



Ensuring Compliance: Regulatory Guidance for Virus Clearance Validation

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Introduction

To assure virological safety of biological therapeutics, regulatory guidance advocates an approach in which virus 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 product 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 or herpesvirus) or nonendogenous to the cell line (e.g., Epstein Barr virus used to immortalize cells), and adventitious viruses (e.g., parvovirus). To date, biotechnology products derived from cell lines have not been implicated in the transmission of viruses. Nevertheless, to assure product safety, validated virus clearance steps should be a part of

the manufacturing process. For plasma products, the threat of virus contamination is more apparent in that plasma is known to carry a number of human pathogenic viruses and virus transmission (e.g., HAV, HBV, HIV and B19) has occurred through administration of virus-contaminated products.

As is typical of pharmaceutical manufacturing, the manufacturer is required to validate the effectiveness of virus clearance steps and to document the effectiveness and adequacy of the manufacturing process to achieve an appropriate level of virus safety. The validation approach is generally modeled after the current regulatory guidance, which is the subject of the present document. This document provides a brief introduction to the regulatory agencies most influential in the area of virus safety regulations and a general summary of the more broadly referenced regulatory guidance documents that pertain to virus safety of biotechnology and plasma derived products. Specific emphasis is placed on the validation of virus clearance.

A Brief Working Summary of the Virus Clearance Guidance

The regulatory guidance for virus safety promotes a three-tiered approach in which:

1. Raw materials are controlled and qualified for use through virus screening measures
2. Specific steps in the manufacture of the product are included in the process line and validated to clear virus
3. Final product is screened for the presence of virus

Because cell lines used as protein expression systems commonly contain and produce viruses (retrovirus-like particles are a common example), it is essential for the manufacturer to have in place a means for clearing these known contaminants. Virus clearance steps must be validated before Phase I product may be administered to humans. To meet this requirement, it is common for biotech manufacturers to validate virus clearance steps for the removal of murine leukemia virus, which serves as a specific model virus for the known retrovirus-like particles produced by murine cell expression systems. It is also common for the manufacturer to insert and validate a parvovirus clearance step into the manufacturing process for Phase I product. For plasma derivative manufacturers, the number of known virus contaminants is greater, and the need for early and more extensive virus clearance validation is commensurately greater.

A subsequent virus validation study typically will be done before production of Phase III material. In this validation study, it is common for the Phase I validation to be repeated. This is especially true if the manufacturing process has changed as the manufacturer has progressed from Phase I to Phase III. In addition to retrovirus and parvovirus, the manufacturer will generally choose two to four additional viruses for testing. The purpose of testing these nonspecific model viruses is to assess the robustness of the virus clearance step. Robustness provides added assurance that the virus clearance step will be unaffected by process perturbations and will be capable of removing adventitious and unpredictable virus contaminants.

The reduction factor requirement generally is established specifically for viruses that are known to contaminate the process. The most common example of this is retrovirus-like particles produced by murine protein expression cell systems. With a process-specific reduction factor target in mind, the manufacturer will add virus clearance steps to the process and will run validation studies to document the virus clearance effectiveness of several unit operations. In addition, the manufacturer will take into account the mechanism of virus clearance for each step, and will be required to incorporate orthogonal clearance steps, i.e., steps that rely on complementary mechanisms of virus clearance.

This general approach is advocated world wide as a means of ensuring the virological safety of therapeutic products of recombinant or plasma origin.

Virus Clearance Regulatory Agencies

EMA

The EMA (The European Agency for the Evaluation of Medicinal Products) comprises a number of Centers. The Center for Proprietary Medicinal Products (CPMP) is concerned with virus clearance validation. This agency has jurisdiction over much of Europe.

