Clearance Services

A guide to planning your Viral or TSE Clearance Study

BioReliance® by SAFC

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Contents

Introduction

Why undertake a viral clearance study?

Manufacturers of biopharmaceutical products, such as blood products, monoclonal antibodies, recombinant proteins, tissue derived products, and some medical devices, are required to assess the ability of the manufacturing process to produce a product that is safe for use in humans. A major concern for many biological materials is viral contamination. There are three principal complementary approaches used to control for the potential viral contamination of biologicals:

- Selecting the source material (see section 4.1.1)
- Testing of source materials and products from various stages of the manufacturing process for the absence of detectable virus
- Testing the capacity of the manufacturing process to remove or inactivate viruses

The last approach is referred to as a viral clearance or viral validation study (or evaluation) and plays an important role in establishing the safety of biopharmaceuticals. The regulatory definition of a study of this type is:

"A documented programme, which provides a high degree of assurance that a specific process will consistently manufacture a product, meeting predetermined specifications." [1]

A viral clearance study should reflect the viral risks associated with the product, and include a selection of viruses that vary in size, shape, genome type, structure, and resistance to various methods of physicochemical inactivation.

The ICH Q5A regulatory guideline[1] indicates that a manufacturer of biological products for human use should demonstrate the capability of the manufacturing process to remove or inactivate known 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.[1–4] These data are used to give a quantitative estimate of the overall level of virus clearance obtained by the manufacturing process. A similar approach can be used to demonstrate the removal of other potential contaminants, e.g., DNA, mycoplasmas, bacteria, and endotoxins.

Due caution is also warranted if biological materials from species affected by transmissible spongiform encephalopathies (TSEs), especially bovine species, are used for the manufacture of medicinal products.[5]

This document outlines the detailed approach BioReliance has developed over the past 20 years to address all the issues that can arise in the execution of viral clearance studies.

The BioReliance approach to viral clearance studies

Our experience

BioReliance is a leader in the field of biologics safety testing, and has been involved in assisting many clients worldwide with licensing pharmaceutical products derived from biological fluids or tissues. BioReliance has been conducting viral clearance studies for European, Asian, and US clients since 1984. Our Regulatory Affairs group ensures that we are kept up to date with the latest regulatory requirements, and we also communicate with the regulatory agencies to ensure that areas such as study design, virus selection, and statistical analysis comply with the current regulations. Many biologics such as plasma derivatives, monoclonal antibodies, recombinant proteins, and medical devices have been licensed around the world using data generated from BioReliance viral clearance studies.

Summary of studies performed by BioReliance

The BioReliance's global viral clearance division has performed thousands of clearance studies, including several hundred TSE clearance studies and a number of mycoplasma and DNA clearance studies. This extensive experience helps us to design the most effective studies for our clients, which include many of the world's major pharmaceutical and biotechnology companies. We have performed both virus and TSE clearance studies on a wide range of different products and process steps. Our experience allows us to advise our clients on potential problems with these types of

studies, and on the most appropriate process steps to validate in order to satisfy regulatory requirements. Examples of products for which clearance studies have been performed include:

- Monoclonal antibodies
- Recombinant proteins
- Tissue-derived products
- Human and bovine blood products and coagulation factors
- Equine and ovine blood products
- Human and animal urine–derived products
- Medical devices (including collagen-derived products)
- Enzymes

There is an increasing requirement from regulatory authorities to study the ability of reused column resins to clear viruses after multiple runs. To address this need, we regularly perform column reuse studies to satisfy this regulatory request. We have also performed cleaning studies (laboratory cleandown procedures and equipment sanitization) and a number of studies looking at carryover of viruses during a column chromatography step.

Regulatory authorities are now also asking to see data on the robustness of a process, looking at extremes of conditions such as pH, temperature, and protein concentration. These data give an indication of the virus reduction capabilities of a manufacturing process under worst-case conditions.

New technologies

Infectivity assays are the preferred method to determine the effectiveness of a process step in removing and/or inactivating infectious agents. Alternative assays, however, may be used to provide useful information in certain circumstances. For example, valuable additional data on the partitioning of viruses can be obtained by the use of a quantitative polymerase chain reaction (qPCR) assay.[6] In some chromatography processes, for example, the buffers used may result in virus inactivation. Although the inactivation kinetics of the buffer may be studied as an independent step, it may not be possible to demonstrate how the virus partitions during the chromatography process using infectivity assays. In other words, any physical removal of virus due to the chromatography process itself could not be studied. However, with the introduction of qPCR assays, many chromatography processes may now also be studied, at least with respect to removal or partitioning of virus genomes. Such studies would be performed in parallel with infectivity studies.

Another assay that has become more widely used is the western blot assay for evaluation of removal of TSE agents. This assay specifically detects the disease-associated prion protein, PrP^{Sc} (or PrP^{res}). Levels of PrP^{Sc} have been shown to correlate closely with levels of infectivity. The western blot assay provides a rapid means of making a preliminary evaluation of the effectiveness of steps in the removal of TSE agents, and can reduce the need for more expensive, time-consuming bioassays.

