A Viral Safety Strategy for Gene Therapy Viral Vectors

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Introduction

Biopharmaceuticals carry with them a risk of contamination by adventitious viruses, and manufacturers must ensure that raw materials, process intermediates and final products do not contain viruses. For traditional biopharmaceuticals (e.g., monoclonal antibodies, other recombinant proteins, etc.), the approach to viral safety is three-fold: 1) preventing entry of viruses into the production process; 2) verifying the absence of viral contaminants in process intermediates; 3) including viral inactivation and removal steps in the manufacturing process. For these conventional biological products, this approach has been successful in preventing the transmission of an infectious virus to a patient.

For novel biological therapeutics, like viral gene and cell therapies, viral clearance by the manufacturing process can not always be used. Steps that are designed to inactivate adventitious viruses may inactivate a lipid enveloped viral vector or cell therapy. Since enveloped viral vectors and cell therapies are much larger than virus filters, virus reduction filtration cannot be used with these products. Consequently, viral safety is dependent on preventing viral contaminants from entering the manufacturing process and detection of the presence of potential contaminants (Figure 1).

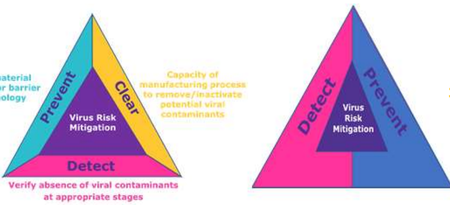
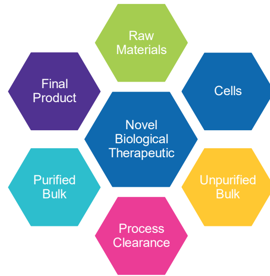


Figure 1. In the absence of opportunities for viral clearance, viral risk mitigation is dependent on PREVENTION and DETECTION of viral contaminants

Overview of Testing

Figure 2. The goal of testing for a viral vector, like any biopharmaceutical is to confirm the identity of the vector, ensure its purity and potency and verify that any process impurities have been removed.

This testing occurs at multiple places in the manufacturing process.



1. **Raw Materials:** Manufacturers of traditional biologics (e.g., monoclonal antibodies, recombinant proteins, etc.) can reduce the potential for viral contamination by eliminating human- or animal-derived materials, like bovine serum or porcine trypsin, from their process. For many new therapies, especially cell therapies, it is very difficult to remove human or animal-derived products from the manufacturing process. If these materials are used in the process, then it is essential that they be thoroughly tested to ensure the absence of adventitious agents.
2. **Cells:** Cell banks used in the manufacture of a biological therapy must be thoroughly characterized according to regulatory expectations for biologics and tested to ensure the absence of adventitious agents. This testing may include verification of the cell type; tests for bacteria, fungi, mycoplasma and possibly mycobacteria; broad specificity viral assays as well as assays for specific viruses; and assays for retroviruses.
3. **Unpurified Bulks:** Testing of unpurified bulks, which may include transfected producer cells, should include verification of identity, and may involve confirmation of the gene of interest. The titer of the vectors should be determined at this stage as well. The absence of replication competent viral vectors should also be confirmed. Tests for microorganisms (e.g., bacteria, fungi, mycoplasma, etc.) are appropriate for this material as are broad specificity assays for adventitious viruses.
4. **Process Clearance:** Unlike traditional biopharmaceuticals, many novel therapies such as enveloped viral vectors and cell therapies, cannot claim viral clearance for their manufacturing processes. Given the nature of the product, it is not possible to separate or inactivate potential viral contaminants without adversely impacting the product. Viral inactivation and removal steps can be included in the manufacturing process for non-enveloped viral vectors, and the viral reduction can be claimed for many such processes.
5. **Purified Bulks:** Purified bulks should include a verification of identity, such as confirmation of the gene of interest, confirmation of AAV serotype, etc. Quantitation of infectious titer, genome titer and potency is appropriate at this stage as well. The absence of microbial contamination and replication competent vector should be confirmed. Verification of the absence of residual product and process impurities should also be done.
6. **Final Product**: The identity of the final vector product should be confirmed, and this testing may include the sequence of the gene of interest, infectivity titer, genomic titer and potency. Sterility and absence of endotoxin should be determined. In addition, characteristics of the final product should be determined. This may include vector aggregation, pH , osmolality, appearance and particulates.

Table 1. Recommended testing for AAV, Lentivirus, Retrovirus and Adenovirus vectors.

