An Integrated Approach to Ensure the Viral Safety of Biotherapeutics

Mark Planic

Testing product and process intermediates alone is helpful, but does not provide a complete solution to viral safety. This article proposes integrated solutions for systemic and proactive viral risk mitigation.

Use of continuous cell lines in the manufacture of biological therapeutic products, such as vaccines, recombinant proteins, and monoclonal antibodies, is associated with the concomitant risk of process/product contamination with endogenous retroviruses, latent viruses, or new and emerging adventitious agents. Cell-culture applications are impossible without the use of nutrient media for cell multiplication and subsequent product generation. Although several serum-free and chemically defined nutrient media formulations are available for commercial use, many cell-culture applications require use of nutrient media supplementation with serum or other animal-derived components. Use of serum-supplemented cell-culture media is considered a point of entry for the introduction of adventitious agents into a manufacturing process. Other raw materials used in the manufacture of biotherapeutics, especially those of animal and human origin, could also present a viral safety risk.

Traditionally, the management of inadvertent virus contamination is achieved through the incorporation of various measures aimed to predict, detect, and inactivate adventitious viral agents from the biological products (i.e., selection, testing, and clearance).

Although a plethora of regulatory guidance documents have been enacted governing product safety from adventitious agents (1-16), complete
risk elimination has not yet been achieved. Several examples of bioproduction process contamination have been documented over the years, implicating various agents such as E. coli 0157:H7, E. coli 0157:H7, Shiga toxin-producing Escherichia coli (STEC), and Salmonella typhimurium. The use of endotoxin-negative liposomes in vivo and in vitro studies showed that these agents are not toxic to human cells.

A recent study by the FDA revealed that 37.5% of respondents—who were from the biotechnology, manufacturing, and quality assurance departments—said that their organization was not satisfied with the solutions they currently have in place. These strategies to appear to be working on the whole, in 80% of companies, and have not been successful in dealing with contamination.

The purpose of this article is to discuss some holistic, interlocking approaches across the manufacturing chain to reduce the risk of adventitious viral agent contamination and to ensure uninterrupted supply of safe biological products to patients in need.

**Product Safety and Quality by Design (QbD)**

Traditionally, product safety has relied on the incorporation of three key measures into the manufacturing process: selection, testing, and viral control. These measures are collectively known as the “safety triangle” (Figure 1). The elements of the safety triangle include the selection of source materials, as well as those based on prior material and supplier qualification.

![Figure 1: Illustration of the biopharmaceuticals safety triangle](image)

By approval and validation testing for adventitious contaminants, testing for various adventitious contaminants at appropriate stages of the manufacturing process from raw materials, starting materials (e.g., cell banks, viral and bacterial seeds), and manufacturing intermediates, and viral clearance, employed either in raw materials control or evaluation of the capacity and capability of the downstream purification process to clear removed or inactivated potential adventitious contaminants.

Although the safety triangle still represents an integral part of the viral safety of biological products, it has been generally accepted that the safety triangle alone may not be sufficient, and some enhancements may be warranted. Today’s industry and regulatory expectations require that an effective viral risk mitigation strategy be built into the whole manufacturing chain, starting from the suppliers of crucial raw materials and components and extending throughout the manufacturing plant. Where applicable, (Figure 2) in this context, viral risk mitigation should be an integral part of the overall quality system and quality risk management strategy (13). Viral safety needs to be designed into the overall drug-design, development process, and regulatory approach.

The concept of product “safety by design” (QbD) represents an integrated, holistic approach to viral safety across the manufacturing chain. The goal of QbD is to provide a manufacturing process from interventions caused by viral contamination and ensure product and patient safety by preventing viral introduction, ensuring early detection, and enabling rapid response to ensure containment and elimination of viruses if introduced into the manufacturing process. It typically spans the following five areas:

- Raw materials (RM)
- New process/product development (PP)
- Manufacturing process
- Quality system (QS)
- Detection testing

In the context of this article, animal-origin (AO) and chemically defined (CD) raw materials are defined as follows:

Animal (including human origin) materials: materials derived from various species of animals,
The following sections address viral risk mitigation across the four areas in more detail.

**Raw Materials**

Raw materials have been regarded as one of the most significant viral entry points into a manufacturing environment. The main goal of viral risk mitigation at this level is to prevent virus introduction into a manufacturing process via raw materials. The following measures should be considered in addressing this level of viral risk remediation:

- Implement a process of identification and segregation of all critical (e.g., animal and human-origin) raw materials.
- Introduce a risk assessment for animal- and human-origin components.
- Develop a policy of “three Rs” (reduction, reutilization, reuse) for animal- and human-origin components.
- Maintain solid knowledge of three Rs for animal- and human-origin materials.
- Establish a detailed supplier auditing and qualification program that includes biosafety considerations.