At BioReliance, we have taken an active role collaborating with biotech and biopharma companies in the development of new and improved assays for viruses and TSEs. We were one of the first companies to use the western blot assay to study the effectiveness of manufacturing processes to remove TSEs. This assay has been in routine use since 1997 and has become increasingly important as the need for TSE safety assessments of biopharmaceuticals increases.

Regulatory affairs

A major undertaking in preparing a submission to regulatory authorities for a biopharmaceutical product is an in-depth understanding of the regulatory requirements for different countries and product types at the different phases of development. BioReliance's Regulatory Affairs group can assist in keeping you up to date with regulatory developments. We regularly have contact with the EU, US, and Asian authorities, and

our technical specialists are well placed to offer advice on current regulatory issues.

All BioReliance studies are performed in full compliance with the relevant regulatory standards and the respective regulatory authorities (US FDA and UK MHRA) perform regular inspections of our facilities. All viral clearance studies are designed taking into account the most current regulatory guidelines published by the European EMEA, US FDA, ICH, and Japanese Ministry of Health, Labor, and Welfare (MHLW). Our Regulatory Affairs group will be able to assist with the following:

- Advice on regulatory requirements and procedures
- Advice on regulatory strategies
- Expert report writing
- Preparation of documentation
- Data review and evaluations
- Response to authorities
- Regulatory information services

Planning a clearance study

Clearance studies are a costly and time-consuming component of the biosafety portfolio of any biopharmaceutical product. Increasing emphasis is being placed by regulatory authorities on the correct implementation of these studies, in order to ensure that the data generated accurately represent the ability of the manufacturing process to remove or inactivate viruses and/or TSEs. Consequently, it is extremely important to ensure that the design, planning, and implementation of the clearance study are discussed well in advance, to ensure compliance with the various regulatory guidelines.

Because there are many factors to be considered when planning a viral clearance study, and no two products or processes are the same, our approach is to view projects as a collaborative study withour clients. Our aim is to identify the most cost-effective method to generate the necessary data to ensure safe passage of your product through to licensing. To achieve this, it is important to involve all relevant personnel in extensive discussions as early as possible.

The manufacturing process should be designed with virus safety in mind. It is much easier to alter manufacturing process steps at the development level than to try to introduce changes to a process once it is established. Ideally, you should include at least one, and preferably two, robust virus clearance steps with different modes of action, within a manufacturing process. Examples of different types of clearance steps often taken in viral clearance studies are shown in Table 1.

These viral clearance processes have been studied with a wide range of biopharmaceutical and plasma products, including:

- Monoclonal antibodies and recombinant proteins from rodent and human cell lines
- Human Factor VIII, Factor IX, thrombin, fibrinogen, anti-thrombin III, albumin
- Other products of animal origin, such as enzymes, collagen, and muscle tissue

Process steps

Many different types of process steps may be effective for virus removal/inactivation (Table 1). Robust steps are those steps considered to be effective under a wide range of conditions (e.g., different buffers, pH conditions, protein concentrations, temperatures, etc.) and include steps such as heat, and for enveloped viruses only, low pH and solvent/detergent treatments. Partitioning steps are generally considered to be less robust, although virus removal filtration processes, where separation is considered to be predominantly on the basis of size, are generally considered robust for viruses significantly larger than the nominal pore size of the filter. Chromatographic partitioning steps can, nevertheless, be effective for virus removal, i.e., repro-

Table 1. Processes studied for viral removal/inactivation

Inactivation	Partitioning
Heat Treatment	**Precipitation**
Pasteurization	Ethanol
Lyophilization/dry heat	Polyethylene glycol
Solvent Detergent	**Chromatography**
	Ion exchange
pH Treatment	Affinity
Low pH (column elution)	Hydrophobic interaction
High pH (sanitization)	Reverse phase
	Virus Removal Filtration

ducibly allow the removal of a high level (>4 log orders of magnitude) of virus within the range of the manufacturer's process conditions.

Not all biopharmaceutical products can tolerate conditions which result in inactivation of viruses (or other contaminants), and partitioning processes are often required to contribute to virus removal during the manufacture of a product.

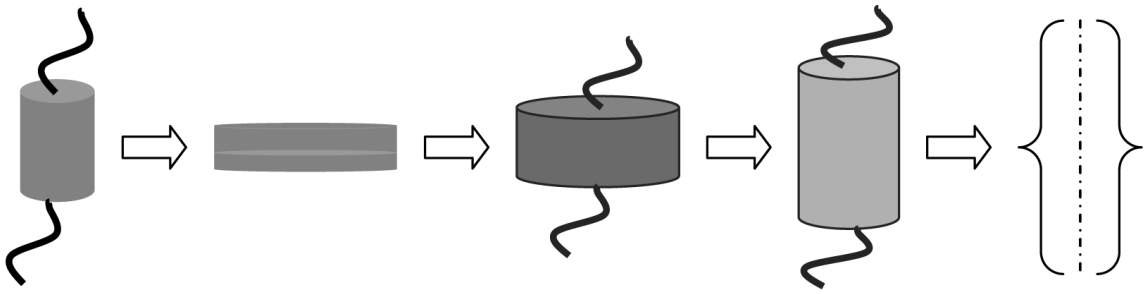
The aim of a viral clearance study is to look at the manufacturing process as a whole. Each process step to be studied is spiked (or challenged) with an appropriate preparation of high-titer test virus. The data for each individual process step can then be combined to give a reduction value for the chosen virus over the whole manufacturing process. This is outlined in Figure 1, which shows a typical manufacturing process for a monoclonal antibody, indicating the potential virus log reduction values for each individual step, for a retrovirus model (murine leukemia virus (MLV)).

