Ensuring Compliance: Validation of Virus Filtration

Introduction

Virus safety is a critical consideration in the manufacture of biological therapeutics. The concern over virus contamination is related to viruses endogenous to the manufacturing cell line as well as adventitious viruses that can be introduced during manufacturing. Manufacturers rely on a multistep approach to control the risk of virus contamination in their processes including:

1. Selection of low risk raw materials and screening of source materials for the presence of virus
2. Testing the biological therapeutic at appropriate stages of production for freedom from detectable viruses
3. Virus spiking or clearance studies to test the capacity of the production process to remove or inactivate viruses

Most biological therapeutic production processes include a virus filtration step, which is not required for purification, but is a dedicated step in the production process for removal of both endogenous and adventitious virus. Virus spiking or clearance studies are performed to evaluate the levels of virus removal across the virus filter using scale down models. Typically at least two separate virus spiking studies are performed: the first is performed before Phase 1 trials and focuses on assessing reduction of a limited subset of viruses over critical virus removal unit operations, including the virus filter. The second study is more comprehensive and occurs later in clinical development before late stage clinical trials or license application, and involves testing multiple unit operations expected to contribute to virus clearance with a broader panel of viruses of different physico-chemical properties.

There are specific considerations for all virus filtration clearance studies and understanding these before initiation of the study will help assure a successful outcome where the results meet the targets.
Choice of Viruses

The choice of virus for testing is dependent on the particular biological being manufactured, the cell line used for production and other historical data available to the manufacturer. Guidance documents outline the rationale for selection of the virus panel (Refs 1-6), however as virus removal by filters is based on size, all manufacturers typically include a parvovirus in the testing panel. At approximately 20 nm, the parvovirus represents the ‘worst case’ for the virus filter as the other viruses typically evaluated in the filtration step are larger and would not be expected to pass through the virus filter.

Quantity and Quality of the Virus Spike

Regulatory guidance states that "the amount of virus added to the starting material for the production step that is to be studied should be as high as possible" and "should be added to the product in a small volume so as not to dilute or change the characteristics of the product." A reduction factor of 4 – 6 may be achieved with virus spike percentages ranging from 0.1% to 1.0%.

Higher virus loads upstream can represent a rigorous challenge to virus filters, potentially enabling larger virus log reduction values (LRVs), assuming no virus downstream. However, high concentrations of virus can have adverse effects on the unit operation: impurities from the cell culture process that are present in the virus spike preparations can contribute to premature filter fouling, which may prevent the target throughput (L/m²) from being reached. Industry standards for virus spike selection and details of virus purification and characterization methods are described in Technical report No 47. In addition, if the spike level is too high, the virus particles themselves can negatively impact performance of the virus filter by causing premature fouling.

Feedstock

The feedstock quality can be a critical component of the spiking study. Ideally, it should be identical to the feedstock that will be processed in the manufacturing setting. Feedstock is usually frozen before the spiking study to facilitate shipping and storage. Freezing and thawing a protein feedstock may produce aggregates, therefore the effect of these on the small scale model should be addressed. Aggregates can cause premature filter plugging, which will compromise the ability of the virus filter to reach process scale throughput (L/m²). Methods to eliminate or reduce the levels of aggregates in the feed and restore the feed to ‘fresh’ condition by using a prefilter, should be investigated and assessed before the virus spiking study. Irrespective of whether a prefilter is needed for feed restoration, or an integral part of the manufacturing process, where possible it should be decoupled from the virus filter during the spiking study in order to assess virus removal across the virus filter only.

Key Virus Filtration 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,” by reproducing the key operating parameters that have an effect on purification and on virus clearance including:

- Filter membrane/media
- Feedstock
- Scalability of retention and throughput (L/m²)

In addition, process parameters may impact virus retention or throughput (L/m²) such as:

- Flow rate or pressure
- Process interruption, planned or unplanned
- Prefiltration options

Since each commercial virus filter has individual specifications for operation, the study sponsor should consider how these process parameters might impact performance of the filter in the scale-down virus spiking study.

