A New Approach to Virus Safety

Andy Bailey at ViruSure GmbH provides an overview of the new CPMP guidelines on virus safety and investigational medicinal products

February 2009 saw the introduction of new regulatory guidance governing the virus safety of investigational medicinal biotechnology products (IMPs) (1). The development of this guideline has enabled a more pragmatic approach to ensuring virus safety of IMPs, seated firmly in the principles of risk-based management. The process of developing the guideline started in 2004 with the publication by the EMEA of a concept letter detailing the proposed scope of the new guideline. The need for such guidance stemmed from a lack of clear regulation regarding the point in development that full ICH Q5A compliant testing should be applied. The ICH Q5A guidelines are specifically applicable to products proceeding into marketing authorisation, but the requirements for products in clinical development have never been clearly specified (2).

Following extensive discussion at a joint European Medicines Agency (EMEA)/FDA/Parenteral Drug Association (PDA) forum on the virus safety of investigational products at the end of 2005, the first draft was published in June 2006. Subsequent consultation with industry resulted in refinements that led to the final guideline, published towards the end of 2008. The new guidance is a leading example of how industry and regulators have worked together to provide a workable solution to assure the virus safety of IMPs. The nature of this collaboration between industry and regulatory bodies is embodied in the companion document to this guidance, which takes the unique approach of publishing both industry's comments, as well as the regulators' responses as to how any concerns raised were addressed, enabling a more comprehensive understanding of the contents of the guidance (3).

This article provides an overview of the key components of this new guidance, and how the application of risk-based management was used to reach the final requirements for manufacturers when testing IMPs.

THE REGULATORY/INDUSTRY COLLABORATION

From the outset, the development of the new IMP guideline was intended to involve both industry and those at the CHMP Biotech Working Party (BWP), as well as other regulators (such as the FDA) in an iterative process to define the scope of the guideline and the final requirements. The initial PDA meeting in Langen, Germany, in December 2005 provided the platform for launching discussion between all interested parties, which led to publication of the first draft by the BWP in June 2006. As with all draft guidance, a process of comment and feedback with industry and industry organisations was initiated, but the desire of the BWP to involve industry was highlighted by two questions which the BWP specifically posed to manufacturers:

- Under what circumstances might it not be appropriate to test end of production (EOP) cells as recommended in the guideline?
- Under what circumstances might it not be appropriate to complete virus clearance studies prior to initiation of Phase III studies; what particular aspects of Q5A do not need to be addressed at this point in time, and in the opinion of industry, what minimum data would assure the viral safety of Phase III material?

The foundation that prompted these two questions had been laid down in discussions initiated during the Langen meeting. The responses from industry in relation to these two questions, and other aspects of the draft guidance were summarised by the BWP in the companion document to the IMP guideline (3). The impact of this open discussion with industry resulted in a final guideline significantly altered in relation to the two specific questions posed above, namely:

- The removal of the requirement for EOP cell bank testing for products in clinical development (while maintaining a requirement to test unprocessed bulks)
- A reduction in the requirement for full ICH Q5A compliant virus clearance studies prior to Phase III clinical studies

SCOPE OF THE IMP GUIDELINE

The scope of the IMP guideline is comparable to that defined for the ICH Q5A guidelines, namely:

- Monoclonal antibodies
- Recombinant DNA derived IMPs including recombinant subunit vaccines

Specifically excluded from the scope of the guidance are recombinant viruses or bacteria (either replication or non-replication competent), live attenuated or inactivated vaccines, IMPs derived from hybridoma cells grown *in vivo* and products from human or animal blood/tissues. Thus, the guideline is applicable to those products which have come to be described as 'well-characterised biologicals'. Those products excluded from the scope cannot be considered as well-characterised systems, and thus the complexity and difficulties in defining a path for ensuring the virus safety that would apply to all such products would have been an impossible task.

THE DEVELOPMENTAL NATURE OF IMPS AND VIRUS SAFETY

The developmental nature of products going into clinical trials presents a number of issues which hinder full application of testing according to ICH Q5A guidelines. Many of the parameters which are fixed by the marketing application stage are often not defined until later in the development process, including:

- Number of population doublings for the cells during routine manufacture
- The scale at which commercial manufacturing will be performed
- In process limits for manufacturing steps

The new IMP guideline for virus safety has taken these aspects into consideration, and where such parameters are not defined, alternative strategies which still provide for an adequate assurance of virus safety can be employed.

USING GENERIC DATA TO REDUCE VIRUS SAFETY TESTING

The use of published data to support virus safety may assist in gaining an understanding of the risks associated with a particular product, or in understanding parameters critical for virus removal, for example. But it is clear that each new IMP has its own unique risk profile, and thus the application of published data alone to support virus safety would entail a demonstration of comparability at a level of detail which is simply not feasible in practice. It is therefore not possible to escape the conclusion that each new MCB must be fully tested to ICH Q5A requirements in order to provide sufficient assurances of safety.