Downscaling the manufacturing process

Downscaling of a manufacturing process for a clearance study is an essential part of the design and planning of the study. Process steps chosen for study must have operating parameters and physical characteristics that can be scaled down, and the downscaled process must be validated to demonstrate it mimics as closely as possible the full-scale manufacturing process. The validity of any viral clearance data is dependent on the accuracy with which the downscaled process mimics the full-scale process. You will need to have a valid downscale model in place prior to commencing the viral clearance study.

BioReliance's viral clearance team is familiar with downscaled processes, including column chromatography, Cohn fractionation, virus removal filtration, chemical inactivation, and treatment at extremes of pH.

Figure 1. An example of a manufacturing process for a monoclonal antibody showing possible values for virus removal at each step (virus example: MLV)

Process step	Affinity Chromatography	Low pH	Ion Exchange	Gel Filtration	Virus Removal Filtration
					
Log reduction values	Not tested	4–5	2–5	Not tested	4–5

For the total process a log reduction of 10–15 may be achieved for MLV.

Viruses of concern

The choice of virus for a clearance study will depend on the product being manufactured. The virus panel chosen should include selected model viruses that differ in their sensitivity to physical and chemical agents. These models should be representative of all possible virus contaminants for a product. It is not possible to predict all potential contaminants, and it is possible that the product (or starting materials) could contain as yet unknown contaminants. Using a large panel of viruses with varying size, genome type, and resistance to physicochemical inactivation will help to address unknown as well as predicted virus contaminants.

In selecting a virus panel, it is important to consider the origin of reagents used in the manufacturing process, as well as the starting material. For example, affinity chromatography columns often contain monoclonal antibodies; cell culture medium may contain bovine serum. Operating personnel may also contribute to the contamination of a product, so the virus panel chosen should include examples of virus families that would cover potential contaminants from an operator. An additional practical consideration in choosing a virus panel is that the virus panel should have

Table 2. Virus selection for a human blood product clearance study

Model	Contaminant	Properties
HIV-1	Retroviruses HIV-1 and 2 HTLV 1 and 2	Enveloped, ssRNA, 80–130 nm Low resistance to physicochemical inactivation
HSV-1 PRV BHV-1	Herpesviruses HHV 1-8	Enveloped, dsDNA, 150–200 nm Low to medium resistance to physicochemical inactivation
BVD Sindbis	Togaviruses Pestiviruses Flaviviruses HCV HGV Tick-borne encephalitis	Enveloped, ssRNA, 40–70 nm Low to medium resistance to physicochemical inactivation
No Model*	Hepadnoviruses HBV	Enveloped, dsDNA, 42 nm Low to medium resistance to physicochemical inactivation
HAV EMC Polio	Picornaviruses HAV	Non-enveloped, ssRNA, 28–30 nm Medium to high resistance to physicochemical inactivation
SV-40	Papovaviruses JCV BKV	Non-enveloped, dsDNA, 45–55 nm High resistance to physicochemical inactivation
FCV	Calici-like virus HEV	Non-enveloped, ssRNA, 35–40 nm High resistance to physicochemical inactivation
PPV CPV	Parvoviruses B19	Non-enveloped, ssDNA, 18–26 nm High resistance to physicochemical inactivation

* Since there is no universally accepted model for HBV, the panel of viruses chosen for a clearance study of human blood products should encompass a range of physicochemical properties similar to those of HBV.

a reliable assay with a defined end point. It should also not pose an undue risk to the operator. For further details on selecting a virus panel, see Appendix I.

Examples of virus panels that might be used in different types of studies are shown in Tables 2 and 3. For a blood product, a typical panel might include HIV-1, BHV, BVD, HAV, and PPV (Table 2). Where neutralizing antibodies to HAV might be present in the sample, an alternative picornavirus such as EMC could be used. When studying the manufacturing process for a monoclonal antibody or recombinant protein product, a typical virus panel might include MLV, PRV, Reo 3, and MMV (Table 3). For a CHO cell–derived recombinant product, PI3 is generally used instead of a herpesvirus since incidences of PI3 contamination have been observed in CHO-derived cell lines.

Further details of model viruses can be found in Appendix II.