Establish a supplier development and improvement program addressing key areas of quality, biosafety, and risk management.

Treat raw materials (e.g., through aldehydes [G-P-C] and UV-C irradiation, gamma irradiation, heat, etc.) to reduce the risk of viral contamination. Although treatment options are helpful in mitigating viral contamination, they are not equally effective against all viruses. The use of appropriate concentration of chemical treatment provides a certain level of risk mitigation, but no complete risk elimination.

Some examples of raw materials (animal origin) and chemically derived treatment options include:

- When serum is used in manufacturing processes, replacement with recombinant proteins can be considered.
- Alternatively, liquid porcine trypsin solution can be used instead of trypsin to reduce the risk of viral contamination. Alternatively, liquid porcine trypsin solution could be nanofiltered using 15–25-nm pore size filters (G-P-C) or treated by UV-C.

- Bulk powder material of animal- or human-origin can be gamma irradiated in its final packaging. Alternatively (or additionally), nanofiltration (20-nm pore size) of liquid solutions can be considered at the point of use.

- Liquid cell-culture media can be treated by nanofiltration (20-nm pore size), UV-C, or heat (e.g., high-temperature for
short-time treatment, H1ST) at the point of use. Gamma irradia-
tion of the media powder before reconstitution may also be
investigated realizing that certain media components may
be incompatible with gamma irradia-
tion.
• Other raw materials including formulation buffers can be
nano-filtered (20 nm pore size) as liquid solutions.

BUILDING VIRTUAL SAFETY INTO A QUALITY SYSTEM
Effective virus risk mitigation should be part of the overall
quality system. That way, the safety is “system-driven”
instead of being people-dependent. Prevention of virus intro-
duction, viral risk understanding/mitigation, and effective response
to potential viral contamination are the main objectives of this
step. When building viral safety into the overall quality system,
a written, comprehensive virus mitigation program is neces-
sary. The main purpose of this program is to promulgate a sus-
tainable, long-term policy as a foundation for viral safety based
on the SHD principles. It will form the basis for incorporation
of viral safety into the overall quality system for both commer-
cial processes and new products in development.

Additionally, a viral risk assessment must be performed. The
purpose of a viral risk assessment is to promote ongoing and pro-
active viral risk identification and management. It should be
conducted using a risk analysis tool suitable for viral risk (e.g.,
failure mode and effect analysis [FMEA], preliminary hazard anal-
ysis [PHA], and risk ranking and filtering [RRF]), with periodic risk
re-evaluation, addressing the following areas at minimum:
• Risk of virus entry with appropriate controls:
  o Starting materials (e.g., cell banks, viral and bacterial
  seeds, animals used in production)
  o Raw materials (e.g., cell culture media, serum, plant
  extracts)
  o Personnel
  o Equipment
  o Manufacturing process (e.g., type of cells, type of process,
  open vs. closed cell-culture steps, duration, containment)
  o Manufacturing plant internal environment and utilities
  o Outside plant environment
• Specific virus controls in the product manufacturing process:
  o Virus testing (bulk harvest, drug substance, drug product)
  o Virus clearance afforded by
  downstream purification

A written emergency (contamination) response plan is an impor-
tant part of the SHD method. The objective of a viral response plan
is to specify necessary steps in the response process, delineate
clear roles and responsibilities, and ensure rapid response to a
suspect or confirmed viral contamination. It ought to be spe-
cific in terms of clearly addressing the questions of what, why who,
how, when, and where. A successful response plan achieves effec-
tive area containment, allows rapid virus elimination through
effective disinfection, and enables speedy facility return to the rou-
tine manufacturing regimen.

Existing procedures may need to be modified to incorporate
viral safety. Certain quality procedures may need to be refined to
integrate elements of viral safety, as appropriate. Such procedures
can include aseptic training, purchasing of suitable raw materials,
raw material supply-chain management, and cleaning and sanitiza-
tion, for example.

Biosecurity should be incorporated into a company's quality
audit program. Incorporation of viral safety elements into the
internal and external (supplier) audits helps identify weaknesses
and strengths of the firm's qual-
ity systems. Importantly, it helps drive improvements in the prac-
tices of the suppliers of critical raw materials.

Lastly, a training module on viral safety and its impact on
product and patients would be prudent. This training brings
awareness and drives employee behavior in specific units of
operations that are more suscepti-
able to viral contamination. In
concert, these measures would greatly enhance a quality-based
viral risk mitigation program and a firm's readiness to respond to a
contamination event.

