed on a unit operation may have two objectives:

- **Specific virus clearance:** To demonstrate and document the clearance capability of the unit operation with respect to endogenous or known adventitious viruses.
- **General virus clearance:** To evaluate the more general capability of the unit operation to clear novel or unpredictable viruses that might contaminate the process or the feedstream.

Ultimately, the clearance capabilities, expressed as logarithmic reduction factors, of a number of unit operations in the process are combined to yield a composite measure of the effectiveness of the process to clear viruses.

Scale-down considerations

Validation studies are performed on a scaled-down model of the process step. In order to do this, it is necessary to determine which process variables are likely to have an impact on the effectiveness of the clearance step. To ensure test validity, it is essential that the scaled-down model accurately preserves the critical process variables of the full-scale system. The critical process variables, which will be different for different clearance technologies, may be divided into the following two categories:

- **Mechanical parameters** such as flow rates, mixing rates, column dimensions, column reuse, etc.[1]
- **Physicochemical parameters** such as protein content, pH, temperature, moisture content, etc.[1]

It is generally not possible to reduce a full-scale process to small-scale with complete accuracy. In light of this, key differences between the small-scale model and the full-scale process should be identified, and the impact of these differences on the validity of the study evaluated.

Test controls

The clearance study should include controls to help demonstrate the validity of the study. Essentially, these controls serve to detect and quantitate loss of virus titer that is not attributable to the specific clearance technology under evaluation. Specific controls "include parallel control assays to assess the loss of infectivity of the virus due to such reasons as the dilution, concentration, filtration or storage of samples before titration."[2] Such non-specific titer loss, if attributed to the clearance technology, would lead to an overestimation of the clearance technology capability. Additionally, "buffers and product should be evaluated independently for toxicity or interference in assays used to determine the virus titer, as these components may adversely affect the indicator cells."[2]

The choice of viruses

Viruses that contaminate biological process streams are divided into three categories:

- **Endogenous virus:** Virus "whose genome is part of the germ line of the species of origin of the cell line and is covalently integrated into the genome of animal from which the parental cell line was derived [...] Intentionally introduced, non-integrated viruses [...] fit in this category."[2]
- **Non-endogenous virus:** "Viruses from external sources present in the Master Cell Bank"[2]
- **Adventitious virus:** "Unintentionally introduced contaminant viruses"[2] Contamination with adventitious virus may occur through the addition of contaminated raw materials or through extraneous contamination.

Viruses used in spiking studies are categorized as follows, depending on the relationship of the virus to an actual virus contaminant:

- **Relevant virus:** "The identified virus, or of the same species as the virus that is known, or likely to contaminate the cell substrate or any other reagents or materials used in the production process."[2]
- **Specific model virus:** "Virus which is closely related to the known or suspected virus (same genus or family), having similar physical and chemical properties to those of the observed or suspected virus."[2]
- **Nonspecific model virus:** "A virus used for characterization of viral clearance of the process when the purpose is to characterize the capacity of the manufacturing process to remove and/or inactivate viruses in general, i.e., to characterize the robustness of the purification process."[2]

Viruses should be chosen for clearance studies on the basis of the following considerations:

1. Viruses "should be chosen to resemble viruses which may contaminate the product,"[1] which inlcude viruses endogenous to the expression system as well as viruses that may be added as part of the production process. An example of the latter is viruses that may contaminate growth medium components.
2. Viruses should be chosen "to represent a wide range of physico-chemical properties in order to test the ability of the system to eliminate viruses in general."[1]

3. Viruses should be chosen that may be grown to high enough titer in order to effectively challenge the virus clearance step under evaluation. However, "care should be taken in preparing the high-titer virus to avoid aggregation which may enhance physical removal and decrease inactivation, thus distorting the correlation with actual production."[2]
4. Viruses should be chosen that do not pose an undue safety hazard to operators.[2] The exception to this rule is HIV, which is often used to test plasma products.
5. A panel of viruses will be established that includes relevant/specific model viruses and non-specific model viruses. For biotechnology products, a typical panel may include:[2]
    - Murine retrovirus (e.g., MuLV, for products derived from murine cell lines)
    - SV40, poliovirus or an animal parvovirus as small non-enveloped virus
    - A parainfluenza or a murine retrovirus as large enveloped RNA virus
    - A herpes virus as a large DNA virus

Common virus contaminants and model viruses

Table 1. provides a summary of viruses that are pertinent to virus spiking studies because they are typical contaminants or common model viruses. The table is not all-inclusive; other viruses may be found as contaminants and may serve as model viruses in validation studies.

