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Technical Sheet

Viral Clearance and TSE Clearance Studies

A critical part of the biologic manufacturing process is demonstrating that the process appropriately removes or inactivates potential known and unknown contaminants. Manufacturers of biopharmaceutical products derived from animal or human tissues, such as blood products, recombinant proteins, vaccines and even some medical devices, are required to demonstrate the ability of their purification and manufacturing processes to inactivate or remove viruses and, for some products, transmissible spongiform encephalopathy (TSE) agents. Such studies are required by regulatory authorities as an integral part of a submission report prior to administration of a product to humans.

At Charles River, three complementary approaches are offered to ensure the viral safety of a biotechnology product:

- Source cell lines and raw materials are tested for freedom from viruses.
- In-process testing programs are used to assess the presence of virus in the crude product and to ensure their absence in the final product.
- Viral clearance is assessed during the purification process.

Before performing a viral/TSE clearance study, a thorough analysis of the potential for contamination of the manufacturing process should be conducted. This involves research on the history of the source material and a testing program. For instance, for recombinant proteins produced on eukaryotic cell lines, the history of the cell substrate is analyzed. Master cell banks, working cell banks, end of production cells and bulk harvests are tested for viruses. This includes testing of raw material used in the production and purification process of the recombinant product. If virus contamination is indicated, analysis of the bulk harvest material, for instance by electron microscopy (EM), will be performed to obtain a measure of the viral load prior to purification.

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What are viral clearance studies?

Clearance is a measure of the capacity of the dedicated manufacturing process steps to primarily inactivate or remove viruses and TSE agents. These studies involve deliberate spiking of virus or TSE agents into process intermediates and then demonstrating their inactivation or removal during the subsequent processing steps. This is usually done on a down-scaled version — a laboratory version — of the selected process steps. The virus/TSE load of the spiked process intermediate and product-relevant process samples is determined and a reduction factor is calculated. For virus titer determination (virus quantification), virus-specific cell-based infectivity assays and, in some cases, quantitative PCR (qPCR) analysis are applied. TSE agents are quantified by western blot analysis.

The scope and design of a viral/TSE clearance study involves:

- Selection of the process steps capable of removing/inactivating viruses
- Verification of the scaled-down process steps
- Selection of viruses or TSE spike solutions
- Determination of process sample cytotoxicity (virus) and interference (virus/TSE)
- Performance of the process steps in the presence of virus/TSE spike
- Evaluation of the virus/TSE removal and inactivation

Selection of Process Steps

It is not necessary to test all steps in a purification process if sufficient clearance can be shown by analysis of fewer steps. Process steps should be selected where there is a reasonable expectation of viral/TSE clearance. Additional factors for consideration are the ease of scale-down of the procedure and the reproducibility of the procedure under process conditions. Results from such steps will provide greater confidence that the results of the viral/TSE clearance studies will translate to the full-scale manufacturing process.

Verification of the Scaled-Down Process

With any clearance study, it is critical to establish the validity of the scaled-down version of the purification steps. The scaled-down process should accurately reflect the actual manufacturing process prior to the implementation of the viral/TSE clearance study. This laboratory process is then evaluated in a biosafety laboratory for its capacity to inactivate or remove viruses/TSE. The validity of the scaled-down process should be demonstrated by comparing process parameters for similarity with the manufacturing-scale process and by verifying that the authenticity and stability of the process intermediates used in the study are the same as those produced in the manufacturing process. Process parameters should be selected to represent specific operating conditions and acceptable tolerances to the process of the manufacturing scale. Our considerable in-process experience (over 2,000 studies) allows us to partner with you in the development and validation of the scaled-down versions of the steps, as well as supply much of the standard equipment that is commonly used in protein purification, such as the equipment needed for inactivation studies and nanofiltration, chromatography devices and lyophilizers.

Selection of Viruses or TSE Spike Materials

Viruses that are intended for use in viral clearance studies should be model viruses that contaminate or may contaminate the product. The model viruses should also present a wide range of physicochemical properties in order to evaluate the robustness of the purification process to remove or inactivate a range of biophysically different viruses.

Depending on the process step to be investigated, a variety of TSE preparations are available for spiking experiments. The physicochemical nature of TSE agents in blood or other body fluids is still unknown, but they are assumed to be present in low concentrations in a soluble monomeric or oligomeric form.