Within the IMP guidelines, generic data may be used to reduce virus safety testing where:

- A manufacturer has extensive experience with a particular cell line
- A manufacturer has extensive experience with a specific virus reduction procedure

EXPERIENCE WITH WELL-CHARACTERISED CELL LINES

Extensive experience with a particular cell line may assist in refining the risk assessment with regards to the anticipated risks with a new IMP. Various testing strategies have been developed over the years to address the numerous potential adventitious viral contaminants that may be encountered in biological start materials and significant experience has been gained over the years with the application of these tests and how they are best used to control risk (4, 6-12).

Rodent cells are used extensively to manufacture recombinant proteins for pharmaceutical use in humans and animals. The expression of endogenous retroviruses by all rodent cell lines requires that appropriate testing regimes for identification and characterisation are implemented. Expression of retrovirus demonstrates considerable variability, particularly when assayed by electron microscopy and reverse transcriptase assays (11). However, infectious retrovirus has only been reported in mouse myeloma and hybridoma cell lines, but never in cell lines of hamster (such as CHO) or rat origin (11).

Table 1 provides an overview of those tests required for unprocessed bulks (now used as a substitute for full EOP testing), and shows how the extent of testing for retroviruses and other adventitious agents increases with the degree of cell line characterisation. Thus, EOP cell bank testing is not required for well characterised IMPs produced in CHO cells or hybridomas until marketing authorisation. The requirement for continued testing of the bulks as shown in Table 1 provides assurances with regard to the absence of virus, without the need for full cell bank testing as defined in the ICH Q5A guidelines. As can be seen from Table 1, recognition is made of the extensive experience over the years with characterised cell lines such as CHO, where the absence of infectious retrovirus-like particles is well documented. Thus there is no requirement to test for retroviruses beyond the characterisation of retrovirus that is performed in association with testing of the MCB.

For cell lines used extensively in the past (for example, CHO, NS0, Sp2/0) the testing requirements are lower than for other cell lines where experience may be limited. This reduced programme is only applicable to IMPs produced in what can be considered well-characterised cell lines, meaning those defined in the ICH Q5A as 'Case A' (demonstrated to contain no viruses, virus-like particles or retrovirus-like particles) or 'Case B' (cell lines where only rodent retrovirus or non-pathogenic retrovirus-like particles are demonstrated to be present). The reduced testing requirements for CHO cells with respect to retroviruses in the unprocessed bulks is a clear example of how experience with a given cell line can result in a reduced testing programme. Manufacturers are required to evaluate any reduction in the

Table 1: IMP testing requirements for unprocessed bulks

	In vitro testing	Tests for infectious retroviruses	*In vivo* testing
CHO	Yes, all bulks	No	No
NS0 and Sp2/0	Yes, all bulks	Yes, once for given scale	No
All other cell lines	Yes, all bulks	Yes, once for given scale	Yes, once for given scale

testing programme based on prior experience on a case-by-case basis.

REDUCING VIRUS VALIDATION REQUIREMENTS FOR IMPS

One of the most significant clarifications in the new IMP guideline is to define where and how manufacturers can apply a reduced package of virus clearance studies. The question of the extent of validation for virus removal required for IMPs has never been clearly addressed in regulatory guidance. ICH Q5A defines the requirements for products proceeding to marketing authorisation, with validation using a full range of model viruses required. Validation requirements for products going into early phase clinical trials has usually followed the approach of validation with a retrovirus model, often together with a more robust model such as Mice minute virus (MMV). The approach of validating with these two viruses for early phase trials developed in response to guidance such as the FDA PTC document on monoclonal antibodies, where validation with only a retrovirus model is suggested (13). Later, potential concerns around MMV and the desire to demonstrate removal of a small robust model virus initiated a desire to ensure adequate removal with this type of model virus. However, the point at which manufacturers should switch from a limited model virus validation study to validation with a full panel of viruses was never addressed adequately in regulatory guidance.

Table 2: Virus validation requirements for products going into clinical trials or marketing authorisation

Validation requirement	Required for Phase I – Phase III?	Required for marketing authorisation?
Demonstration of sufficient retrovirus removal to ensure retrovirus sterility	Yes	Yes
Validation with retrovirus model and small robust virus (such as Parvovirus)	Yes	Yes
Validation with a full range of model viruses (usually at least four)	No*	Yes
Validation of dedicated virus removal steps	Yes	Yes
Validation of contributing virus removal steps (such as chromatography)	Possibly**	Yes
Re-validation where the manufacturing process changes	Yes	Yes
Evaluation of the robustness of virus removal	No***	Yes
Column re-use studies	No	Yes

*Once the process is finalised, full ICH Q5A compiant validation is expected

**Depending on the level of retrovirus clearance required in order to ensure sterility

***Studies to support clinical studies should be performed under worst case conditions where known

The early drafts of the IMP virus safety guidelines advocated the approach of a switch to validation with a full panel of model viruses prior to Phase III clinical studies, similar to FDA guidance (13). Following consultation and comments from industry, the final version of the IMP guidance advocates a more risk-based approach where the extent of validation should take into account factors such as:

- The nature of the cell line used for manufacture
- The use of raw materials of bovine or animal origin
- The potential levels of contamination
- The number and type of manufacturing steps with potential for virus clearance

The question of how much clearance is sufficient will be dependent on the type of clearance steps incorporated. Steps such as solvent/detergent treatment for the inactivation of enveloped viruses or virus filtration have a long history and are well accepted (14,15). Chromatography steps or precipitation steps are considered more as contributing steps, not necessarily dedicated to virus removal. Current standards dictate that the manufacturing process should implement virus clearance steps effective in the removal of both enveloped and non-enveloped viruses. Non-enveloped viruses tend to be more difficult to inactivate or remove than enveloped viruses (16,17). Non-enveloped viruses also tend to be smaller, making them more of a challenge for size-based removal (such as virus filtration). Manufacturing processes that fail to provide for the 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 and removal steps, at least one of which should be effective against both enveloped and non-enveloped viruses.

Thus, for products where no significant risks are introduced through the use of animal derived components, a validation of virus removal with only a retrovirus model and MMV should be sufficient for most products entering Phase III studies. The acceptability of this approach stems largely from past experience with virus validation studies investigating the potential removal of robust model viruses like MMV (or other parvovirus models). Parvoviruses are often referred to as a worst case challenge for virus removal (they are one of the smallest virus families and demonstrate a high resistance to inactivation procedures), and experience has shown that processes demonstrating robust removal of MMV are usually effective for the removal of other virus models or families. Thus the inclusion of a virus filtration step, effective for Parvovirus removal, will also demonstrate effective removal of all other viruses to which it is challenged. It should be noted that manufacturing processes which fail to include orthogonal dedicated effective virus removal steps are likely to be subject to more intense regulatory scrutiny, and may therefore precipitate the requirement for additional virus clearance studies with other model viruses in order to provide sufficient assurances of safety.

Table 2 provides an overview of the extent of validation for virus removal likely to be required for those products in clinical development as compared to the requirement for products proceeding through marketing authorisation (where full ICH Q5A compliant testing would be required). It may be possible to reduce the testing using platform process technologies.

PLATFORM PURIFICATION PROCESSES

Significant potential for reducing the extent of validation for virus clearance exists through the use of generic data generated with specific virus removal steps. The use of platform technologies for the production of different monoclonal antibodies, for example, enables the collection of a significant body of data for the

effectiveness of steps such as low pH, ion exchange or virus filtration. Such data can be used:

- To eliminate the need for validation of virus removal by a specific step for a new product. This approach requires extensive demonstration of comparability of any data with the new product
- To reduce the extent of robustness studies required for marketing authorisation
- To assist in defining worst case conditions for validation of virus clearance

In all instances, the manufacturer is required to provide extensive data to demonstrate why the application of such generic data is applicable to the new product.

THE VIRUS SAFETY RISK ASSESSMENT

In addition to the provision for submitting testing and clearance data for potential virus contaminants, manufacturers are required to submit a virus safety risk assessment providing a risk-based evaluation of the residual risk for the product. This assessment should include evaluation of:

- The nature and history of the cell line
- Extent of testing and characterisation of the cell line (cell banks)
- Use of raw materials of human or animal origin and any testing performed to control potential virus contamination
- Potential exposure of the product to other adventitious virus contamination
- Virus clearance data
- Any generic data from the manufacturer either in relation to:
  - Experience with a given cell line
  - Platform process technologies and generic virus clearance data
- Calculation of the estimated particles per dose (such as for retroviruses based on the retrovirus particle load in the bulk harvest as determined by electron microscopy or other methods)
- Published data supporting any argumentation
- Clinical indication (not a primary decision parameter)

The new IMP guideline on virus safety has therefore enabled a clear and transparent process for identifying and evaluating virus risk, and the subsequent implementation of risk control measures that assist biopharmaceutical manufacturers in anticipating and meeting regulatory requirements for products in clinical development. Such an approach can only be viewed as a positive development in the continued move towards risk-based management of biopharmaceutical products.

About the author

Andy Bailey has been actively involved in the pathogen safety of biopharmaceuticals for many years, first at Q-One Biotech (UK) as Director of Virus Validation, and later at Baxter (Vienna, Austria). Andy is the CEO of ViruSure (Austria), a company specialising in the virus and prion biosafety testing of biopharmaceutical products and has held this position since 2005. Andy has presented at numerous regulatory agencies in support of products and as an invited speaker at expert workshops, including the UK MHRA, German PEI, French AFFSAPS, US FDA, EMEA, JMHLW (Japan) and Korean FDA, and serves as an external expert for the EU for prion and virus safety related issues.
Email: andy_bailey@virusure.com

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