Table 3. Virus selection for a mammalian cell–derived product clearance study

Model	Contaminant	Properties
MLV	Retroviruses MLV	Enveloped, ssRNA, 80–130 nm Low resistance to physicochemical inactivation
HSV-1 PRV BHV-1	Herpesviruses	Enveloped, dsDNA, 150–200 nm Low to medium resistance to physicochemical inactivation
PI3	Paramyxoviruses PI3	Enveloped, ssRNA, 150–300 nm Low to medium resistance to physicochemical inactivation
BVD	Flaviviruses	Enveloped, ssRNA, 40–50 nm Low to medium resistance to physicochemical inactivation
Reo 3	Reoviruses Bluetongue virus	Non-enveloped, dsRNA, 60–80 nm Medium resistance to physicochemical inactivation
MVM CPV PPV	Parvoviruses	Non-enveloped, ssDNA, 18–26 nm High resistance to physicochemical inactivation

Study design and process

There are several stages in the design and performance of a viral clearance study (TSE studies follow a similar design):

At the earliest stage in study design, detailed technical discussions are necessary to allow the implementation of the most appropriate study design. It is important at this stage that as much technical information as possible is provided, to allow the most suitable process steps, samples, and viruses to be selected.

Following detailed discussions, a quotation is prepared, indicating the suggested process steps, viruses, and information relating to the sampling regime proposed. An indication of timelines may also be included.

Typically, once a study design has been agreed upon and a clearance study contract has been signed, a time slot is allocated in the lab for the preliminary studies and the spiked process runs. It is usually possible to reserve a lab slot in advance with a lab reservation agreement. Once a signed contract is received, we assign a Study Director who will be responsible for all aspects of the study. At this point a Statement of Work (SOW) is prepared. The SOW describes the purpose of the study, the viruses used, the process being studied, assays used, and a description of the methods being used for the calculation of results (Figure 3).

Once you have approved the SOW, the lab work associated with the study can be scheduled. The conduct of the study is explained in more detail below. We encourage you to be present during the performance of the spiked process runs. However, our viral clearance teams are very experienced in a wide variety of processing techniques and can, if necessary, conduct studies in their entirety if this is preferred. However, for all chromatography steps we strongly advise you to be present throughout.

Dedicated viral clearance labs are available during the spiked process runs. In addition, designated offices are also available for your use. During the term of the study, your key contact person, either the Study Director or a Project Manager, will provide regular updates to you on the progress of the study. You are welcome to contact this person at any point during the study to discuss relevant issues.

Once the virus titration assays are complete, preliminary results are calculated and sent to you. The results are then compiled into a QA-audited draft report, which is dispatched to you for comments.

Reports for clearance studies are very extensive and include all of the raw data from the titrations, interference and cytotoxicity studies, and all of the calculations used in preparing the results. All correction factors for significant changes in volume resulting from pH neutralization or processing are clearly indicated in the report, in addition to any other controls (e.g., ultracentrifugation control). All QA audits are also detailed in the report. This provides you with all the information required for a submission to the regulatory authorities. Once you have had an opportunity to review the draft report and relevant comments have been incorporated, an audited Final Report will be issued. A copy of an example of a Final Report can be provided upon request.

Because of the many stages involved in performing a virus clearance study, the length of time required for a study can range between approximately 3 and 6 months from preparing the SOW to issuing a report.

Preliminary studies

Preliminary studies are performed to demonstrate whether samples to be tested in the virus clearance study may interfere with the assay systems used. Control samples, representative of the process samples to be tested, should be used. It is advisable to test up front the effect of the process samples on the cells and viruses used in any particular assay (it is important to understand that such samples must include the product, and should not simply consist of the buffers used in the process). The samples should ideally come from a full-scale manufacturing process, and if possible, from the same production batch as to be used in

the spiking studies. The regulatory authorities throughout Europe, the US, and Japan have raised the issue of addressing potential cytotoxicity and interference effects and require that such preliminary studies be performed in a clearance study.[1,2]

Cytotoxicity

Cytotoxicity assays are performed to demonstrate whether process materials are toxic to the indicator cells used in the virus titration assays. Samples representative of the key process samples and intermediates should be tested for cytotoxicity on each of the cell lines to be used.

Serial dilutions of each sample are incubated with the indicator cell lines and the cells are examined regularly for signs of cytopathology. The first dilution of a sample showing no visible effect on the cells is selected as the noncytotoxic dilution.

Should a sample exhibit a very high level of cytotoxicity, it may be necessary to manipulate that sample further to remove the toxic effect. Under extreme circumstances it may be necessary to select a mock product should the levels of cytotoxicity prohibit use of the product in the study.

Interference

Interference assays are performed to determine whether samples interfere with the ability of the relevant virus to infect the indicator cell lines or prevent detection of the appropriate virus-induced cytopathic effect (cpe).

Serial dilutions of samples are spiked with virus, and inoculated onto the relevant indicator cell lines. The appearance of virus cpe in the sample dilutions is compared to that for virus spiked into tissue culture medium alone. The first dilution for which there is no obvious interference with the appearance of virus cpe is taken as the noninterfering dilution.

