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Viral Risk Mitigation

A GLOBAL REGULATORY PERSPECTIVE

- Guidance for Raw Materials and Cell Lines
- Preventing Contamination
- Detecting Contamination
- Viral Clearance Evaluation

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BioProcess™ SPECIAL REPORT

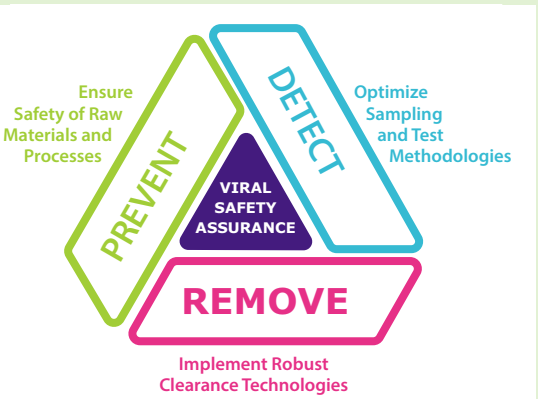
Virus Risk Mitigation in the Development of Monoclonal Antibody Products

A Global Regulatory Perspective

Martin Wisher

The production of biological products using animal cells and human operators always will have the risk of microbial and viral contamination. Manufacturers have developed a multitiered approach — tailored to individual processes — to prevent adventitious viruses entering production processes, detect contamination in raw materials and process intermediates, and remove viruses in downstream purification. This article provides an overview of the global regulatory framework to ensure the viral safety of biologics.

Figure 1: Developing a comprehensive risk mitigation strategy: a multifaceted approach



Contamination of Biological Products

Past contamination events have resulted in corrective and preventative actions to reduce the risk of viral contamination in biologics. A number of viral contamination events have been reported in different production processes.

Many of those events involved Chinese hamster ovary (CHO) cells, a commonly used cell line for monoclonal antibody (MAb) production, and in some cases, bovine serum was identified as the likely source of contamination. The most frequently reported viral contamination of CHO cells has been with minute virus of mice (MVM), and the most likely sources were media components such as glucose. Porcine trypsin was the likely source of porcine circovirus contamination of rotavirus vaccines.

Unfortunately, not all adventitious virus contaminations are easily detected. For instance, most insect viruses silently infect insect cells. Nodavirus or rhabdovirus infections of insect cells are common, and although scientists generally believe that such viruses are not of concern to humans, nodavirus can produce morbidity after injection into suckling mice.

Regulatory Guidance for Raw Materials and Cell Lines

Box 1 lists regulatory guidance documents that provide the framework for viral safety of raw materials and cell lines used in biologic production. The US FDA guidance on the characterization and qualification of cell substrates for viral vaccine production provides details of viral detection assays and a rationale for why such assays are used and which viruses are detected. Earlier FDA guidance documents, such as the 1997 Points to Consider (PTC) on monoclonal antibodies document, also contain useful information. The 2010 World Health Organization (WHO) technical report augments the International Council for Harmonisation

(ICH) Q5A and Q5D documents on Quality of Biotechnological Products. The approach outlined in the 2015 edition of the Chinese pharmacopoeia is similar to those of US FDA, ICH, and WHO documents, with some differences in the specifics of individual assays.

These guidance documents rely on a common strategy of risk mitigation to ensure viral safety built on three complementary approaches:

- preventing viral contamination by using high quality raw materials in production
- using a panel of assays to detect the presence of viral contaminants in raw materials, cell lines, and process intermediates
- implementing virus reduction technologies in the production process to improve the safety of biological product (Figure 1).

Preventing Contamination

Preventing contamination of production processes is the foundation of every viral safety strategy, and the approach taken is different for each type of biologic and for each process. Although each process is unique, general principles can be applied to all processes to reduce the risk of viral contamination.

Wherever possible, animal-derived cell culture media components should be replaced with recombinant proteins or animal-component–free media alternatives. If animal-derived components must be used, they should be sourced from regions with risk of low bovine spongiform encephalopathy (BSE). Because of the inherent risk of viral contamination in biologics production, different viral inactivation technologies have been developed to treat high-risk cell culture components. Bovine serum or porcine trypsin that cannot be replaced by recombinant products should be gamma or UV irradiated before use. EMA guidance documents (**1**, **2**) and US 9 CFR (*Animals and Animal Products*) describe general testing strategies.

If suitable for a production process, the preferred option is to adopt chemically defined, animal-derived-component–free media. Some components that have a high-risk of rodent virus contamination (e.g., glucose) can be treated with high temperatures/short time (HTST) (e.g., at 102 °C for 10–15 seconds) to inactivate potential parvovirus contaminants.