AAV	Lenti/Retrovirus	Adenovirus
Identity • PCR for Genomic Region of Interest • AAV Serotype • Vector Sequence (NGS)	**Identity** • PCR for Genomic Region of Interest • Vector Sequence (NGS)	**Identity** • PCR for Genomic Region of Interest • Vector Sequence (NGS) • Restriction Endonuclease Analysis for ID of Purified Adenovirus Vector
Titer • $TCID_{50}$ Assay • Genomic Titer by Droplet Digital PCR	**Titer** • $TCID_{50}$ Assay • Genomic Titer by Droplet Digital PCR	**Titer** • FFU Assay • Genomic Titer by Droplet Digital PCR
Potency • r-AAV Expressed Protein	**Potency** • r-LV/RV Expressed Protein	**Potency** • r-Adeno Expressed Protein
Purity • Sterility (also **rapid method**) • Mycoplasma/Spiroplasma (also **rapid method**) • Endotoxin • *In Vitro* Adventitious Viruses • *In Vivo* Adventitious Viruses • Replication Competent AAV	**Purity** • Sterility (also **rapid method**) • Mycoplasma/Spiroplasma (also **rapid method**) • Endotoxin • *In Vitro* Adventitious Viruses • *In Vivo* Adventitious Viruses • Replication Competent LV	**Purity** • Sterility (also **rapid method**) • Mycoplasma/Spiroplasma (also **rapid method**) • Endotoxin • *In Vitro* Adventitious Viruses • *In Vivo* Adventitious Viruses • Replication Competent Adenovirus
Process/Product Residuals • PCR for helper viruses or transfected plasmids • Host Cell DNA • Host Cell Protein • DNA Size Distribution • Empty/Full Capsid by AUC • Residual AAV Ligand • Residual Benzonase® • Residual BSA	**Process/Product Residuals** • PCR for transfected plasmids • Host Cell DNA • Host Cell Protein • DNA Size Distribution • Residual Benzonase® • Residual BSA	**Process/Product Residuals** • PCR for transfected plasmids • qPCR Detection of AAV • Host Cell DNA • Host Cell Protein • DNA Size Distribution • Residual Benzonase® • Residual BSA
Final Product Characterization • Vector Aggregates by DLS • Osmolality • pH • Appearance • Particulates	**Final Product Characterization** • Vector Aggregates by DLS • Quantitation of LV Particles Using Virus Particle Counter • Osmolality • pH • Appearance • Particulates	**Final Product Characterization** • Vector Aggregates by DLS • Osmolality • pH • Appearance • Particulates

Viral Clearance

Viral clearance studies are not possible for gene therapy enveloped viral vectors or cell therapies, simply because inactivation or removal of potentially contaminating viruses may have a deleterious effect on the product. Non-enveloped viral vectors, however, may be resistant to viral inactivation measures, and there may also be removal steps that differentiate between the product and a non-enveloped adventitious virus.

We have evaluated the viral clearance potential of manufacturing processes for numerous non-enveloped viral vectors, and data for example cases are shown below in Table 2.

Table 2. Examples of enveloped virus reduction for non-enveloped virus-like particle or viral vector products

Process Step	Log_{10} Virus Reduction		
	BACV	VSV	WNV
Low pH Inactivation	3.48	1.16	3.44
Detergent Inactivation	≥5.15	≥4.41	ND
Chromatography 1	≥6.70	≥6.08	≥4.93
Chromatography 2	2.67	3.93	NR
Chromatography 3	1.82	3.84	ND
Virus Reduction Filtration (35nm)	≥4.41	≥4.83	ND

These data demonstrate that the manufacturing process for a non-enveloped virus can achieve effective reduction of enveloped viruses.

- Low pH can achieve inactivation of enveloped viruses; however, the level of that reduction is dependent on the pH and the susceptibility of individual viruses to low pH.
- Detergent can achieve effective inactivation of enveloped viruses.
- As with any product, the level of reduction achieved by a chromatography step is dependent on the chromatography resin, the conditions under which the chromatography step is run and the virus.

The manufacturing process for a non-enveloped viral vector can provide an additional safety factor for these biological products. Using enveloped viral inactivation steps, large pore virus filters and even chromatography steps the viral safety of these products can be enhanced.

Summary

Novel therapies, like viral vectors and cell therapies present a challenge to traditional approaches to viral safety. For enveloped viral vectors and cell therapies, viral clearance by the manufacturing process cannot be claimed.

These novel therapies must then rely on careful selection and testing of materials used in the manufacturing process and thorough testing of the product and its intermediates.

This approach will ensure that these new biopharmaceuticals that may bring new opportunities for healing to patients remain safe from adventitious viral contamination.

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