In our experience, it is important to allow the interference assays to run for the full length of the assay, since the interference effect may not necessarily be observed until the last few days of the assay.

Spike recovery

A number of additional preliminary studies, termed spike recovery assays, will be performed depending on the nature of the study. Spike recovery assays are performed to determine whether the starting materials (and in some cases other buffers or samples) could have an effect on the viruses used, during the process step. These assays would typically involve spiking selected samples with a high level of virus, and incubating for a given period prior to titration.

For example, virus may be spiked into starting material, or relevant process buffers, to determine whether these may inactivate virus during the course of the viral clearance study. For inactivation studies, selected samples may be spiked with virus after dilution or quenching of the inactivant, to ensure that the inactivant under study has been effectively quenched and does not continue to inactivate virus following sample collection.

All the preliminary studies must be performed and completed before commencing the spiked process runs. This requires close discussion with you regarding the timing of the studies and the quantities of materials necessary to complete these preliminary tests.

Spiked process runs

Once the preliminary studies are complete, the spiked process runs can be performed. It is our client's responsibility to ensure that they have a validated downscale model of their process for the virus clearance studies. It is important that this is available well in advance of the virus clearance study, as preparation of these models can take longer than first anticipated.

For each individual step to be studied, you will need to ideally provide starting material from your full-scale manufacturing process. This material is then spiked with a high titer

of virus, and then taken through the individual downscaled process steps. Relevant process samples are collected, diluted to the appropriate noncytotoxic and noninterfering dilution (see above), and then assayed. The total virus load in the start material can then be compared with the total virus recovered in the relevant product fraction (Figure 2).

BioReliance's labs are specifically designed to run downscaled processes for viral clearance studies. These dedicated viral clearance laboratories contain a range of specialized equipment including fully automated ÄKTA™ FPLC™ systems and a dedicated walk-in low-temperature laboratory. A list of equipment dedicated to the viral clearance labs is available and can be supplied upon request. Spiked process runs for chemical inactivation, low pH, virus removal filtration, or heat treatment steps can generally be performed by BioReliance personnel. We would, however, encourage you to be present for the more complex processes, such as chromatography steps, where you will have a better understanding of the process should any technical issues arise. BioReliance personnel, however, would perform all manipulations involving the handling of viruses. You will have a dedicated office for use during your visit to the BioReliance facility.

Titrations

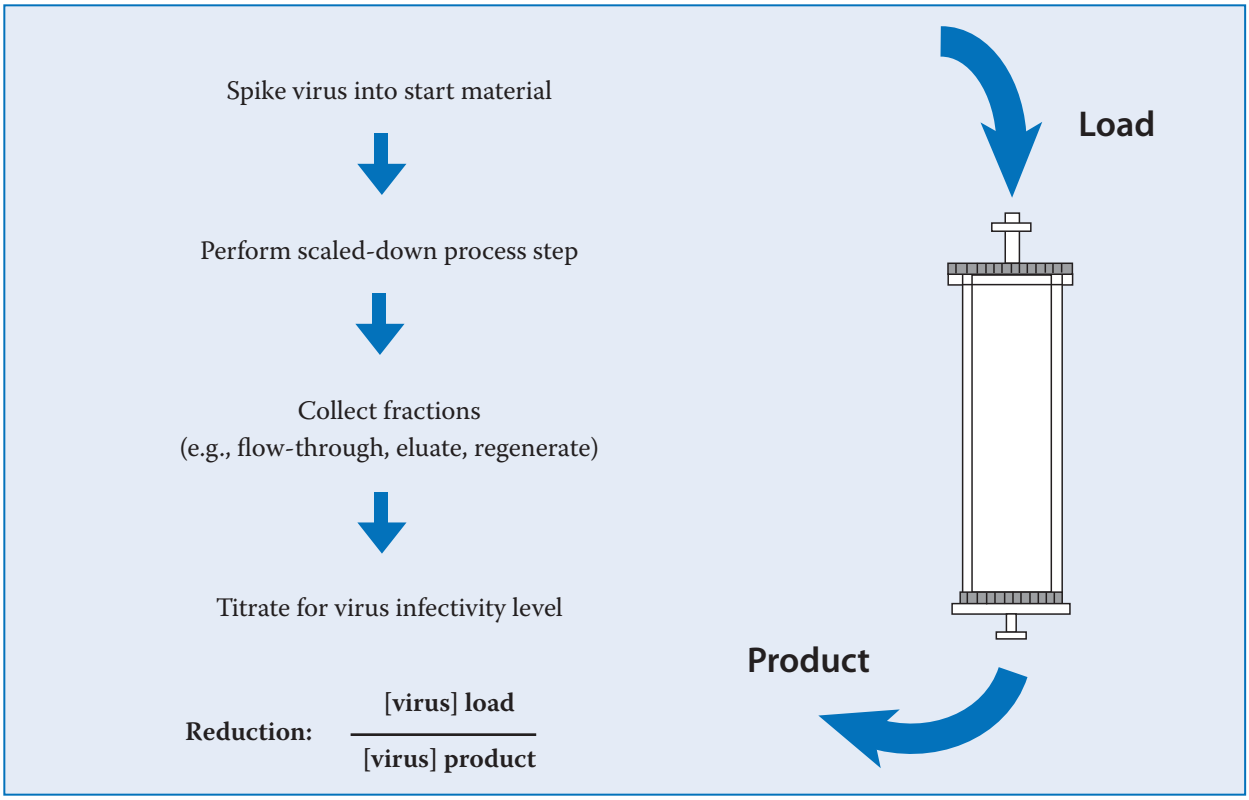
Samples generated during a spiked process run are generally assayed directly (i.e., without freezing). The length of the titration assays will vary, depending on the virus used, and range from around 5 to 23 days.

