Viruses: Managing the Risks

Andy Bailey at ViruSure GmbH discusses risk-based quality management for virus safety with biopharmaceutical products

The regulatory shift towards risk-based quality management has been triggered by the desire to provide a more balanced, flexible, transparent, responsive and, ultimately, a more predictable approach to biopharmaceutical quality. This shift is evident in numerous new guidance documents, including:

- ICH Q8 guideline on pharmaceutical development (including, for example, Process Analytical Technology (PAT) and Design Space) (1)
- ICH Q9 guideline on Quality Risk Management (QRM) (2)
- ICH Q10 guidelines on the Pharmaceutical Quality System (3)

The application of these approaches for already licensed products has presented manufacturers with the challenge of implementing a new method of pharmaceutical lifecycle management for those products already approved via the more outdated regulation through guidance. A more realisable goal is the implementation of the principles of risk-based management to developmental products, where the more classical approach of definition of the risk, evaluating potential impacts and then implementing measures to control the risk, can follow a clearer structure. With an integrated approach to QRM, principles for risk management are implemented throughout the quality system, and define a clear and transparent approach for dealing with risk.

This article provides a foundational overview of the principles of risk-based management in virus and prion safety, and the contribution of the various strategies relating to risk minimisation in the final product.

QRM AND VIRUS SAFETY

Effective risk management for virus safety should be a transparent process that enables appropriate decisions regarding risk control for the product to be made and effectively communicated to all concerned. The application of risk-based management to the virus and prion safety of biopharmaceutical products has been in operation for many years, but successful implementation requires an in-depth knowledge of the sources of potential risk, available measures for reducing and controlling the baseline risk, as well as an understanding of the regulatory history upon which the testing requirements have been built. For example, the requirement for testing of all cell lines for simian retroviruses in the 1997 FDA PTC on the testing of monoclonal antibodies was implemented because of historical exposure of certain parental cell lines to cells of simian origin (4). Today, the principles of risk management should be used to determine if such testing is really necessary, and, where suitable arguments can be made, both the workload and costs to manufacturers can be reduced.

Risk can be defined as the probability of occurrence of a particular harm combined with the severity of that harm were it to occur. Ultimately, risk evaluations for virus safety should be based on sound scientific knowledge, and the extent of documentation and evaluation commensurate with the magnitude of the risk. Establishing systematic procedures for evaluating risks is an essential component of QRM, and procedures therefore need to be established to ensure that the virus safety of the product is effectively evaluated and appropriate risk control measures implemented throughout the lifecycle of the product.

PRODUCT COMPLEXITY AND RISK

Whilst the virus safety testing requirements for monoclonal antibodies and other recombinant proteins, for example, are relatively well established, emerging technologies continue to challenge the regulatory requirements for virus and prion safety. Table 1 provides an overview of some of the more complex regulated products, the challenges they present and potential risk-control measures.

Table 1: An overview of potential risks and their control measures for new or developing biopharmaceutical product classes

	Potential risks	Risk control measures
Active pharmaceutical ingredients	For animal derived products: • Animal virus risks • Incorrect sourcing • Incorrect testing prior to manufacture • Insufficient segregation from other high risk products • Limited virus removal	Elimination of animal derived components Sourcing from low risk materials Effective segregation and sanitisation procedures Testing for animal derived viruses Incorporation of effective virus clearance steps
Human cell-based therapies and tissue engineered products (including stem cell products)	Human virus contaminants Endogenous retroviruses Latent virus infections Selective virus tropism exhibited by progenitor cells	Donor selection and screening Testing for active and latent viruses Co-cultivation assays
Gene therapy vectors	Replication competent virus contamination Interference in *in vitro* and *in vivo* adventitious agent assays Recombination events in the clinical setting	Testing for replication competent viruses Neutralisation of virus prior to testing Patient monitoring
Live virus vaccines	Interference in *in vitro* and *in vivo* adventitious agent assays No dedicated virus removal steps	Neutralisation of virus prior to testing Use of virus specific PCR assays
Plasma or urine derived medicinal products	Emerging human or zoonotic viruses TSE risks (For example, vCJD)	Donor selection, screening and testing Effective virus and TSE clearance steps
Transgenic products	Animal derived viruses	Herd monitoring Testing for animal derived viruses Effective virus clearance
Xeno-transplantation and xenogeneic cell-based therapies	Animal derived viruses Endogenous retroviruses Recombination events between animal and human viruses	Herd monitoring Testing for animal derived viruses Co-cultivation studies with permissive human cell lines

Table 2: Virus risk identification

Identified risk	Example risk control measures
Historical exposure to known virus risks	Donor history Close herds (for animal derived components) Minimisation of exposure of cell lines to animal derived components
Sourcing policy	Minimisation of geographical TSE risks (for example, BSE and vCJD) Donor selection API selection Vendor audits
Testing	Testing of animal derived components Cell bank characterisation Lot release testing Virus co-cultivation assays
Regulatory compliance	For example, ICH Q5A requirements (8) FPERT testing for vaccines (FDA) Bovine virus testing (for example, FDA 9CFR and CPMP bovine virus testing requirements)
Platform technologies	Generic virus clearance studies Selection of parental cell lines with improved virus safety profiles
Limited virus removal by the manufacturing process	Incorporating dedicated virus inactivation steps (**Note:** where no virus clearance is available, then the focus on demonstrating virus safety shifts to selection of appropriate start materials and testing programs)

