

# Ensuring Regulatory Compliance: Validation of Virus Filtration

## *Virus spiking study design*

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### **Abstract**

Biological therapeutics of recombinant and plasma origin are commonly manufactured using biological source material that is intrinsically contaminated with viruses. Moreover, some manufacturing processes are, by their nature, susceptible to virus contamination from extrinsic sources. Accordingly, manufacturers of biological drug products are required to incorporate sufficient virus clearance steps into their manufacturing processes to ensure that their products are virus contaminant-free. Spiking studies are designed to validate the effectiveness of each virus clearance step. This brief highlights many of the key study design considerations and the basic elements of a well-designed virus filtration spiking study. It is intended to serve as an aid for spiking study sponsors to design and execute a successful spiking study and to remain in compliance with current regulatory requirements.

### **Background**

The regulatory strategy<sup>1, 2, 3, 4</sup> applied to virus safety of recombinant or plasma-derived drug products comprises the following four complementary approaches:

- Selecting and testing source materials to be virus-free or sufficiently low in virus load
- Testing the capacity of the production process to remove or inactivate viruses
- Producing product in conformance with GMP and testing the product, at appropriate stages of production, for freedom from detectable viruses
- Monitoring drug-product recipients for viral diseases potentially caused by virus-contaminated product

To implement the second of these approaches, manufacturers perform validation studies, i.e., virus spiking studies, on scale-down models of each virus clearance unit operation. After the virus spiking studies, the manufacturer is able to evaluate the overall virus reduction capability of the manufacturing process and determine whether the process is capable of providing an appropriate level of virus clearance to assure finished drug product safety.

Usually, at least two virus spiking studies will be performed for a given process. The first study, performed prior to manufacture of product for Phase I clinical safety trials, emphasizes documenting effective and adequate clearance of known virus

contaminants. The second study, performed prior to manufacturing for Phase III clinical trials, is more comprehensive. This study is designed to document known virus-contaminant removal as well as removal of a wide range of viruses that represent potential virus contaminants.

### Spiking Study Objectives

The overall aim of a virus spiking study is to assess the effectiveness of a virus clearance unit operation in clearing viruses from the product feedstream. This aim can be divided into two specific objectives.

*Specific-virus clearance:* "to provide evidence that the production process will effectively inactivate/remove viruses which are either known to contaminate the starting material or which could conceivably do so."<sup>2</sup> These are designated as "relevant" viruses.

*General-virus clearance:* "to provide indirect evidence that the production process might inactivate/remove novel or unpredictable virus contamination."<sup>2</sup>

## Key Study Design Considerations

### Scale-down Validity

Spiking studies are intended to reflect the virus clearance capability of the process-scale unit operation.

Therefore, "the level of purification of the scaled-down version should represent as closely as possible the production procedure,"<sup>3</sup> by reproducing the key operating parameters that have an effect on purification and on virus clearance. The critical operating parameters will be different for different virus clearance technologies, primarily because of differences in mechanism of action. For filtration, scale-down will focus on such parameters as:

- Identity between process and validation study filter media
- Flow rate or pressure
- The ratio of volume processed to filter surface area (V/A)
- Equivalence of process and validation study feedstocks
- Product yield and quality
- Equivalence in virus retention between process and scale-down filter devices

Typically, the study sponsor may rely on the filter manufacturer to supply some of the data required to support the claim of scale-down validity.

Although not specified in regulatory guidance, some manufacturers choose to employ worst case process-variable settings in the design of the spiking study.

### Feedstock

The nature of the feedstock is critical to creating a test system that accurately represents the true manufacturing process. Ideally, feedstock should be identical to the feedstock that will be processed by the unit operation in the manufacturing setting. Commonly, because of shipping or storage requirements, the manufacturer will freeze feedstock prior to the spiking study. Because freezing and thawing a protein product may produce aggregates, the effect of aggregates on the validity of the spiking study should be addressed. Aggregates can cause premature filter plugging, which will compromise one's ability to maintain the process scale V/A. To eliminate the problem of protein aggregates, it is possible to use a purification method to remove aggregates, or generate fresh feedstock at the site of the virus spiking study.

### Choice of Viruses

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

- Endogenous
- Non-endogenous
- Adventitious

In biotechnology processes, endogenous viruses exist as a part of the cell line used to express the protein product. In plasma-derived products, endogenous viruses are natural contaminants of donor blood.