With any biological assay, however, it is possible that repeat assays may be required. For this reason, backup samples will also be frozen and stored as a precaution.

Appropriate controls must also be incorporated for any sample manipulations that do not form part of the process step (for example, if a sample is frozen prior to titration, or if a sample is ultracentrifuged to remove toxicity).

For all viruses used routinely in Bioreliance's viral clearance studies, a 50% tissue culture infectious dose ($TCID_{50}$)

Figure 2. Example of the spiking process for a chromatography step



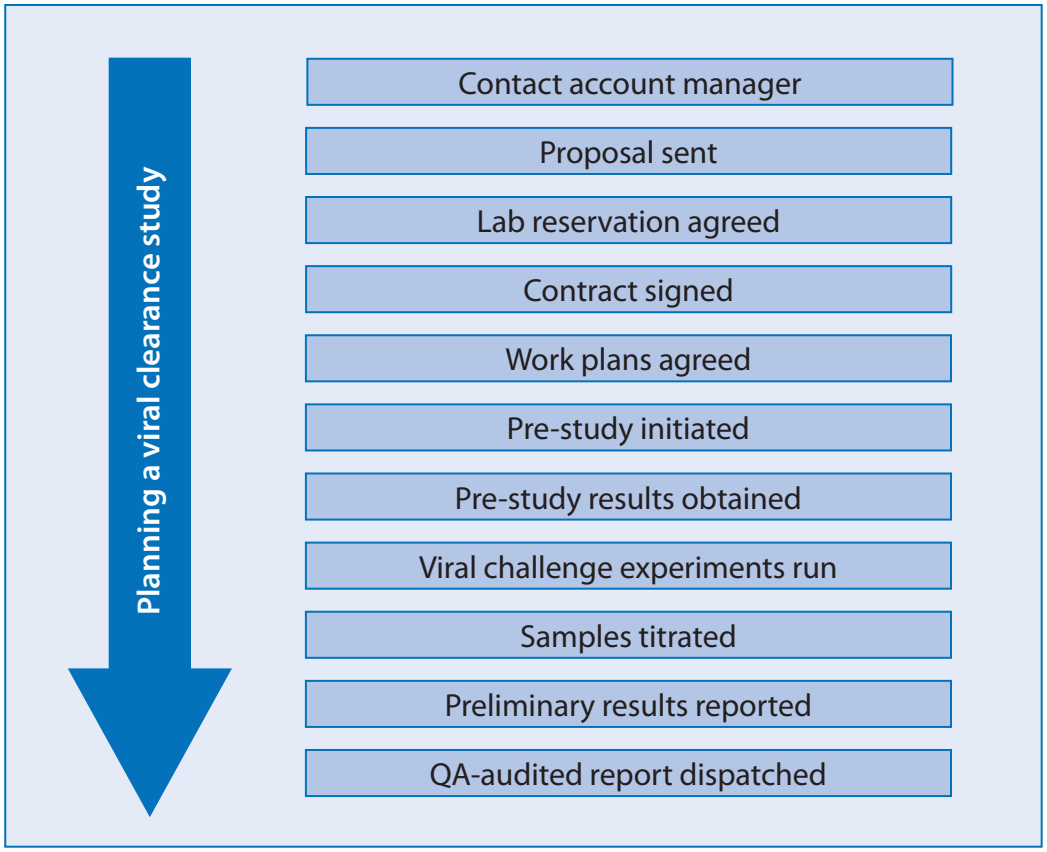
assay is used. Serial dilutions of sample are inoculated onto the appropriate cell line. Assays are incubated for the appropriate time, and each well is scored as either positive or negative, and the proportion of positive wells then determined. The virus titer is then calculated, using the method of Karber.[7]

The sensitivity of the titration assay is directly related to the volume of material used. Put simply, if no virus is detected in 1 ml of sample, it could be considered that there is <1 iu/ml. If no virus is detected in 10 ml, it could be considered that there is <1 iu/10 ml, or <0.1 iu/ml. Thus, a 10-fold increase in the tested sample volume results in a 10-fold increase in sensitivity of the assay. Therefore, where it may be necessary to increase the sensitivity of the assay, we would suggest large-volume plating of the first dilution for product fractions.