PRODUCT DEVELOPMENT
New product development presents an ideal opportunity to incorpo-
rate all the relevant principles of SHD into the new process. Here,
product safety is intentionally designed into the new process with
the goal of preventing introduc-
tion of adventitious viruses into
the process and designing mean-
ingful product testing strategy, while enabling rapid detection and
containment in any area where a problem has occurred. The follow-
ing points should be considered when building SHD into new pro-
duct/ process development:
• Selection and engineering of
  cell lines
• Development of animal-origin-
  free/chemically defined cell
  banks (e.g., from transfection to maturation, and working
  cell bank [MCB, WCB] generation)
• Development of animal-origin-
  free/chemically defined manufac-
  turing processes, devoid of
  animal and human origin com-
  ponents
Incorporation of an appropriate and meaningful testing strategy
Use of upstream viral barrier technologies (e.g., UV-C, HEPA, nanofiltration) for media/raw material treatment
Use of closed process systems where appropriate
Incorporation of effective, validated viral clearance steps in downstream processing (including two orthogonal viral clearance steps, e.g., an inactivation step and a viral removal by nanofiltration) for drug substance generation
Design of closed processes units of operation, making them inaccessible to environmental adventitious agents
Use of disposable, single-use equipment wherever feasible
Regularization of a true sampling plan and well-defined testing plan for adventitious agents
Inclusion of process analytical technology (PAT) to enable early detection of cell culture contamination
Implementation of all the raw materials and quality systems principles discussed in the previous sections

COMMERCIAL PRODUCT MANUFACTURING
Control measures at this level serve primarily to prevent virus introduction and to ensure virus containment. The controls employed at this level may include:
Continuous process improvement, considering some of the elements discussed for new product development
Effective facility and equipment cleaning and sanitation procedures using process validation and sporadic chemicals
Annual facility improvement plan and proper facility design to prevent contamination, including area containment to prevent virus spread from the affected area segregation of various activities such as raw material handling, media and buffer preparation, cell culture operations, downstream operations, and post viral clearance operations (air pressure differential, HEPA treatment of production water, isolation, and appropriate filtration of production gases, equipment, and personnel; and a pest control program);
Proper employee training and grooming, including policy on managing employees with apparent communicable respiratory, gastrointestinal, cutaneous diseases in GMP areas

VIRAL TESTING/DEFINITION (IUPHAR)
As the name indicates, the main purpose of this level is to detect adventitious viruses. Early detection is essential in order to deploy an adequate response to contain and eliminate the virus. Various testing methods are used at several stages of process/product development and manufacture.

The following testing principles should be considered:
Testing design should be suitable for the process and, at a minimum, in compliance with the current regulatory requirements
Testing should be conducted at the most meaningful process steps using appropriate samples, sample schemes, and suitable testing methods
Limitations of the existing testing methods used for raw materials, cell banks, seed, harvests, and process testing should be understood and addressed appropriately.
• Risk-based testing augmentation: specific virus testing (e.g., by polymerase chain reaction [PCR]) or other technology should be implemented, if justified by risk assessment. This testing should include new and emerging agents.

• New detection technology (10) with broad detection and identification capabilities (e.g., deep sequencing, microradiography) should be adopted to augment the overall testing and characterization program, to address certain testing gaps, or to support risk assessment program.

SUMMARY OF VARIOUS VIRUS RISK MITIGATION OPTIONS

Although the safety triangle plays a central role in ensuring viral safety of the final product, various other options (Figure 5) are available to further augment the safety trip and reduce the overall risk of viral entry into the product stream. When properly integrated, these measures can bring residual viral safety risk to a very low level.

CONCLUSIONS

Viral safety is an important quality attribute of a drug product. Endogenous or adventitious viral agents are generally regarded as product impurities and are not acceptable in the final drug dosage form. Ensuring freedom from adventitious or endogenous viral agents is therefore crucial for the safety of biological drug recipients. Traditionally, the safety triangle (infection—testing—viral clearance) has been crucial to the safety of biological drug recipients. Traditionally, the safety triangle (infection—testing—viral clearance) has been crucial to the safety of biological drug recipients. However, it may not be sufficient enough to meet today’s expectations. An integrated viral risk mitigation program (i.e., safety by design across the supply chain) provides an important method to provide a high level of viral safety of biological products. Safety by design should not only be incorporated into new product development, it should also be part of a larger process-control strategy for both development and commercial products.

Zero risk in the manufacture of biological products is not achievable; aiming for a risk that is “as low as reasonably achievable” (ALARA) should be an acceptable goal—and it can be achieved through use of a holistic, integrated, and product-specific safety by design program.

REFERENCES