Non-endogenous viruses are “viruses from external sources present in the Master Cell Bank.”<sup>3</sup> This category is therefore especially relevant to recombinant drug products. Adventitious viruses are introduced into the product during the manufacturing process either through the addition of contaminated raw materials or through extraneous contamination.

Viruses used in virus spiking studies are divided into three categories: relevant, specific model and non-specific model. A “relevant” virus is the same as, or the same species as, an endogenous virus or a likely adventitious virus. These viruses could be contaminants of “the cell substrate or any other reagents or materials used in the production process.”<sup>3</sup> A “specific model” virus is closely related to, and has similar physical and chemical properties to, a known virus contaminant. Lastly, “non-specific model” viruses represent a wide range of virus physical and chemical properties.

To satisfy the “specific virus clearance” objective, viruses in the “relevant” category are the first choice. If use of a relevant virus is not possible, the manufacturer chooses the best specific model virus to serve as a model for the relevant virus. To satisfy the “general virus clearance” objective, the study sponsor will evaluate clearance of a group of non-specific model viruses (generally two or three). These viruses are combined with the relevant and specific model viruses to create a four- or five-virus panel that represents viruses of different genomes (DNA and RNA), sizes and surface properties (enveloped and non-enveloped). The following table provides a summary of viruses pertinent to virus spiking studies, because they are typical contaminants or common model viruses. (Table 1).

## Common Virus Contaminants and Model Viruses

**Table 1.**

<b>Virus</b>	<b>Category</b>	<b>Size (nm)</b>	<b>Notes</b>
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

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## Clearance Mechanism

Clearance mechanism refers to the means by which the technology effects virus clearance. Current regulatory philosophy holds that reduction factors from multiple steps employing the same mechanism may not be considered additive. The rationale for this position is that virus not cleared by a given mechanism is more likely to pass through additional virus clearance steps that employ the same mechanism. This concern is the basis of the need for manufacturers to define the mechanism of each virus clearance step and to employ "orthogonal" clearance steps, steps that rely on complementary mechanisms.

The two basic clearance categories are inactivation and removal. Inactivation technologies may be divided into those that inactivate due to non-specific chemical interaction with the virus (e.g., solvent detergent), specific chemical interaction with the virus (e.g., nucleic acid intercalators), or the physical effects of heat (e.g., HTST treatment or pasteurization), irradiation or pressure. Removal technologies are further categorized as those that remove by partitioning (e.g., chromatography) and those that remove by size exclusion. Although some filters are specifically derivatized to contain chemical ligands that remove viruses and other biomolecular impurities through partitioning mechanisms, robust, dedicated virus removal filters rely primarily on size exclusion.

## Reproducibility

Reproducibility is a concern in two areas. First, virus assays "should have adequate sensitivity and reproducibility and should be performed with sufficient replicates and controls to ensure adequate statistical accuracy of the result."<sup>2</sup> Second, in accord with good scientific practice, the overall study results should be reproducible. Accordingly, "an effective virus removal step should give reproducible reduction of virus load shown by at least two independent studies."<sup>3</sup>

## Virus Stock Preparations

Virus stock preparations may be characterized as to their purity, degree of aggregation and titer. Because viruses are prepared by using cell culture methods, the preparations are inherently susceptible to a large amount and variety of impurities. These impurities contribute to filter fouling and may prevent one from reaching the predetermined V/A. There is no specific regulatory guidance or standard industry practice related to virus purity characterization or quantitation.

Higher virus titers allow larger virus reduction factors to be demonstrated and allow a more rigorous challenge to the clearance unit operation. For this reason, regulatory guidance states that "the amount of virus added to the starting material for the production step which is to be studied should be as high as possible."<sup>2</sup> However, so as not to alter the product composition unacceptably, the volume of spike should be kept low, typically less than 10%. The guidance also voices concerns over virus aggregation that could be induced by deliberately concentrating the virus. The use of aggregated virus could lead to underestimation of inactivation effectiveness and overestimation of size-exclusion effectiveness. In this vein, there is no quantitative regulatory guidance for the acceptable level of virus aggregation or specific means to evaluate virus aggregation. The common practice is to use size-based prefiltration to remove virus aggregates from a spiked feed stream prior to performing the clearance study.