Schedule for a viral clearance study

Figure 3 provides a summary of the steps taken to complete a viral clearance study at BioReliance. The details and timeline of each step will vary from project to project and are contained within the SOW.

Figure 3. An overview of the flow of a clearance study from initial contact to final report dispatch



Appendix I

Factors to consider when selecting a panel of viruses

The factors detailed below should be considered when choosing a panel of viruses or contaminating agents to study the clearance potential of steps in a manufacturing process.

The source of the starting material

Different starting materials pose a risk for different viruses to differing degrees. Human blood and blood products are known to transmit infectious agents including hepatitis viruses and human immunodeficiency virus (HIV). Regulatory authorities stipulate that the study of HIV in a viral clearance study of a human blood product is a mandatory requirement.[2,4] Human T-cell lymphotrophic virus type I and II (HTLV-I and HTLV-II) and human parvovirus B19 are also transmitted in blood, although HTLV-I and -II are cell associated and only transmitted in fractions containing mononuclear cells. In transplant patients the transmission of herpes viruses (cytomegalovirus, Epstein-Barr virus, and human herpes viruses 6–8 (HHV-6, HHV-7, and HHV-8)) and adenoviruses in whole blood are also of concern. The risk of transmission of viruses and other pathogenic organisms via blood and blood products can be reduced, but not eliminated, through donor selection and screening. Moreover, viruses like hepatitis A and human parvovirus B19 are particularly resistant to inactivation by conventional manufacturing processes.

When considering products derived from animal cell culture, the cells themselves may harbor endogenous viruses (e.g., rodent cell lines may harbour endogenous retroviruses). Bovine serum or porcine trypsin, commonly used in the culture of animal cells, may be contaminated with several viruses. Bovine viral diarrhoea virus (BVD) and bovine polyomavirus, a potentially zoonotic virus, are commonly encountered agents in bovine serum. A major concern for other products of bovine origin is the agent responsible for bovine spongiform encephalopathy (BSE). Porcine parvovirus (PPV) and porcine circovirus are prevalent infections of pigs and are often found to be contaminants of porcine trypsin.

Raw materials

Many raw materials used in the manufacture of biopharmaceuticals are potential sources of viral contamination. Some examples are:

- Antibodies coupled to chromatography media
- Cell culture media
- Enzymes used in the manufacturing process, such as porcine trypsin

Operating personnel

Contamination may also enter the manufacturing process via a breakdown of GMP, such as viral contamination by the personnel operating the facility.

Route of administration of product

The finished product may be administered to a patient in one of several ways, e.g., orally, intravenously, by inhalation, or as a topical ointment. How the product is administered to the patient will determine the risk of infection to the patient from viruses, and the families of viruses that would be of most concern.

Number of patient doses

The amount of bulk harvest material that goes into a product and the number of times a product is administered to a patient during treatment influence the risk of infection to a patient. For example, if a patient

receives only a single dose of a product there will be less of a risk to the patient than if they receive multiple doses of the same product.

All of the factors above should be considered when choosing the panel of viruses or contaminating agents used to study the clearance potential of steps in a manufacturing process.

For a viral clearance study of a product entering phase I/II clinical trials, a minimum study should be performed on an enveloped and non-enveloped virus, assessing the virus removal capabilities of two robust orthogonal steps where possible.[8] For biopharmaceuticals derived from human blood products, the enveloped virus chosen would be HIV. MLV would be the non-enveloped virus chosen to study products derived from rodent cell lines. Increasingly, regulatory authorities have been asking for a second robust virus to be included in such early-phase studies to show, at an early stage, the overall robustness of the process. We would, therefore, often incorporate a parvovirus model.

Where a product is due to enter phase III clinical trials, a study should demonstrate the capacity of a process to reproducibly remove a range of viruses with different physicochemical properties. Thus, a study would typically be performed in duplicate and include a panel of 4 (for recombinant proteins and monoclonal antibodies) or 5 (for human blood products) viruses.