Determination of Process Sample Cytotoxicity

During process performance, process samples are taken and quantitatively analyzed for virus concentration using cell based infectivity assays. A cytotoxicity test must be performed to determine if and to what degree the process sample matrix must be diluted or treated so that it has no toxic effects on the indicator cells. After incubation with the process samples, the indicator cell lines are inspected microscopically for cytopathic effects including changes in cell morphology, loss of cell adherence, syncytia formation and cell death.

Determination of the Process Sample Interference to the Virus/TSE Quantification System

The effect of the process sample on the virus/TSE quantification system (infectivity assay, qPCR, western blot) must be determined by comparing the virus/TSE titer in the presence and absence of the process sample. Like in the cytotoxicity test, the process sample must be diluted or treated to demonstrate that the process sample matrix does not impact the quantification system.

Performance of the Process Steps in the Presence of Virus or TSE Spike

Virus/TSE is spiked at high titers into the process intermediate prior to performing the scaled-down manufacturing process step. The virus/TSE titer of the spiked process intermediate solution and of the process samples taken during the process itself is determined. For viruses, an endpoint titration method or qPCR analysis is applied; for TSE, a western blot analysis is commonly applied. Only animal-based bioassays are currently available for TSE agents, which are expensive, and results are not available until after a six- to twelve-month incubation period. A TSE bioassay is only applied for highly critical source material and selected process samples.

The setup of all quantification assays is influenced by the cytotoxicity and interference results. These assays must be conducted prior to performing the process steps with spiked process intermediates.

Evaluation of Virus/TSE Removal or Inactivation

A TCID50 assay or plaque assay is used to determine the viral titer of spiked process intermediates and process samples. The actual number of samples that should be analyzed depends on the process step itself and must be individually defined within each study. Alternative methods such as qPCR may be employed to understand the mechanism of viral clearance in more detail. To identify key steps of TSE removal or inactivation, the samples are analyzed using a western blot to detect the TSE protein in its abnormal confirmation.

A logarithmic reduction factor based on the virus/TSE titer determined in the load (spiked process intermediate) and the relevant process sample (product containing final sample) is calculated. The reduction factor is a numerical value indicative of the virus/TSE removal/inactivation capacity of the analyzed process step. Reduction factors of single process steps in a whole manufacturing process can be summed up to an overall reduction factor for the manufacturing process.

Summary

Clearance studies are an essential part of a manufacturer's program to ensure product safety. In conjunction with characterization of starting materials and a program of bulk and final product testing, a careful analysis of the capability of the manufacturing process to remove or inactivate a wide range of model viruses or TSE agents plays an important role in establishing the safety of biological products.

Viruses Available for Viral Clearance Studies				
Type	**Viruses**	**Family**	**Size (nm)**	**Physicochemical Resistance**[1]
RNA enveloped				
	Human immunodeficiency virus-1	Retroviridae	80 - 110	Low
	Xenotropic, amphotropic, and ecotropic murine retroviruses	Retroviridae	80 - 110	Low
	Bovine viral diarrhea virus	Flaviviridae	40 - 60	Low - Medium
	Vesicular stomatitis virus	Rhabdoviridae	70 - 150	Low
	Parainfluenza type 3	Paramyxoviridae	100 - 200	Low
	West Nile virus	Flaviviridae	40 - 50	Low
RNA non-enveloped				
	Reovirus type 3	Reoviridae	60 - 80	Medium
	Poliovirus type 1	Picornaviridae	23 - 30	Medium - High
	Encephalomyocarditis virus	Picornaviridae	25 - 30	Low - Medium
	Hepatitis A virus	Picornaviridae	25 - 30	Medium - High
	Feline Calicivirus[2]	Caliciviridae	35 - 39	Medium
DNA enveloped				
	Pseudorabies virus	Herpesviridae	120 - 200	Low
	Herpes simplex virus (HSV-1, HSV-2)	Herpesviridae	120 - 200	Low
	Infectious bovine rhinotracheitis virus	Herpesviridae	120 - 200	Low
DNA non-enveloped				
	Human adenovirus type 5	Adenoviridae	70 - 90	Medium
	Simian virus 40	Polyomaviridae	40 - 50	High
	Porcine parvovirus	Parvoviridae	18 - 24	High
	Murine minute virus[2]	Parvoviridae	18 - 24	High
	Bovine parvovirus	Parvoviridae	18 - 24	High

[1] Resistance is highly dependant on mode of treatment; overall resistance is stated.

[2] Two strains.