ICH

The ICH (The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use) "is a unique project that brings together the regulatory authorities of Europe, Japan and the United States (EC, Japanese MHLW, FDA) and experts from the pharmaceutical industry (European Federation of Pharmaceutical Industries Associations, Japanese Pharmaceutical Manufacturers Association, Pharmaceutical Research and Manufacturers of America) in the three regions to discuss scientific and technical aspects of product registration. The purpose is to make recommendations on ways to achieve greater Harmonisation in the interpretation and application of technical guidelines and requirements for product registration in order to reduce or obviate the need to duplicate the testing carried out during the research and development of new medicines. The objective of such Harmonisation is a more economical use of human, animal and material resources, and the elimination of unnecessary delay in the global development and availability of new medicines whilst maintaining safeguards on quality, safety and efficacy, and regulatory obligations to protect public health." The ICH has created a number of documents that have been adopted or are in the process of being adopted by various geographies.

FDA

The FDA's Center for Biologics Evaluation and Research (CBER) is responsible for most, if not all, of the drug products that would be considered at risk for virus contamination. Their mission "is to protect and enhance the public health through regulation of biological products including blood, vaccines, therapeutics and related drugs and devices according to statutory authorities."

The Paul Ehrlich Institut

The Drug Law of the Federal Republic of Germany cedes to the Paul Ehrlich Institut (a.k.a., the Federal Agency for Sera and Vaccines) the role of "competent higher federal authority" for "sera, vaccines, blood preparations, test allergens, test sera and test antigens". The competent higher federal authority is the body who grants manufacturing and marketing authorization to the drug manufacturer. The PEI has been very influential over the past several years in establishing validation practices for virus clearance.

Regulatory Agencies Web Sites

<http://www.emea.eu.int/>

<http://www.ich.org/>

<http://www.fda.gov/cber/>

<http://www.pei.de/>

Summary of Regulatory Guidance for Virus Clearance Validation Studies

The following is a composite summary of regulatory guidance^{1, 2, 3, 4} on virus clearance, with emphasis on how this guidance relates to virus filtration.

Objective

Virus clearance studies are performed with the overall intent of quantifying risk of virus contamination in the final dosage form. 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 are not known to contaminate the process or the feedstream.

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

Scale-down Considerations

Validation studies are performed on a scaled 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 model system 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, one should identify key differences between the small-scale model and the full-scale process, and evaluate the impact of these differences on the validity of the study.

Test Controls

The validation 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. Such non-specific titer loss, if attributed to the clearance technology, would lead to an overestimation of the clearance technology capability. 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 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 that depend on the route of contamination:

- *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
In plasma-derived products, endogenous viruses are natural contaminants of donor blood.
- *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 includes 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 such as bovine or human blood products. Viruses chosen for this purpose should be in the “relevant” or “specific model” virus category.
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. For plasma products, this virus is considered to pose enough of a product-contamination risk that the use of model viruses in clearance validation studies is not generally allowed.

Common Virus Contaminants and Model Viruses

Table 1.

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	70 x 175	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

5. A panel of viruses will be established that includes relevant/specific model viruses and non-specific model viruses. For plasma-derived products, a typical panel may include³:
- HIV-1
 - A model for hepatitis C virus (Sindbis, Yellow fever or BVDV)
 - Non-enveloped virus (Hepatitis A virus or B19)
 - Enveloped DNA viruses (Herpesvirus such as pseudorabies virus)
6. For biotechnology products, a typical panel may include²:
- Murine retrovirus (eg, MuLV, for products derived from murine cell lines)
 - Viruses that represent a wide range of properties
 - SV40, poliovirus or an animal parvovirus as small non-enveloped virus.
 - A parainfluenza or a murine retrovirus as large enveloped RNA virus.
 - A herpesvirus as a large DNA virus.

Table 1 on the previous page 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 Assays

The validity of the virus clearance study is dependent on the validity of the virus assays 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 A standard means by which to demonstrate virus assay reliability is to calculate the 95% confidence limits for the assays of the spiked feedstock and the virus-filtered material⁴. These confidence limits, designated as "+s" and "+a", respectively, should be within 0.5 log of the mean.