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## Reduction Factor Requirements

The overall process reduction factor requirement is established for known virus contaminants, a common example being retrovirus-like particles produced by murine cell protein-expression systems. For known or anticipated virus contaminants, the manufacturer should quantitate the expected virus load in the unprocessed bulk. Typically, this is done through use of transmission electron microscopy or infectivity assays. The manufacturer should design a process that provides a high level of assurance that final product will be virus free. Typically, this is done by documenting a  $10^{-6}$  probability that a unit dose will contain a virus. Therefore, the overall process reduction factor should be 6 units greater than the  $\log_{10}$  of the total virus load. For example, 100 L of an unprocessed bulk containing  $10^6$  virus/mL will have a total virus load of  $10^{11}$  viruses. If this bulk is used to produce 1000 vials of finished product, the potential virus load per vial would be  $10^8$ . In this case, the required reduction factor would be 14 ( $\log \{10^8/10^{-6}\}$ ). With this approach, an overall process reduction factor requirement in the range of 15 is common.

The manufacturer will achieve this target by validating existing purification steps (e.g., chromatography) for virus clearance, and by adding dedicated, "effective" virus clearance steps, such as filtration, to the process.

As discussed below, an "effective" virus clearance step provides a reduction factor of at least 4. Generally, manufacturers expect to claim a reduction factor between 4 and 6 for each dedicated step, although lower reduction factors may be acceptable.

For viruses tested to achieve the general virus clearance objective, there is no specific guidance as to the minimum reduction factor that one should achieve per virus. In lieu of specific guidance, many manufacturers adopt an internal reduction factor target of 4.

## Appropriate Controls

To ensure scientific validity, "the study should 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."<sup>3</sup> The controls may be used to quantitate loss of virus titer that is not due to specific action of the clearance step under consideration. The three most common controls are:

- Hold control: evaluate the loss of virus titer over time, following introduction of the virus into the feedstock.
- Prefiltration: evaluate the loss of virus titer following passage of the spiked feedstock through the prefilter.
- Cytotoxicity/interference: evaluate the inhibitory effect of the feedstock on the virus assay.

Each of these controls is discussed in greater detail in the section entitled "Preliminary Testing."

## Basic Spiking Study Design

A basic spiking study for virus filtration has the following characteristics:

- The feedstock attributes (concentration, temperature, chemistry, etc.) are comparable to those of process scale feedstock.
- The relevant scale-down filter device specifications are equivalent to those of the process scale filter device.
- The filtration is operated in a manner that mimics process-scale operation.
- The V/A is scaled linearly from process scale to the scaled-down model of the unit operation.
- The pooled filtrate is sampled for virus.
- The spiked feedstock is filtered; there is no post-use buffer rinse.

The following is based on this general approach. However, more complicated studies may be designed (e.g., collection and assay of filtrate fractions and rinse of the virus filter following filtration of the feedstock).

## Requisite Information

In order to design a successful spiking study, the following information should be known.

1. *Process volume*: The volume of feed stock that will be filtered
2. *Process filter surface area*: The surface area of the membrane housed within the filter device
3. *Volume to area ratio*:  $V/A$ , the process volume divided by filter surface area
4. *Volume of filtrate to assay for virus*: Some virus testing laboratories have constraints on the volume of filtrate that can be assayed. Because this volume will have an impact on the reduction factor that may be claimed from the study, it is important to determine the volume of filtrate that will be assayed following the virus filtration.
5. *Target reduction factor*: Generally, this value will be between 4 and 6.
6. *Virus titer*: Because virus titer will be used to determine the amount of virus spike required to reach the target reduction factor, the titer must be determined before the validation study is performed. Due to the inherent variability of virus assays, this titer will be an approximation of the titer obtained during the spiking study. Nevertheless, a reasonable estimate of the titer by the virus producer is necessary to allow preliminary calculations to be made.