Appendix II

Table 4. Model viruses used by BioReliance for viral clearance studies

Virus	Family	Structure/ genome	Size	Physicochemical resistance	Model/host
Human immunodeficiency virus	*Retroviridae*	Enveloped, single-stranded RNA	70–100 nm	Low	Model for HIV 1, HIV 2, HTLV-1 and HTLV-2. Mandatory for products derived from human plasma/ blood.
Murine leukaemia virus (ecotropic and xenotropic)					Represents a non-defective C type retrovirus. Mandatory for biological products derived from CHO cell lines and monoclonal antibody products.
Maedi visna virus					Model for lentiviruses. May be used as a model for HIV, and/or a model for potential contaminants of ovine-derived material, particularly ovine blood products.
Herpes simplex virus type 1	*Herpesviridae*	Enveloped, double-stranded DNA	120–200 nm	Low to medium	Models for human or animal herpesviruses, e.g., *human herpes virus* 1–8, which may be transmitted in blood and plasma products; bovine or equine herpesviruses present in animal blood products. Models for *Epstein-Barr virus* used in hybridoma generation.
Infectious bovine rhinotracheitis virus (bovine herpes virus type 1)					
Porcine pseudorabies virus					
Parainfluenza virus type 3	*Paramyxoviridae*	Enveloped, single-stranded RNA	150–200 nm	Low to medium	Potential contaminant of bovine serum. Incidences of contamination of CHO-derived recombinants with this virus family observed.
Vesicular stomatitis virus	*Rhabdoviridae*	Enveloped, single-stranded RNA	50 x 200 nm	Low to medium	Model for rhabdoviruses such as *Rabies virus.*
Bovine viral diarrhoea virus	*Flaviviridae*	Enveloped, single-stranded RNA	40–60 nm	Low to medium	Models for potential *togavirus* or *flavivirus* contaminants. BVD is the preferred model for *hepatitis C virus* in human blood and plasma derivatives. Alternatively, *Sindbis virus* may be used.
Sindbis virus	*Togaviridae*	Enveloped, single-stranded RNA	60–70 nm		

Table 4. Model viruses used by BioReliance for viral clearance studies, continued

Virus	Family	Structure/ genome	Size	Physicochemical resistance	Model/host
Contagious pustular dermatitis virus (ORF)	*Poxviridae*	Enveloped, double-stranded DNA	220–400 nm	Low to medium	Model for potential pox virus contaminants, model for potential contaminants of ovine derived material.
Adenovirus type 2 and 5	*Adenoviridae*	Non-enveloped, double-stranded DNA	70–90 nm	Medium	Model for human and animal adenoviruses.
Reovirus type 3	*Reoviridae*	Non-enveloped, double-stranded RNA	60–80 nm	Medium to high	Infects human and animal cells, potential contaminant of hybridomas and recombinant cell lines. Model for orbiviruses, rotaviruses, and *bovine bluetongue virus.*
Encephalomyocarditis virus	*Picornaviridae*	Non-enveloped, single-stranded RNA	25–30 nm	Medium to high	Model for *hepatitis A virus* contamination of human blood/plasma, or *picornavirus* contamination of other products.
Human hepatitis A virus					
Human poliovirus					
Theiler's mouse encephalomyelitis virus					
Feline calicivirus	*Caliciviridae*	Non-enveloped, single-stranded RNA	~40 nm	Medium to high	Model for *hepatitis E virus*, a Calici-like virus. It is also a model for *rabbit haemorrhagic disease virus*, a Calicivirus family member.
Simian virus 40	*Papovaviridae*	Non-enveloped, single-stranded DNA	~45 nm	High	Model for polyomaviruses including *bovine polyomavirus,* and JCV and BKV (both human polyomaviruses)
Bovine parvovirus	*Parvoviridae*	Non-enveloped, single-stranded DNA	18–25 nm	High	Model for *human parvovirus B19*, representing a severe test of the downstream process. Parvoviruses are known contaminants of CHO cell fermenters, and are also potential contaminants of rodent-derived biopharmaceuticals.
Canine parvovirus					
Murine minute virus					
Porcine parvovirus					

List of abbreviations

B19	human parvovirus B19
BHV	bovine herpes virus
BKV	BK virus
BSE	bovine spongiform encephalopathy
BVD	bovine viral diarrhea virus
°C	degrees Celsius
CHO	Chinese hamster ovary
CMV	cytomegalovirus
CP	Client Protocol
cpe	cytopathic effect
CPV	canine parvovirus
DNA	deoxyribonucleic acid
ds	double-stranded
DSP	downstream process
EBV	Epstein-Barr virus
EMC	encephalomyocarditis virus
EMEA	The European Agency for the Evaluation of Medicinal Products
EtOH	ethanol
FDA	The US Food and Drug Administration
FPLC™	Fast Protein Liquid Chromatography
FCV	feline calicivirus
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HAV	hepatitis A virus
HBV	hepatitis B virus
HCV	hepatitis C virus
HEV	hepatitis E virus
HGV	hepatitis G virus
HHV	human herpes virus
HIV	human immunodeficiency virus
HSV	herpes simplex virus
HTLV	human T-cell lymphotrophic virus
ICH	International Conference on Harmonisation
iu	infectious units
JCV	JC virus
l	liter
M	molar
MHLW	Japanese Ministry of Health, Labor, and Welfare
MHRA	Medicines and Healthcare products Regulatory Agency
MLV	murine leukemia virus
MMV	murine minute virus
µl	microliter
nm	nanometer
ORF	scab mouth orf virus
PEG	polyethylene glycol
PPV	porcine parvovirus
PRV	pseudorabies virus
QA	quality assurance
qPCR	quantitative polymerase chain reaction
Reo 3	reovirus type 3
RNA	ribonucleic acid
SOW	Statement of Work
ss	single-stranded
SV-40	simian virus 40
$TCID_{50}$	50% tissue culture infective dose
TNBP	trinitrobenzyl phosphate
TSE	transmissible spongiform encephalopathy

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