Virus	Category	Size (nm)	Notes
Parvoviruses (e.g., canine, porcine)	Specific model virus for B19 Non-specific model virus	15 – 24	Plasma products Plasma and recombinant products
Mouse Minute Virus	Adventitious/Relevant	18 – 26	Recombinant products
Parvovirus B19	Endogenous/Relevant	18 – 26	Plasma products
Poliovirus Sabin 1	Non-specific virus	24 – 30	Recombinant or plasma products
Encephalomyocarditis Virus	Endogenous	25 – 30	Recombinant products
Hepatitis A Virus	Endogenous/Relevant	27 – 32	Plasma products
Hepatitis C Virus	Endogenous virus	30 – 60	Plasma products
Hepatitis B Virus	Endogenous/Relevant	42	Plasma products
Duck Hepatitis B Virus	Specific model for HBV	40 – 48	Plasma products
Japanese Encephalitis Virus	Specific model for HCV	40 – 50	Plasma products
Simian Virus 40 (SV40)	Non-specific	40 – 50	Recombinant and plasma products
Bovine Viral Diarrhea Virus	Specific model virus for HCV	40 – 70	Plasma products
Sindbis	Specific model virus for HCV	60 – 70	Plasma products
Reovirus 3	Non-specific model virus	60 – 80	Plasma and recombinant products
Vesicular Stomatitis Virus	Non-specific virus	70x175	Recombinant and plasma products
Murine Leukemia Virus	Specific model for C- or A-type retrovirus like particles	80 – 110	Recombinant products
HIV	Endogenous/Relevant	80 – 120	Plasma products
Pseudorabies Virus	Specific model for herpesvirus	120 – 200	Plasma products
Epstein-Barr Virus (mononucleosis)	Endogenous	120 – 200	Used to immortalize antibody-producing B lymphocytes
Herpes Simplex Virus	Endogenous	150 – 200	Recombinant
Parainfluenza (flu)	Non-specific virus	150 – 300	Recombinant or plasma products
Cytomegalovirus	Endogenous	180 – 200	Plasma products

Virus Assays

The validity of the virus clearance study is dependent on the robustness of the test system used to quantitate virus loads. For this reason "virus infectivity assays used to quantitate the virus titer should be sensitive, reproducible and conducted with sufficient replicates to demonstrate statistical accuracy."[4] If the feedstock is "toxic to the indicator cells, dilution, adjustment of the pH, or dialysis of the buffer containing spiked virus might be necessary."[2]

Virus assay sensitivity is inherently limited by a test design in which a small percentage of virus-filtered material is subjected to the assay. Increasing the sample volume tested increases the probability of virus detection in cases of low virus concentration.

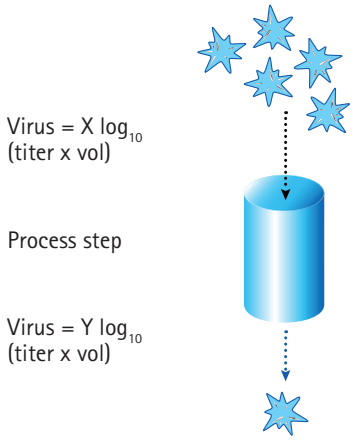
Reduction Factor Calculations

The reduction factor is the $\log_{10}$ of the ratio between the total virus load before the clearance operation and the total virus load after clearance step.

The principle of a viral safety study is to spike a process step with higher titer virus and **quantify the inactivation/removal capacity of the individual process step** expressed as:

Logarithmic Reduction Factor (LRF)

$$LRF = X \log_{10} - Y \log_{10}$$



The current guidance states that "sufficient sample volumes should be tested to ensure that there is a high probability of detecting virus in the sample if present." Nevertheless, it is common for post-clearance samples to be free of detectable virus. In this case, it is necessary to calculate a theoretical virus concentration in the post-clearance sample before a reduction factor may be calculated.

Assuming the volume of sample tested is much smaller than the total volume of material tested, the probability that the sample volume does not contain infectious virus can be approximated by the Poisson distribution and a theoretical concentration of virus in the sample can be determined.

Interpretation of Validation Study Results

Test validity

The final output of a clearance study will be a reduction factor which should be considered in the context of all clearance information available from the manufacturer. Scientific validity is also dependent on aspects of the test design, execution and control of test systems including:

- Validity of the scale-down model
- Appropriateness of the viruses chosen to accurately model actual or likely virus contaminants
- Assurance that the feedstock accurately represents actual process feedstock
- Assurance that the virus stock was prepared appropriately
- Appropriateness of the controls and verification that the control results support the conclusion of test validity
- Validation that the virus assay methods allow accurate quantitation of the viruses in spiked feedstock and in filtrate
- Correctness of calculations

Unit Operation Robustness

In virus safety guidances, "robustness" is a term used to refer to two related concepts. The first is the capability of a virus clearance technology to clear a wide range of viruses.[2] The second is the sensitivity of the clearance technology effectiveness to perturbations of process variables.[3]

Effectiveness

On the basis of the reduction factor (generated via a valid test design and execution) and an assessment of robustness, a unit operation may be classified as effective, ineffective or moderately effective. "Effective" steps provide a reduction factor of at least 4 and are unaffected by small perturbations in process variables. "Ineffective" steps provide a reduction factor of 1 or less, and "moderately effective" steps fall between these two extremes.[1]

Evaluation of process clearance requirement

Overall process clearance requirements are determined by how much virus is likely to be present in the unprocessed bulk, since the process must be designed with excess capacity to remove viruses.[2]

Furthermore, as the industry moves towards increasing understanding of the impact of small changes in manufacturing operations on product quality, biologics manufacturers are expected to understand how these changes impact the virus reduction assessments of the individual operations. Small-scale, well-designed replicated studies with a suitable panel of viruses are crucial to understanding the virus clearance capabilities of each step in the manufacturing process and their robustness with respect to virus safety. Successful completion of these virus validation studies is key for both regulatory approval and ultimately patient safety.

References

1. 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.
2. Q5A Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human of Animal Origin, ICH, 1997 (Adopted as a guidance document and published in Federal Register September 1998).
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4. Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use, CBER FDA, 1997.

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