## Preliminary Testing

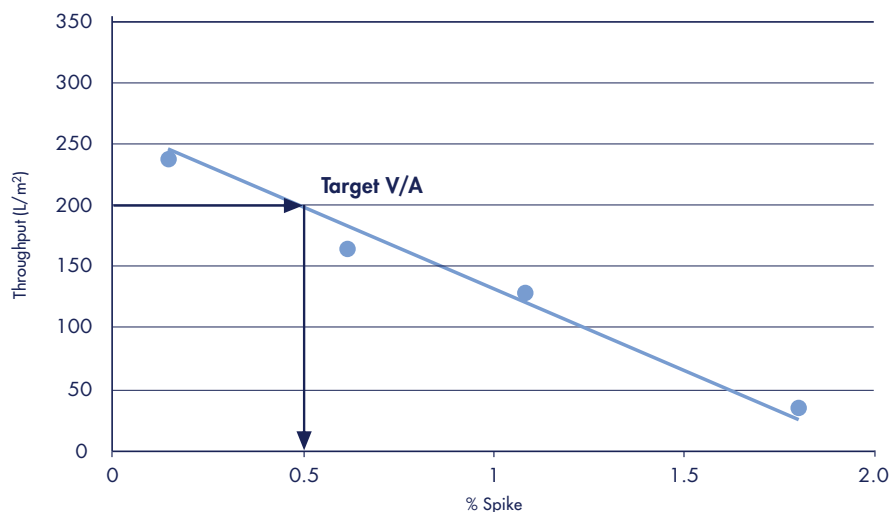
Performing the following tests prior to the actual validation study will allow better characterization of the test system and better design of the spiking study. This will reduce the risk of an invalidated or unsuccessful spiking study. The first three of the following tests are also controls which should be performed during the actual validation study. The need to perform any of these tests as a prelude to the validation study is not absolute. The study sponsor should evaluate the potential costs and benefits of these tests on a case-by case basis.

1. *Hold control*: Virus inactivation following introduction into the feedstock will compromise the validation study. To eliminate this risk, a "hold" control may be performed. In this test, virus is added to feedstock and the virus titer is checked periodically through a period of time at least equal to the anticipated duration of the spiking study. The relationship between virus titer and time will provide an indication as to the impact of the feedstock on virus viability.

2. *Prefiltration*: Generally, the spiked feedstock will be prefiltered, with the purpose of removing virus aggregates and other relatively large biomolecular impurities from the feedstream. It is common to use 0.1 or 0.2 micron filters for this purpose. Although these filters are not expected to remove significant amounts of virus, there are occasions when several logs are removed. If this is the case, the spiking study will be invalidated due to an insufficient virus challenge. To eliminate this risk, a preliminary prefiltration study may be performed, in which actual feedstock is spiked with virus and filtered through the prefilter by following the validation study protocol. Virus titers of spiked feedstock before and after prefiltration are used to determine the titer loss due to prefiltration.

3. *Cytotoxicity/interference*: Viruses are commonly assayed by using an infectivity assay in which virus-spiked feedstock is added to cell culture and the virus contacts a host cell to begin the infectious cycle. The infection leads to specific visible effects on the cell culture (e.g., plaque formation or focus formation), and these effects are enumerated. If the feedstock is cytotoxic, meaning that the cell culture is damaged or killed by contact with the product, the virus assay sensitivity will be compromised. Likewise, a feedstock component may interfere in some manner with the virus assay. Either of these occurrences could invalidate the virus assay, and in turn invalidate the entire validation study. To eliminate this risk, a preliminary study may be performed in which known amounts of virus are added to feedstock and to non-cytotoxic/non-interfering buffer. These two spiked fluids are then added to the cell culture and virus

## Determination of Maximum % Spike Compatible With Achieving Target V/A



**Figure 1.** Data are depicted from four trials in which progressively larger spikes were added to protein feedstock and filtrations were performed to quantitate throughput (V/A) of the virus filter. A best-fit line was generated and used to determine that the target V/A of 200 L/m<sup>2</sup> (established during process development) may be achieved with a maximum spike of 0.5%. Larger spikes will lead to a throughput below target.

is quantified by following the standard virus assay protocol. Differences in virus counts will indicate the effect of feedstock on the virus assay.