Virus assay sensitivity is inherently limited by a test design in which a small percentage of virus-filtered material is subjected to the assay. For this reason, "sufficient sample volumes should be tested to ensure that there is a high probability of detecting virus in the sample if present."⁴

Reduction Factor Calculations

The reduction factor is the log₁₀ of the ratio between the total virus load before clearance and the total virus load after clearance¹:

Reduction factor =

$$\log_{10} [(V1 \times T1)/(V2 \times T2)]$$

Where:

V1 = volume of spiked feedstock prior to the clearance step

T1 = virus concentration of spiked feedstock prior to the clearance step

V2 = volume of material after the clearance step

T2 = virus concentration of material after the clearance step

The 95% confidence interval of the reduction factor is calculated as the square root of the sum of the squares of the assay errors designated above as "+s" and "+a".

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.

This theoretical virus concentration is calculated as follows. For samples representing a percentage of the total volume, it is possible that the volume not sampled contains virus. The probability (**p**) of this is dependent on the total volume of filtrate (**V**), the sample volume of the filtrate (**v**), and the number of viruses in the total volume (**n**), and may be quantified by using the following equation:²

$$p = ((V-v)/V)^n$$

If the sample volume is very small as compared to the total volume, this probability (**p**) can be expressed by the Poisson distribution:

$$p = e^{-cv}$$

In this equation, “**c**” is the virus concentration.

By using the convention of applying 95% confidence limits, “**p**” would be set at 0.05.

In solving for “**c**”, the above equation is transformed to:

$$c = 3/v$$

This value for “**c**” is then used as the value for T2 in the first equation, and the reduction factor is calculated as above.

Interpretation of Validation Study Results

Test Validity

The final output of a clearance study will be a reduction factor. The validity of this number depends on the scientific validity of test design and execution. To evaluate scientific validity, several aspects of the test design and execution should be examined. In addition to standard requirements of GLP, some specific items include:

- Validity of the scale-down test system
- 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 Unit operations generally categorized as robust are: pH 3.9, heat inactivation, solvent-detergent and filtration (15 to 40 nm).⁴

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

A critical issue to be addressed by the manufacturer concerns the level of virus clearance required of the entire production process. This issue is especially relevant to the “specific virus clearance” objective described above. There is no requirement to establish a process reduction factor for viruses tested to satisfy the “general virus clearance” objective.

To address the process clearance requirement, it is necessary to know how much virus is likely to be in the unprocessed bulk, since the process must be designed with excess capacity to remove viruses.² Typically, virus in unprocessed bulk is quantitated by transmission electron microscopy of concentrated bulk or by infectivity assays. For expression systems known to express retroviruses (eg, murine cells or ascites), limits of detection dictate that a 10⁶ virus/mL load should be assumed if attempts at quantitation yield negative results⁴.

It can be assumed that this virus load, without virus clearance in the production process, would be distributed equally among each of the final filled dosage units. Assuming further a requirement for a 10^{-6} probability of virus in a filled dosage unit, it is possible to calculate the full process clearance requirement. As an example, an unprocessed bulk, found to contain 10^{10} viruses, will be used to produce 1000 vials of final filled product. In the absence of virus clearance, each vial would contain 10^7 viruses. Factoring in the requirement of a 10^{-6} probability of virus contamination per vial, the required reduction factor would $\log_{10}(10^7/10^{-6})$, or 13.

Inclusion of Appropriate Virus Clearance Steps

For plasma products, guidance states that two "effective" and mechanistically complementary steps should be included in the process³. This strategy provides assurance that viruses surviving the first step are likely to be cleared by the second step. However, if one clearance step can be shown to be effective in clearing a wide variety of viruses of differing physico-chemical characteristics, a second effective step would not be required.³ In either case, additional steps that are considered "reliable" or "moderately effective" would need to be included and validated for clearance capability in order to demonstrate the appropriate level of virus clearance. Each of these steps should provide a different mechanism of virus clearance.

References

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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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