Virus filters have been developed with a modified chemistry, enabling cost-effective filtration of large volumes of chemically defined cell culture media and feeds. Such barrier filters achieve high levels of parvovirus removal and provide sterilizing-grade filter performance for bacteria and mycoplasma. Newer gene-editing technologies offer different opportunities for viral safety: A CHO cell line has been engineered without receptors to MVM, thereby making it resistant to MVM infection. Careful consideration of the different options for sourcing, selection, and treatment of raw materials used in upstream processes is an essential part of all viral safety strategies.

Box 1: Guidance for Raw Materials

US Food and Drug Administration (US FDA)
- *Guidance for Industry: Characterisation and Qualification of Cell Substrates and Other Biological Starting Materials Used in Production of Viral Vaccines for the Prevention and Treatment of Infectious Diseases*, 2010
- *Points to Consider in the Manufacturing and Testing of Monoclonal Antibody Products for Human Use*, 1997

World Health Organization (WHO)
WHO Technical Report Series 978, Annex 3 Requirements for the Use of Animal Cells As In Vitro Substrates for the Production of Biologicals, 2010

Chinese Pharmacopoeia
Requirements for Preparation and Control of Animal Cell Substrates Used for Production of Biologics, 2015

International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH)
ICH Q5A: *Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin*, 1997

ICH Q5D: *Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/ Biological Products*, 1997

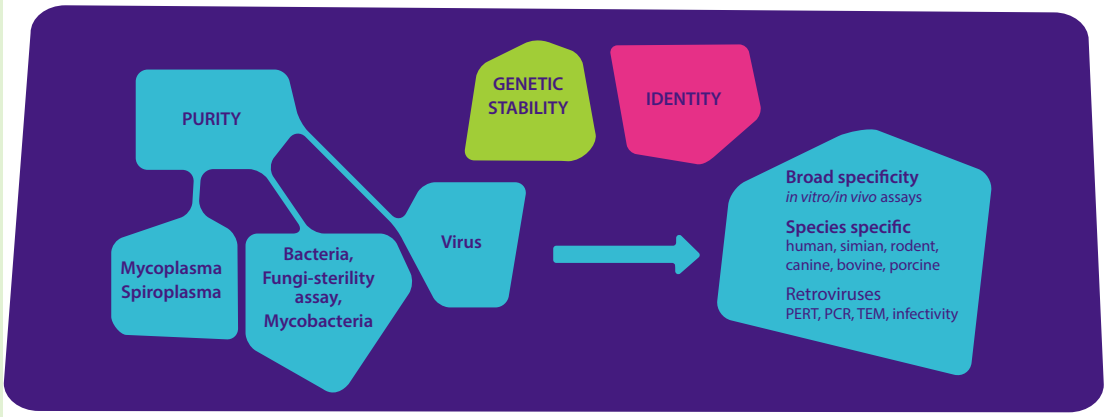
European Medicines Agency

Detecting Contamination

Testing to detect viral contaminants in raw materials, cell banks, and process intermediates is an integral second component of every viral safety strategy. Defining where and how much testing is required can be challenging, and European Pharmacopeia 5.1.7 (**3**) provides a useful risk-based framework to identify viral contamination risks and subsequent testing requirements.

Typically, a one-time characterization of the master cell bank (MCB) is performed to confirm no microbial or viral contaminants are detected, and more limited testing is performed on working

Figure 2: Master cell bank (MCB) characterization



cell banks (WCBs), which are a few passages beyond an MCB. A one-time testing of end-of-production cells (EOPC) or extended cell banks also is expected because those cells represent potential worst-case scenarios for amplification of low-level contaminants that might have been present but not detected in an MCB.

Specifics on when to test and how much testing is required differs according to the region and stage of clinical development. In Europe, EOPC testing for well-characterized cell lines such as CHO is not needed during clinical development and is required only at the time of license application. In the United States, EOPC testing is required before initiating a phase 3 clinical trial. Guidances in other regions are less clear, however EOPC testing is generally performed.

The clear expectation is that no adventitious virus will be detected in a cell bank characterization. Rodent cell lines such as CHO and baby hamster kidney (BHK) express replication defective endogenous retrovirus particles, whereas mouse myeloma cell lines (NS0, Sp2/0) can express replicating retroviruses.

Testing of MCBs should confirm identity. For recombinant cell lines, genetic stability also should be confirmed before license application (Figure 2). Testing also should confirm an MCB is free from detectable bacteria, fungi, and mycobacterium contamination.