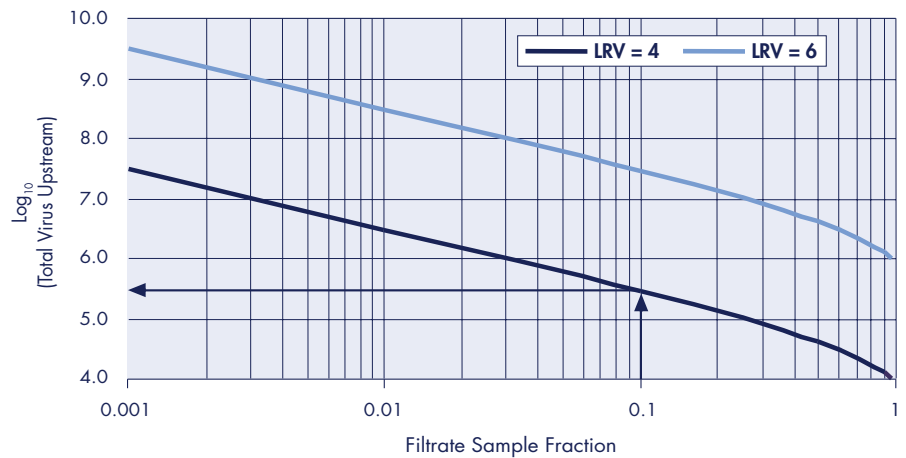
4. *Shipping*: Shipping of feedstock to a contract test facility can have an impact on the quality of the feedstock. Protein stability and propensity toward aggregate formation are key. To ensure that the quality of the protein feedstock is preserved through the shipping process, a shipping study may be performed. In this study, a mock of the shipping process is performed and quality attributes of the feedstock are evaluated. Of specific concern for the filter validation study is the filterability of the feedstock following the shipping process.

5. *Estimating maximum virus spike to achieve target V/A*: As described previously, virus stock preparations may contain impurities that foul virus filters and compromise one's ability to reach the predefined V/A acceptance criterion. To reduce this risk, a study may be performed to determine the relationship between amount of spike and achievable V/A. To perform this study, feedstock is spiked with progressively larger amounts of virus and the spiked feedstock is processed by following the spiking study protocol. The amount of spiked feedstock filtered through the virus filter is determined, the V/A ratios represented by these volumes are calculated and the V/A is plotted against the amount of virus spike (Figure 1). Finally, this relationship is compared to the specific V/A acceptance criterion, to estimate the maximum virus spike.

## Preliminary Calculations

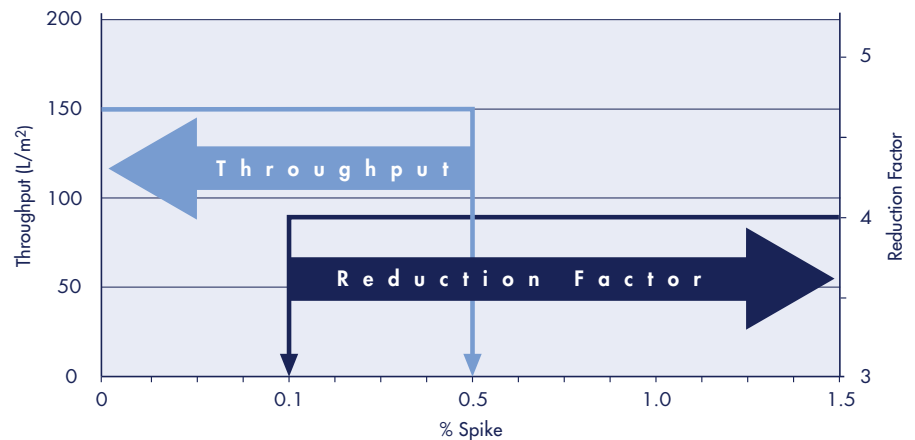
1. *Estimating the minimum virus requirement:* To reach a target reduction factor requires a combination of spiking with some minimum number of virus and assaying of some fraction of the filtrate. The continuum of this relationship between virus spike and filtrate assay fraction is depicted, in Figure 2, for the two common reduction factor targets of 4 and 6. The y axis represents virus spike in terms of total number of viruses and the x axis represents the amount to assay, as a fraction of the total filtrate. To estimate the minimum virus requirement, the target reduction factor and the fraction of the filtrate to be assayed must be established (Figure 2).
2. *Optimal spike determination:* The optimal spike is one that allows one to simultaneously achieve the target reduction factor and the target V/A. Spike may be expressed as a volumetric amount of virus stock added to a given volume of feedstock, as a percentage or fraction of the virus stock relative to the given volume of feedstock, or as the total number of viruses added to the feedstock. The optimal spike may be determined by comparing the spike amount that is required to reach a target reduction factor to the spike amount beyond which the target V/A will not be achieved.