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Basic Spiking Study Design

The following is a general approach that can be modified to reflect practices and assess risks of individual processes. To have confidence in the results, the test should be reproducible. Accordingly, “an effective virus removal step should give reproducible reduction of virus load shown by at least two independent studies.”

Requisite Information

In order to design a successful filtration spiking study, the study targets, in terms of LRV and volumetric throughput (L/m²), in addition to any feedstock specific practices of processing operations including anticipated process interruptions, should be communicated to the virus testing laboratory.

These targets impact the different test considerations including:

1. Process volume: The volume of feedstock that will be filtered on the small-scale virus filter.
2. Volume of virus to spike and filtrate to assay: Assuming no virus is detected downstream of the virus filter, the volume of sample assayed can impact the LRV.

Preliminary Testing

Performing the following evaluations before the spiking study will allow better characterization of the test system and inform study design.

1. Shipping: Understand the impact of shipping on the quality of the protein feedstock: a mock shipping process can be performed and the attributes of the feedstock evaluated with particular focus on analytics than assess changes in aggregation profile.
2. Cytotoxicity/interference/viability: Virus titer is commonly determined using infectivity assays. These prestudies assess the compatibility of the feedstock with the test system and determine if feedstock dilutions are necessary before the titer assay to mitigate potential incompatibilities.
3. Microfiltration: Generally, the spiked feedstock will be microfiltered using 0.1-0.45 micron filters, to remove virus aggregates and other relatively large biomolecular impurities from the feedstream. Assessing titer before and after microfiltration determines the levels of aggregated virus.
4. Estimating level of virus spike to achieve target: Virus stock preparations contain impurities that can foul virus filters and limit the ability to reach the target throughputs. Often, the virus testing laboratory will provide information on the virus prep purity that can be useful in determining which virus preparations are most suitable for the virus filter. Figure 1 shows an example of feed spiked with virus of different purity levels. Scoping studies can help identify the appropriate virus preparation and spike level for the feedstock that allows processing to the target throughput (L/m²). Figure 2 summarizes the factors that impact success of filtration studies.
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.” Although some manufacturers have specialized virus laboratory capabilities, most biologics manufacturers work with independent virus testing organizations. Some of the more common contract testing laboratories are listed here.

Successful virus filtration studies are generally necessary to achieve process clearance targets and collaboration between the contract testing organization, the filter manufacturer and the study sponsor makes it more likely this will be achieved.

Contract Testing Laboratories

- Charles River Laboratories offers TrueSpike™ High Purity Virus Preparations that were specifically developed for optimized performance in filtration studies with monoclonal and recombinant protein products.
  - www.criver.com
- WuXi AppTec
  - www.wuxiapptec.com
- Bioreliance, Inc.
  - www.bioreliance.com
- Lancaster Labs
  - www.pharm.lancasterlabs.com

Factors that Impact Success of Filtration Studies

- Model Viruses Selection
- Scale-down Model Validity
- Effect of Process Perturbations
- Target Reduction Factor
- Virus Prep Quality and Titer
- Test Virus Appropriateness
- Virus Assay Sensitivity

Figure 2.
Virus Study Assessment Criteria
Conclusion

Process clearance validation studies confirm that the manufacturing process can adequately reduce the levels of viruses of different physico-chemical characteristics and that reduction is achieved using orthogonal or complementary methods.

Proper design of clearance studies is critical to ensuring the process can robustly deliver an appropriate level of virus safety. Scale-down validity, choice of virus, feedstock quality, and inclusion of appropriate study controls should all be addressed in the study design.

The virus filter makes an important contribution to the process virus clearance and preliminary scoping studies with representative feed in the virus spiking laboratory helps assure successful virus filtration spiking studies.

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 feedstock and the volume of the feedstock

Reduction factor or reduction value: $\log_{10}$ 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 unit operation.

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

References

1. Note for Guidance on Plasma-Derived Medicinal Products, CPMP EMEA, 2001
3. Q5A Viral Safety Evaluation of Biotechnology Products Derived From Cell Lines of Human or Animal Origin, ICH, 1997
5. Preparation of Virus Spikes Used for Virus Clearance Studies, PDA Technical Report 47
6. Virus Filtration, PDA Technical Report 41
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