Testing an MCB for viruses requires different testing methods, including broad specificity *in vitro* and *in vivo* assays, with methods that are targeted to specific viruses or types of viruses. Testing plans are tailored to individual specifics of each production process and depend on the cell line, origin of raw materials used in production, and production history. An important component of MCB characterization is screening for the presence of retroviruses. They can be detected by performance of infectivity assays (cocultivation assays), detection of an integrated provirus using specific polymerase chain reactions (PCRs), expression of retrovirus reverse transcriptase activity (RTase, PERT assay), and presence of retrovirus particles using transmission electron microscopy (TEM).

Selecting which assay to use depends on the cell line being characterized. Rodent cells (CHO, murine myeloma) are known to express retrovirus particles, so cells should be screened using infectivity and TEM assays, with an RTase assay used only if both other assays are negative. By contrast, human cells should not express retroviruses, so they should be screened using PERT, PCR, and TEM assays, with an infectivity assay used only if any assay produces equivocal results.

The *in vitro* virus assay is the most commonly used broad-specificity assay for adventitious virus

detection. It is used to screen cell banks and batches of bulk harvest for the presence of viral contaminants. Monolayers of at least three different detector cell lines are inoculated with a test article (cell lysate from cell bank or bulk harvest). Following extended incubation, the inoculated cells are screened for the presence of viral cytopathic effect and haemadsorption activity, and the cell supernatant screened for haemagglutinating activity.

The relative sensitivity of such broad-specificity *in vitro* and *in vivo* virus assays was the subject of an extensive study with many different viruses (**4**) and was motivated by the desire to determine whether an *in vivo* test added value to viral safety testing. The sensitivity of an *in vitro* virus assay was evaluated with 15 combinations of cells and viruses at 14-day and 28-day incubation end-points. Results indicated that the 28-day *in vitro* assay was more sensitive than the 14-day assay for all combinations of viruses. Although the 1993 US FDA *Points to Consider in the Characterization of Cell lines used to Produce Biologicals* and 1997 *Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products* state that a time of 14 days is a minimum requirement for an *in vitro* virus assay, more recent FDA and WHO regulatory guidelines (**5**, **6**) recommend performing a 28-day assay, at least for cell banks. If manufacturers use a 14-day *in vitro* virus assay, then the US FDA expects justification for why using a less-sensitive assay does not present a risk to patient safety.

Overall, when researchers compared the relative sensitivity of *in vivo* and *in vitro* assays, they found that for all but two of the viruses tested, the *in vitro* virus assay was more sensitive than the *in vivo* assay.

It's important to note that the results of this study were used as justification for modifying requirements for animal testing for virus in recently published European Pharmacopoeia documents. EP 2.6.16 proposes viral testing strategies built on risk assessment and limits *in vivo* testing to suckling mice (**7**). That guidance also indicates *in vivo* testing is required only if it mitigates risk and advocates consideration of newer technologies such as next-generation sequencing (NGS) as an alternative to *in vivo* or specific nucleic acid tests (NAT). The document also suggests NGS could be an alternative or supplement to *in vitro* virus tests, if local regulatory authorities agree.

Box 2: Regulatory Guidance on Adventitious Agent Testing

Assays to detect adventitious viruses; all documents aligned in recommending use of live cells or cell lysate (at 10^7 cells/mL in conditioned medium)

In Vivo assay

US FDA Guidance
Recommends adult mice (20 observed for 21 days), suckling mice (20, observed for total 28 days with passage at 14 days), embryonated eggs (allantoic and yolk sac inoculation with passage), Guinea pigs (42 days observation to detect Mycobacterium)

WHO
Recommends adult mice (20 observed for 28 days), suckling mice (20, observed for 28 days but no passage at 14 days), Guinea pigs (but can be replaced by in vitro method for Mycobacterium), embryonated eggs (but only if testing avian cell lines or novel cell substrates)

EP 5.2.3
Recommends inoculation of suckling mice (>10, observed for 28 days) only if assessment indicates that it provides risk mitigation taking into account the overall testing package. Embryonated eggs are required only for avian cell substrates.

Similarly, EP 5.2.3 discusses the utility of NGS as an alternative test for virus detection and eliminates the requirement for *in vivo* testing with adult mice and guinea pigs (**8**). Although these recommendations apply to testing for vaccine production, the general approaches are applicable for many instances of biologics testing: risk-based assessments for viral safety and implementation of newer virus testing methods as an alternative or supplement to traditional assays.