The magnitude of the risk evaluation exercise will be determined primarily by the nature of the product. For well characterised biologics such as monoclonal antibodies and other recombinant proteins, the requirements are well established in regulatory guidance, and unless additional virus risks are introduced (for example, through the use of inappropriately controlled APIs) the main focus will be on ensuring regulatory compliance. For higher risk products however (such as human plasma derived proteins, gene therapy vectors, live virus vaccines), the risk evaluation procedure will undoubtedly be more extensive. The use of interdisciplinary teams, including virological experts experienced in the virus safety requirements for biologics, is recommended. Table 2 lists areas often evaluated during virus safety risk assessments, with some of the more important risk control measures.

PILLARS OF SAFETY

The safety tripod has for many years served as the foundational paradigm for virus safety risk assessments. In this model effective sourcing in process testing and effective virus clearance form the pillars upon which virus safety is built.

SOURCING – DEFINING THE BASELINE RISK

When evaluating the nature of the start material and the potential risks, consideration must be given to the following aspects:

The History of the Source Material

For well characterised cell lines this may seem a simple task, but even in this instance the distant history of the cell line may impact on the testing strategy. For example, cell lines that have at some point in their history been cultivated in equine serum are likely to require testing for potential equine contaminants. A thorough investigation of all aspects of collection, processing and storage of the start material should form an important component of the risk management strategy.

The Nature of Potential Virus Contaminants

The nature and likelihood of potential virus contaminants will determine to an extent what tests may be required. Consideration should be given to the following classes of virus:

- Those with potential to cause disease in humans
- Zoonotic viruses, such as Reovirus, Bovine polyomavirus (5)
- Viruses that replicate in or transform human cells but are not known to be zoonotic, such as Herpesviruses, Adenoviruses
- Viruses belonging to groups associated with severe oncogenic or immunosuppressive diseases in their natural hosts, such as retrovirus

In some cases, the geographical sourcing of source materials can also have an impact on the level of risk. For TSEs, a higher risk for BSE and vCJD exists within Europe, and the UK in particular. Certain viruses are also known to present a higher risk in certain geographical locations, and may ultimately impact on the level of attention needed in the risk evaluation.

New Zealand is often favoured as a sourcing region for bovine serum. One of the reasons for this is the geographical location and unique ecosystem that contributes to a lower virus risk. The ecosystem and history of New Zealand presents an exceptional environment which impacts on the potential for emerging infectious zoonoses (6). Geographically, the islands are one of the most isolated in the world, and, until recently, had allowed the development of a unique native fauna in the absence of natural predators. Until the first human incursions to the islands some 700 years ago, the only native mammals were two species of bats, and thus the development of parasitic arthropods capable of spreading disease matched the limited ecosystem dominated by such terrestrial fauna. New Zealand's native fauna does not include species which are in other countries recognised as hosts for many human pathogens, and the strict quarantine laws which have been in place for over 100 years minimise the risk of introducing new pathogens into the ecosystem (7). The integration of such arguments into the overall risk-based evaluation can help in steering risk-based quality decisions.

TESTING – REDUCING THE BASELINE RISK

Various testing strategies have been developed to address the numerous potential adventitious viral contaminants that may be encountered in biological start materials (4, 8-10). These include:

- *In vivo* tests for adventitious viruses
- *In vitro* tests for adventitious viruses
- Retrovirus specific tests (including infectivity tests and generic tests – for example, FPERT)
- Electron microscopic evaluation for virus like particles
- PCR based tests

The advantages and disadvantages of these tests and how they are applied to best

control risk is beyond the scope of this article, but the reader is referred to review articles which provide a more extensive discussion around their application (9,11,12).

Identifying unknown viruses still represents a significant challenge for virologists, and is the main reason why only a small percentage of those viruses circulating have been identified. The *in vitro* assay for detection of viruses uses a selection of cell lines with a proven history in the detection of a wide range of potential virus contaminants (10-12). It is used both in the testing and characterisation of recombinant cell banks, as well as in the testing of raw materials (for example, bovine serum (8,13)). However, no *in vitro* assay can provide guarantees for the detection of all potential contaminants, but rather the aim is to cover a broad spectrum. The term *in vitro* testing is applicable both to tests performed on production material, as well as the testing of raw materials. For example, guidelines have been established in both Europe and the US for the testing of bovine serum prior to use in manufacture (14,15).