## Reduction Factor as a Function of Virus Spike and Virus Assay Volume



**Figure 2.** This graph relates total virus spike, the fraction of filtrate subjected to virus assay and reduction factor. With two of these variables held constant, the third may be determined. For example, if the target reduction factor is 4 and the fraction of filtrate to assay is 0.1, then the minimum virus spike required would be 5.5 logs, or  $10^{5.5}$  virus units. If the stock virus titer is  $3 \times 10^6$  virus/mL, then the minimum volume of stock virus required would be 0.1 mL ( $3 \times 10^6 / 10^{5.5}$ ). If the volume of feedstock was 100 mL, then this would represent a 0.1% spike of virus stock into feedstock.

## Optimal Spike Determination



**Figure 3.** From figure 1, it was determined that the maximum percent spike compatible with reaching the target V/A of 150 L/m<sup>2</sup> is 0.5%. From figure 2, it was determined that the minimum percent spike compatible with reaching target reduction factor of 4 is 0.1%. In this figure, these two boundaries are compared to determine a range of percent spikes that is compatible with reaching both of these objectives, V/A and reduction factor, simultaneously. In this case, the range is 0.1% to 0.5%.

## Execution of the Spiking Study

"Viral clearance studies should be conducted in a separate laboratory equipped for virological work and performed by staff with virological expertise in conjunction with production personnel involved in designing and preparing a scaled-down version of the purification process."<sup>3</sup> Although some manufacturers have sufficient virus laboratory capabilities, most study sponsors contract with an outside laboratory. Some of the more common of these laboratories are listed. Because a successful virus filtration study requires expertise in virology, filtration and the therapeutic drug product, it is often most beneficial for the spiking study execution to run as a collaboration among the contract laboratory, the filter manufacturer and the study sponsor.

### Contract Laboratories that Conduct Virus Spiking Study Studies

AppTec Laboratory Services

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Bioreliance, Inc.

[www.bioreliance.com](http://www.bioreliance.com)

Charles River Laboratories

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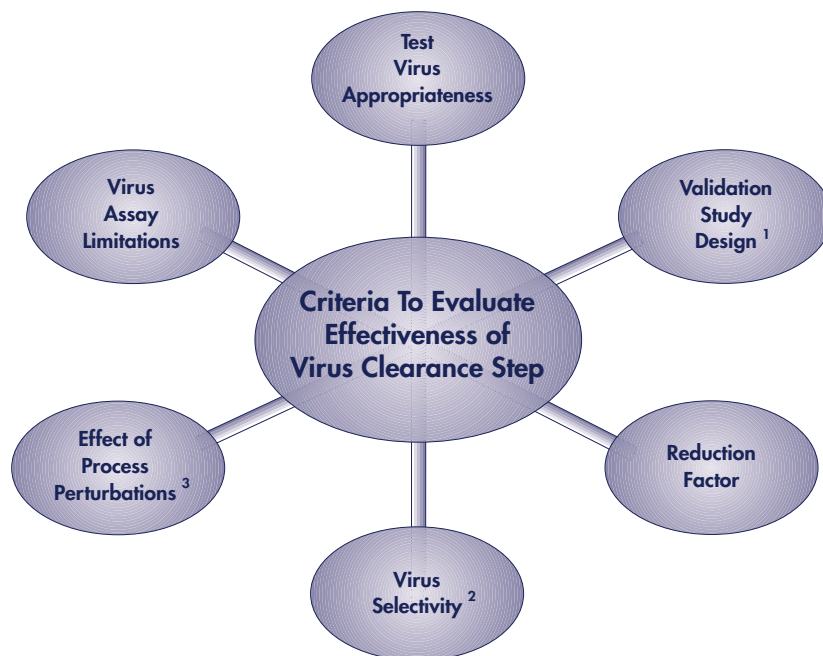
## Interpretation of Spiking Study Data

Virus clearance steps may be placed into one of three categories: effective, moderately effective or ineffective.<sup>2</sup> Virus clearance effectiveness should be evaluated in the context of several criteria, which are presented in the Figure 4. An effective virus clearance step will reproducibly provide a reduction factor of 4 or more. An ineffective step demonstrates a reduction factor of 1 or less, this cutoff being related to the "limitations of virus validation studies."<sup>2</sup> A moderately effective step falls in between the two extremes. Thus, a step that reproducibly yields a reduction factor of 2 is not considered effective but may nevertheless be recognized as reliably contributing to the overall process reduction factor. Commonly, manufacturers will place a lower limit of 3 on the reduction factors that will be combined to yield the overall reduction factor for the manufacturing process.