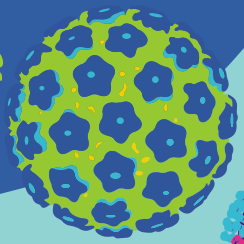
Those changes to the European Pharmacopeia, mean there is no unified global position on regulatory expectations for *in vivo* adventitious virus testing. That is likely to remain the case until ICH Q5A is modified because this guidance stipulates an *in vivo* assay using suckling mice, adult mice, and embryonated eggs for detection of adventitious virus (Box 2).

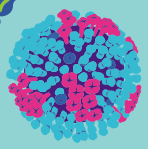
Limitation of Detection Assays

A number of factors can affect the ability of all assays to detect virus contamination. All virus tests have a limit of detection (LOD) that affects how such tests determine whether low concentrations of adventitious agents are present. Assay sensitivity depends on the amount of sample

Viral Safety Assurance for MAbs: Prevent, Detect, Remove

Current and Future Solutions





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A viral contamination can shut down a biopharmaceutical plant for months impacting manufacturing operations, causing significant business disruption and ultimately threatening drug supply. Fortunately, a range of technologies are available today to help prevent viral contamination and assure an efficient and safe biopharmaceutical production process.

1 Adventitious Viruses

A Constant Process Threat

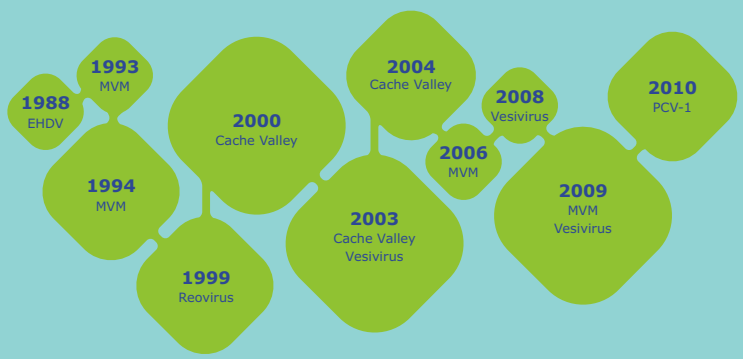
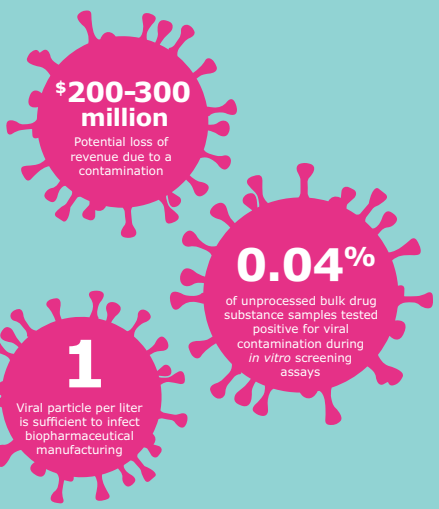
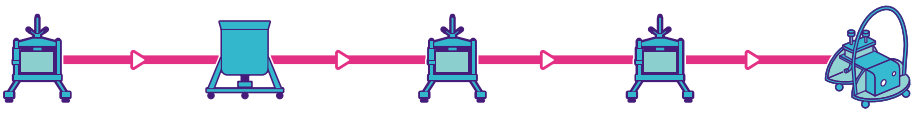


Fig.1 Reported major viral contamination events in biopharmaceutical manufacturing



2 Traditional Solutions

Viral safety solutions that remove virus from monoclonal antibody and recombinant protein production are well understood.



	Affinity Chromatography	Low pH	Cation Exchange Chromatography	Anion Exchange Chromatography	Virus Filtration
Parvovirus LRV	1-3	<1	~1	2-5	>4
Retrovirus LRV	1-5	>5	~2-3	~4-5	>5

Fig.2 Expected viral clearance by manufacturing unit operation, log reduction value (LRV).
Miesegaes G., Lute, S., Brorson K., (2010) Analysis of Viral Clearance Unit Operations for Monoclonal Antibodies. *Biotechnology and Bioengineering* Vol 106, No 2, June 1 2010 p 238-246

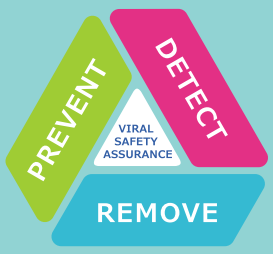
Downstream chromatographic purification

Downstream processing separates the protein of interest from cell culture harvest and results in a purified, concentrated molecule with low levels of impurities. Various technologies with a variety of base media, ligands and formats offer multiple options for purification. Although purification is the primary goal, reliable virus removal is also required to meet the viral safety needs of the downstream process.