One drawback to PCR-based tests for product release testing is the specificity of such assays. Instances exist, however, where PCR based tests have been shown to provide either more reliable data, or enable a more complete viral safety package to be submitted for a product. The *in vitro* and *in vivo* assays have limits of sensitivity that do not 100 per cent exclude the presence or absence of a contaminant. The recent detection of Cache valley virus (CVV – a Bunyavirus contaminant endemic in many countries (16)) – by PCR testing of bovine serum highlights the fact that general infectivity assays (which are performed on serum) have a defined limit of sensitivity that is determined by the nature of the assay and the volume of material tested. In this instance, CVV was present at such low levels that insufficient virus was present in the volume tested by the standard *in vitro* infectivity assay to result in a positive assay. However, the volume of bovine serum used in a standard 1,000 litre bioreactor represents a volume of serum several orders of magnitude greater than that tested for lot release of the serum and where the cell line is permissive for virus growth, such low level contaminants can quickly spread through a bioreactor. For CVV, PCR testing represents a more sensitive method for detection by virtue of the large particle to infectivity ratio, in the order of 100-10,000, that exists for most viruses. A PCR assay may therefore be two to four logs more sensitive than the corresponding infectivity assay, although the exact increase in sensitivity for PCR over infectivity can vary widely depending on the virus.

PATHOGEN CLEARANCE – CONTROLLING THE RESIDUAL RISK

The implementation of virus clearance into the manufacturing process provides a mechanism for controlling any residual risk remaining following effective sourcing and after testing has been implemented. How much clearance is necessary can only be answered by first understanding the nature of the product and the level of residual risk. It is almost certain that any manufacturing process that includes two or three dedicated and effective virus inactivation/removal steps is likely to meet even the most stringent of regulatory reviews. The extent to which a process will be viewed as having sufficient viral clearance will depend on the various strategies adopted during the design phase of the product. A product which completely excludes components of animal derived origin is likely to require less virus removal in order to pass regulatory scrutiny than a product that still uses components of animal origin. The nature of the animal-derived components and the potential virus contaminants may also have an impact on any assessment of how much virus clearance will be considered sufficient. Where animal-derived components are used, it is advisable to seek virological expert advice regarding potential concerns for virus contamination.

The question of how much clearance is sufficient is also dependent on the type of clearance steps incorporated. Steps such as solvent/detergent treatment for the inactivation of enveloped viruses have a long history and are well accepted (17). Other steps may not be viewed as providing a similar level of assurance. Chromatography steps or precipitation steps, for example, contribute to virus removal, but processes that rely solely on partitioning steps have a higher probability of virus transmission historically (18).

Dedicated virus clearance steps with an established history of effective virus removal should therefore be planned into the process to allay any virus safety concerns. The validation of new technologies for virus clearance will require more data in order to convince authorities of the robustness of the step.

Non-enveloped viruses tend to be more difficult to inactivate or remove than enveloped viruses (19,20). Non-enveloped viruses also tend to be smaller, making them more of a challenge for size based removal (such as by nanofiltration). Manufacturing processes that fail to provide for effective removal of non-enveloped viruses will inevitably receive more questions about measures for controlling risk from such viruses. It is therefore advisable to plan for at least two dedicated virus inactivation/removal steps, at least one of which should be effective against both enveloped and non-enveloped viruses.

UNDERSTANDING THE CONTRIBUTION TO RISK REDUCTION OF EACH OF THE PILLARS

Recent years have seen a shift in the paradigm for virus safety, away from a perception that each pillar of the safety tripod contributes equally to the overall virus safety of the product, towards the view that virus inactivation and/or removal may play a more important role in assuring the safety of the product (21). This paradigm shift has been most noticeable in the human plasma products industry, primarily because the actual contribution to risk reduction contributed by donor selection, donor screening and virus inactivation/removal can be mathematically modelled and calculated. Thus, it can be demonstrated that donor screening and donor testing each contribute in the order of a one to two log10 reduction in measurable risk for viruses such as HIV or HCV (21, 22). In contrast, the incorporation of two steps into the manufacturing process, each providing in the order of 5.0 log10 inactivation or removal, can provide a risk reduction in the order of 10 log10. Such data has resulted in greater scrutiny being placed on the manufacturing process, and ensuring that the design of the virus inactivation study and presentation of the data is such that the log reduction factors claimed for a manufacturing process can be relied upon.

Similar mathematical modelling to that presented above is more difficult for recombinant biopharmaceuticals, where the nature of the contaminant is not as clearly defined as in the case of human plasma derived products. However, it is clear that some similarities can be drawn. As discussed above for CVV, the *in vitro* and *in vivo* assays have limits of sensitivity that do not 100 per cent exclude the presence or absence of a contaminant. Recognising the limitations of *in vivo* and *in vitro* testing reinforces the importance of an effectively designed manufacturing process that takes into account potential virus contaminants and which incorporates steps effective for their removal. The reader is referred to several excellent reviews on established and emerging technologies for virus inactivation or removal (11, 23-26).

CONCLUSION

The establishment of a clear and transparent process for identifying and evaluating virus risk, and the subsequent implementation of risk control measures, can assist biopharmaceutical manufactures in anticipating and meeting regulatory requirements for virus safety. Understanding the relative contributions of sourcing, testing and virus clearance to risk reduction should form an important part of the risk evaluation procedure, and ultimately enable more scientific risk-based decisions for effectively controlling the risk. For products where little or no removal is afforded through virus inactivation or removal, then it is clear that the burden of demonstrating a virus safe product shifts towards the sourcing and testing pillars of the safety tripod.

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