An ultimate goal of the validation process is to derive the overall virus reduction factor for the process. "The ability of the overall process to remove infectivity is expressed as the sum of the (reduction factors) at each step."<sup>3</sup> However, the manufacturer is cautioned to consider the following when determining the overall process reduction factor. First, unit operation reduction factors of 1 or less generally are not acceptable. Second, "addition of individual virus reduction factors resulting from similar inactivation mechanisms along the manufacturing process may overestimate overall viral clearance."<sup>3</sup> For this reason, it is necessary to understand the mechanism by which each step effects virus clearance. Lastly, "a single step having a large effect gives more assurance of viral safety than several steps having the same overall effect."<sup>2</sup>

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## Virus Study Assessment Criteria



1. A key aspect of study design is the accuracy with which the validation study apparatus and operation scales to those of the manufacturing scale clearance step.
2. "A process step may be highly effective for some viruses but ineffective against others, e.g., S/D treatment is effective against lipid-containing but not lipid-free viruses."<sup>2</sup>
3. The effectiveness of a virus clearance step will be in question if the virus clearance step capabilities are affected by small changes to process parameters.

**Figure 4.**

### Conclusion

Filter spiking studies are validation studies that are required to demonstrate effectiveness of virus clearance unit operations in the manufacture of biological therapeutics. The data from unit operation virus spiking studies are combined to document that the manufacturing process, in its entirety, effects adequate virus clearance.

Proper design of the validation study is critical to ensuring success of the study. Scale-down validity, choice of virus, feedstock quality, virus stock quality and inclusion of proper study controls are examples of the topics that need to be addressed in the study design. To effectively design the study, it may be necessary to perform preliminary testing aimed at characterizing the interactions among the virus, pre-filter, virus filter and feedstock.

Resources spent in this preliminary characterization allow a more careful setting of test parameters and lead to the greatest opportunity for success in the virus spiking study.

## Definitions

*Feedstock:* The manufacturer's fluid that is filtered in the spiking study

*Percent Spike:* The ratio between the volume of virus stock spiked into the feed stock and the volume of the feed stock, multiplied by 100

*Reduction factor:*  $\log_{10}$  of the ratio of total virus in the feed and the total virus in the filtrate

*Spike:* The virus that is added to a feedstock

*Spiking Study:* A validation study in which scale-down filter devices and process conditions are used to validate the clearance capability of a virus filter. The results of this study are submitted to the appropriate regulatory body as part of a marketing authorization application (e.g., BLA).

*Target reduction factor:* Reduction factor established as that which the study sponsor wishes to claim for the virus clearance unit operation

*Target V/A:* V/A acceptance criterion. Generally, this is based on the process scale V/A that was derived as part of process development.

*V/A:* Volume that is passed through a unit area of filter. It is expressed as L/m<sup>2</sup> in the process or mL/cm<sup>2</sup> in scale down studies.

*Virus Stock:* The stock virus preparation that will be spiked into the feedstock

*Virus Titer:* The concentration of the virus preparation, expressed as virus units per mL

## Virus:

*Adventitious virus:* "Unintentionally introduced contaminant viruses"<sup>3</sup>

*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."<sup>3</sup>

*Non-endogenous virus:* "Viruses from external sources present in the Master Cell Bank"<sup>3</sup>

*Non-specific 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"<sup>3</sup>

*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"<sup>3</sup>

*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"<sup>3</sup>

## References

1. Note for Guidance on Plasma-Derived Medicinal Products, CPMP EMEA, 2001
2. Note for Guidance on Virus Validation Studies: The Design, Contribution and Interpretation of Studies Validating the Inactivation and Removal of Viruses, CPMP EMEA, 1996
3. Q5A Viral Safety Evaluation of Biotechnology Products Derived From Cell Lines of Human or Animal Origin, ICH, 1997
4. Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use. FDA CBER, 1997

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