Downstream virus filtration

Flexible prefiltration options enable increased mass loading on virus filters to meet the demands of today's biomanufacturers. Robust viral clearance should be maintained during virus filtration following planned or unplanned process interruptions, assuring performance and consistency of this critical virus reduction operation.

3 What's next to Minimize Risks?



A risk mitigation strategy that includes the prevention, detection, and removal of viral contamination will help ensure process safety.

Fig.3 Viral safety assurance

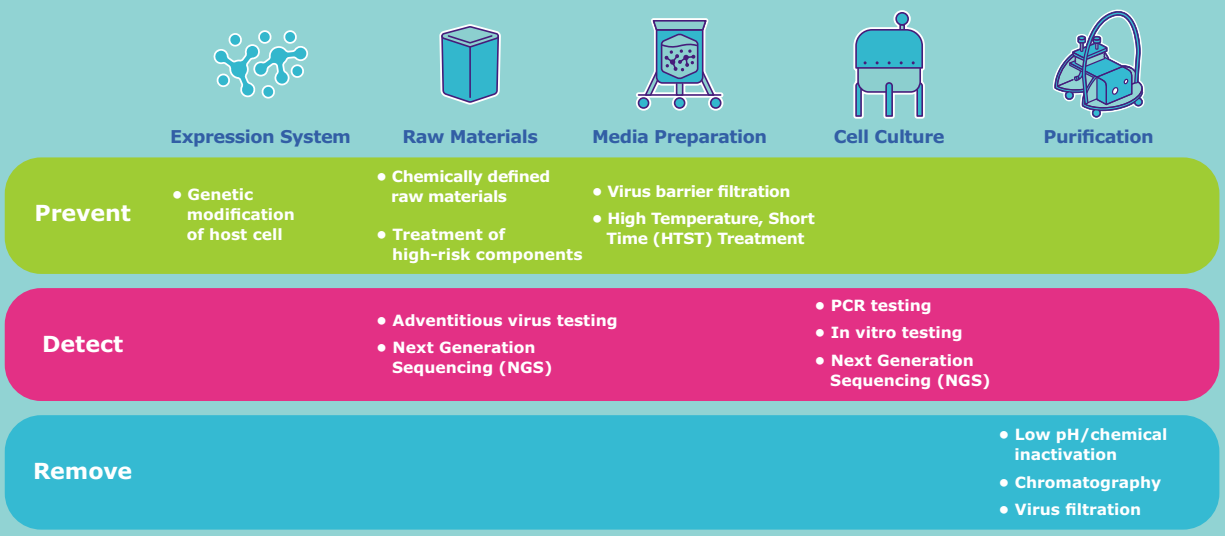


Fig.4 Various technologies help minimize viral contamination risks throughout the process

A diverse range of new technologies further minimizes the risk of introducing viral contamination into biopharmaceutical production, including virus-resistant engineered CHO cell lines, novel filters designed specifically for cell culture media, and innovative technologies for sensitive detection of unknown viruses. More traditional virus filtration technologies have been augmented by new prefiltration options enabling more efficient processing of a broader range of feed streams.

Prevent

Gene Editing Technology created engineered CHO cell lines that are resistant to MVM, one of the main viral contaminants in the industry.

New barrier technologies like **filtration products designed for cell culture media** offer easy to use and implement solutions for risk reduction.

Detect

Next Generation Sequencing opens doors for more rapid, sensitive detection of viral contaminants

Remove

Prefilters remove plugging species from mAb processes significantly improving capacity of the filtration device while maintaining high parvovirus removal and flux.

Learn more
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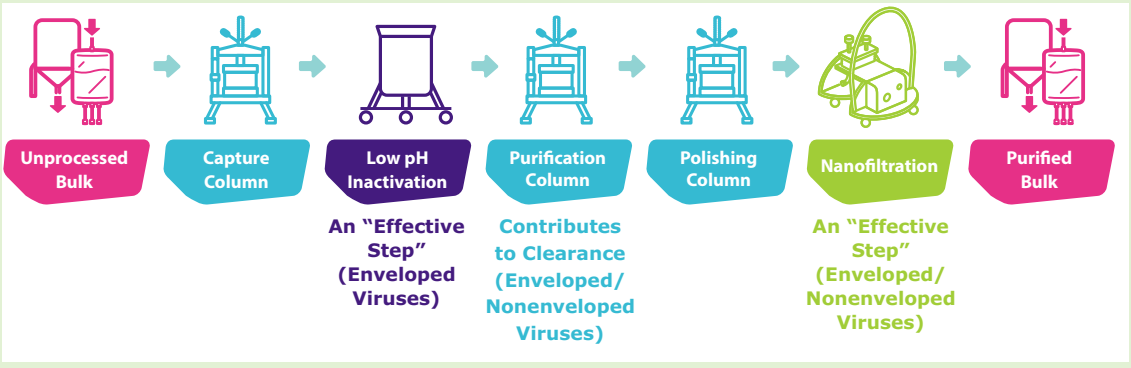
Figure 3: Typical monoclonal antibody purification process



Box 3: Regulatory Guidance Documents

ICH Q5A: *Quality of Biotechnological Products: Viral Safety Evaluation of Biotechnology Products Derived from Cells Lines of Human or Animal Origin,* CPMP/ICH/295/95

Guideline on Virus Safety *Evaluation of Biotechnological Investigational Medicinal Products,* EMEA/CHMP/BWP/398498

Note for Guidance on Viral Validation Studies: *The Design, Contribution, and Interpretation of Studies Validating the Inactivation and Removal of Viruses.* CPMP/BWP/268/95

Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use. FDA/CBER, 1997

Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals, FDA/CBER, 1993

that can be analyzed in that assay as a proportion of the total amount of material. For *in vitro* tests, particular cell lines can be more or less susceptible to infection by known or novel viruses. Some materials may interfere with the ability of an assay to detect viruses or cytotoxic to detector cells used in an assay. A negative test result does not indicate that an adventitious agent is absent, but rather it indicates that an adventitious agent is below the assay LOD. Another limitation of detection assays is that they generally are designed to detect specific virus pathogens, which means you often find only what you're looking for.

Testing for virus is performed on an MCB, cells at maximum use, and bulk harvest. Typically, a minimum of three lots of bulk harvest are tested for bioburden, mycoplasma, and viruses using an *in vitro* virus assay before clinical trials. Those assays are performed on each batch of bulk harvest as part of routine process quality control. For rodent cells, the amount of expressed retrovirus particles is determined by TEM or quantitative PCR assays.

The most recent European Pharmacopoeia chapter (EP 5.2.3) notes the availability of new sensitive molecular technologies with broad detection capabilities such as massively parallel sequencing (MPS), degenerate PCR for whole virus families, oligonucleotide arrays, and mass spectrometry (**8**). As mentioned above, these methods can be used either as an alternative to *in vivo* or specific NAT or as a supplement/ alternative to *in vitro* culture with approval from national regulators.

NGS sometimes is called high-throughput sequencing, deep sequencing, or MPS. NGS generates high millions or billions of sequencing reads from a nucleic acid library template. Those reads are then analyzed using powerful bioinformatics computing processing and compared with databases of known sequences. A critical component of NGS is computational analysis of data. Initially, poor-quality reads are eliminated from the analysis, and then a multistep basic local alignment search tool (BLAST) analysis is performed — where reads from different mammalian cell housekeeping genes act as controls to determine breadth and depth of sequence coverage. Sequences of expected components then are subtracted, enabling analysis of cell line samples to focus on any adventitious virus sequences.

An NGS interest group with members from industry and regulatory agencies is developing some practical guidance and standards for NGS and bioinformatics analysis. In addition, an ongoing study is evaluating the relative sensitivity of NGS testing for viral detection. The power of NGS may not be increased sensitivity but rather

Figure 4: Viral clearance study design at different stages of clinical development

Rodent-derived Cell Line

X-Murine Leukemia Virus
Retrovirus, Enveloped, RNA

Pseudorabies virus
Herpes virus, Enveloped, DNA

Reovirus
Reovirus, Non-Enveloped, RNA

Mouse Minute Virus
Parvovirus, Non-Enveloped, DNA

- **Retrovirus**
- **Parvovirus**
- **Two orthogonal steps**
- **Risk assessment**
- **Duplicate study (EU)**

- **Four virus panel**
- **Evaluation of multiple process steps**
- **Robustness studies**
- **Column sanitization (carry over)**
- **New/Aged resin studies**
- **Risk assessment**
- **Duplicate study**

broad specificity and ability to detect sequences of novel or unknown viruses. New technologies such as NGS and broad sensitivity NAT assays can be integrated into viral safety testing plans to ensure the safety of biologics for patients.

Removal Steps and Viral Clearance

Although careful practices and comprehensive testing can reduce the risk of viral contamination, such actions by themselves generally are insufficient to ensure viral safety. For monoclonal antibodies, robust clearance technologies are implemented during processing to inactivate or remove viruses. The ability of these processes to effectively inactivate or remove viruses is assessed in viral clearance studies.

Rodent cells (e.g., CHO cells) produce retrovirus-like particles. These particles are defective and cannot integrate into the genome of a cell and replicate. Although the risk is small, it is possible that these retrovirus-like particles recombine with retrovirus sequences in the human genome to generate a new human retrovirus. This is a theoretical risk, but it can be mitigated if manufacturers can demonstrate sufficient retroviral clearance in production processes.

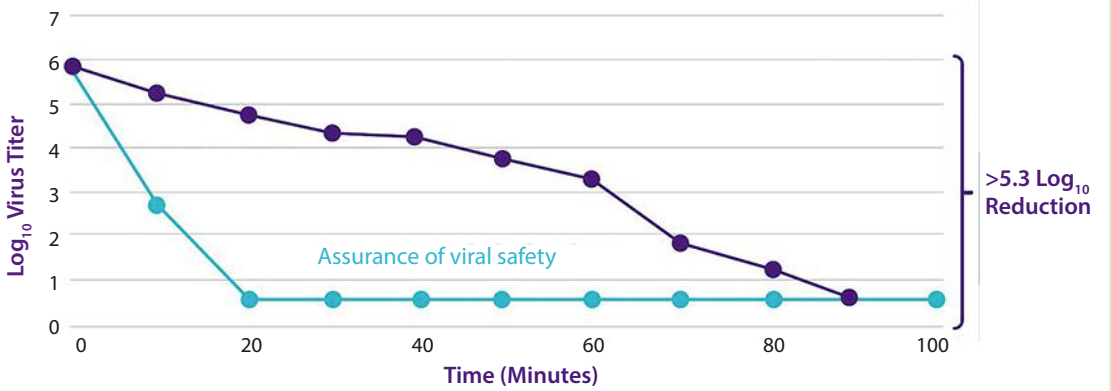
Clearance studies provide indirect evidence that a production process has the capacity to inactivate or remove novel or yet-undetermined virus contaminants. Guidance documents that discuss viral clearance assessments are summarized in the Box 3. Most monoclonal antibody viral clearance studies evaluate clearance across a limited number of steps in a downstream process. Those steps are selected based on their potential to clear virus. Typically, steps that will inactivate viruses (e.g., low pH) and remove potential viral contaminants (e.g., nanofiltration) are evaluated. It is important that the processes evaluated have independent or orthogonal mechanisms of virus inactivation and removal. Figure 3 shows the operations in a standard monoclonal antibody purification process and their likely contribution to viral clearance.

Requirements for viral clearance testing depend on the stage of clinical development. In the European Union, duplicate clearance studies are performed before phase 1 trials using two viruses: an enveloped virus (e.g., murine leukemia virus, which is a model for retrovirus particles) and a small, nonenveloped virus (usually a parvovirus such as minute virus of mice, MVM). Two different, orthogonal steps are typically evaluated using worst-case parameters. The US FDA requires testing with at least murine retrovirus and mentions that studies with parvovirus are useful but not essential for early phase clinical trials. Before licence application and usually during Phase 3 trials, clearance of a full virus panel is evaluated in duplicate tests across multiple process steps. Such studies can include robustness studies of those effective steps, column sanitization, and studies with new and aged resin (Figure 4).

Evaluating Clearance Data

Understanding the results of clearance studies provides manufacturers with information to make risk-based decisions on the safety of their downstream processes. For example, Figure 5 shows two inactivation steps, both of which achieve over five logs of reduction. However, one step

Figure 5: Inactivation: same overall clearance, but different inactivation kinetics



Box 4: US FDA New Viral Clearance Validation Approaches

Discontinuation of the need for aged Protein A resin studies in late-phase virus removal studies
- Data show no reduction in Murine Leukemia (MuLV) removal with >200 cycles of use

Use of retrovirus-like particle (RVLP) quantitative PCR to track RVLPs during capture chromatography in lieu of small-scale validation using MuLV
- Requires high expression of RVLPs by CHO cells

Use of ASTM E2888-12 as a modular claim for low pH inactivation of rodent retrovirus instead of performing validation studies
Can claim 5 $\log_{10}$ reduction factor if
- Temperature >15° C; Time >30 min; pH <3.6; <500 mM NaCl; protein <25 g/L; glycine, citrate, or acetate buffer

For virus removing filters, extrapolate the log reduction factor (LRF) for parvovirus (e.g. Minute Virus of Mice, MMV) to larger viruses
- This may limit claims for MuLV LRF

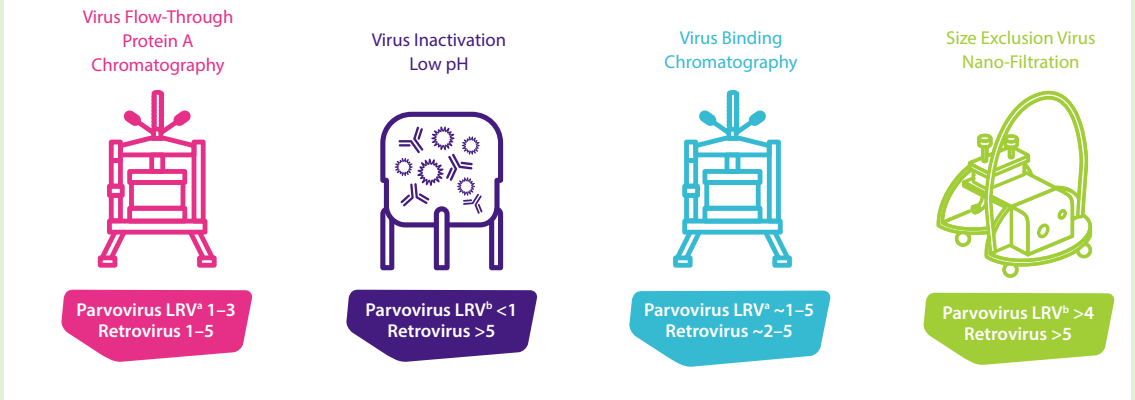
shows rapid inactivation, and the other achieves it 10 minutes before the normal hold time of that step. The step that provides rapid inactivation has a higher virus safety risk-mitigation element because there is a longer period of inactivation.

For evaluating clearance across chromatography columns, biomanufacturers expect that in addition to the clearance levels there is an understanding of where a virus is partitioning. If a virus is not in product eluate, is it in the flow-through and wash, or bound to the column? This infectivity mass balance might be possible for nonenveloped viruses, but it is unlikely to be good for enveloped virus because of viral inactivation in buffers or test temperatures. Clearance across an entire process is the sum of the log reduction from a number of orthogonal steps. Figure 6 shows typical levels of viral reduction that might be achieved for different steps in a monoclonal antibody production process.

Recent changes to US FDA expectations for viral clearance are outlined in Box 4. Clearance evaluations with aged protein A resin studies are no longer required because studies have demonstrated no change in virus removal capabilities with resin age. Recently this approach has been agreed to by European regulators. In addition, a standard has been developed in the United States for low pH inactivation of rodent retroviruses that enables claiming a five-log reduction in virus if the step operation is performed within a specific operating window.

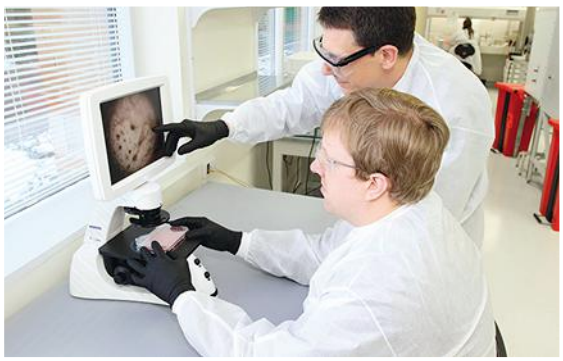
For virus-removal filters, the log reduction factor achieved with parvovirus can be extrapolated to larger viruses without performing a clearance study. However, adopting this approach can limit overall reduction claims for retrovirus removal. Those recommendations have not been published but were presented by Kurt Brorson from US FDA at the PDA Virus Safety meeting in 2015 (9).

Figure 6: Downstream virus clearance technologies



[a] Remington, et. al. Viral Clearance By Protein A, Anion Exchange and Cation Exchange Chromatography Steps. *Amer. Pharm. Review.* 2015

[b] Merck KGAa Darmstadt Germany data



Summary

Biotechnologically derived medicinal products have a very good safety record with no viral contamination of final products. This strong record is a result of the incorporation of virus risk-mitigation strategies throughout production processes. Such strategies include preventing entry of contaminants into upstream processes, implementing sensitive testing for contaminants in raw materials and process intermediates, and incorporating virus removal and inactivation steps into downstream purification processes. Increased awareness of potential contaminants helps manufacturers to define testing strategies and the need for the clearance operations, which consequently should lead to improved safety assurance for patients.

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For an unparalleled experience throughout the life cycle of your therapy, BioReliance® world-class biosafety solutions offer a full range of GMP cell banking services, cell line and virus bank characterization, viral clearance and lot release testing. Merck's complete biosafety testing solutions, paired with our long-standing reputation for quality and expertise, will give you the mission-critical capabilities to bring safe, life-changing medicines to market.

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