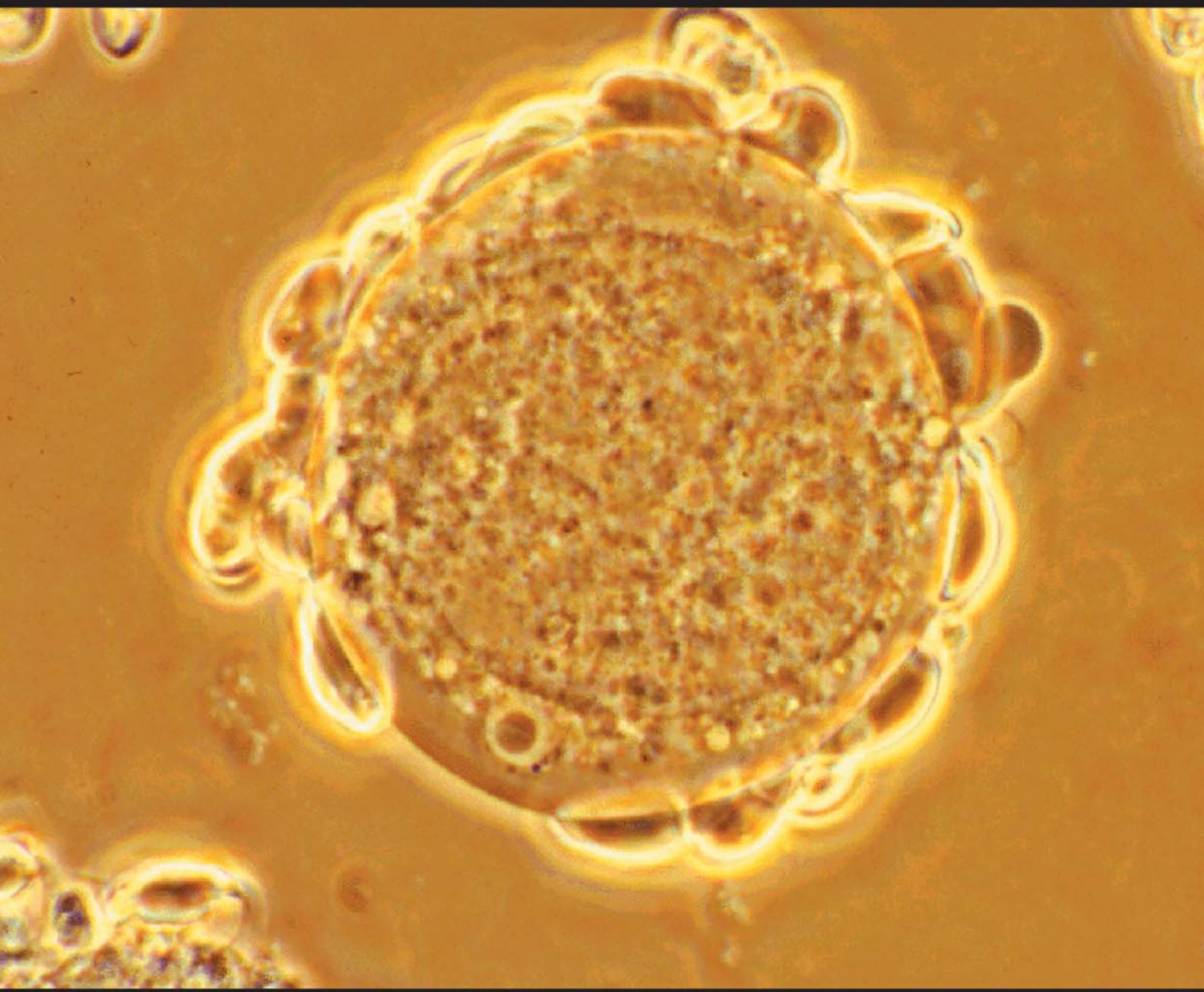
A Publication of
The Williamsburg BioProcessing Foundation

January/February 2003
ISSN 1538-8786

BioProcessing JOURNAL

Advances & Trends In Biological Product Development



Vol. 2/No. 1

www.bioprocessingjournal.com

Strategic Approaches to Viral Safety and Viral Clearance Assessment in Cell Culture-Derived Pharmaceutical Products

BY MARSHALL DINOWITZ

Viral safety and viral clearance evaluation are high-profile areas for product safety. Regulators are keenly focused on viral safety and expect high-quality data to support it, particularly for IND and BLA approvals. Familiarity with process and regulatory requirements, as well as expertise in the key areas of viral clearance, are essential for strategic planning and can yield savings in time, effort, and money.

Familiarity with regulatory guidelines for viral safety and viral clearance is essential. This knowledge provides an overview of regulatory expectations.[1,2,3,4] Experience in developing and implementing studies to support viral safety, as well as an understanding of the rationale behind the guidance, are of equal importance. To attain these objectives, companies must enlist individuals who have the expertise to meet regulatory expectations, and who also will avoid the common pitfalls that can occur during these studies. Some of the key principles and pitfalls of viral safety studies will now be presented.

Utilizing a team of individuals who understand the following components will aid in viral safety planing and execution:

- Manufacturing, including biochemical parameters, hold times, production timelines, and goals;
- Manufacturing improvement goals (short and long term);
- Regulatory expectations and rationale;
- Virology and the scientific study design appropriate for the process;
- Principles of viral clearance evaluation;
- Process and testing safety issues.

Viral safety is a cooperative effort consisting of team members from different departments, contract research organizations and, as necessary, consultants. To work effectively, communication between all team members is essential and should be encouraged.

Concern for effective viral safety

For several reasons, regulators have a high degree of concern for viral safety. Problems have occurred where viruses have been present in products used in human therapeutics (eg, HIV and various hepatitis viruses found in blood and blood products) and vaccines (SV40 virus and veterinary vaccines contaminated with live adventitious virus).[5] Furthermore, the cell culture process used for the production of many biotechnology products is ideal for the growth of adventitious viruses that may be inadvertently introduced via raw materials or personnel. Most other potential microbial contaminants are readily removed by standard microbial filtration, whereas many viruses typically are not removed under the standard conditions validated for microbial removal. The approach for assuring viral safety, therefore, is highly visible and scrutinized at all steps in development.

One well recognized, three-pronged approach for assuring viral safety uses multiple barriers to viral entry. This approach involves the testing of raw materials and cell banks for the presence of virus, lot-by-lot virus testing of cell culture harvests, and the establishment of viral clearance capability in the process. The first two of these three prongs are relatively well understood and guidance for them is well described elsewhere, including references to the U.S. CFR for specific virus testing requirements and study design.[1,2,3] The third prong, viral clearance, is the most problematic and complex.

Viral clearance evaluation studies are often inappropriately referred to as "validation" or "virus validation" studies. However, viral clearance assessment studies are not true validation studies because they do not actually validate the process for its ability to clear viruses. These studies only assess the ability of the process to reduce the level of the specific viruses used. These viruses are usually only surrogates for the many types of viruses that may potentially be encountered in production. It is more appropriate to refer to viral clearance studies as "clearance assessment" or "viral clearance evaluation."

Strategic approach

Strategies for viral safety, especially with regard to viral clearance studies, require an informed scientific approach. The approach should take

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into account the regulatory requirements as they progress through the phases of clinical testing as well as license application, or the requirements for amending an existing license. Viral clearance studies for licensure are more demanding than those for early clinical testing.[6] The planning and study design for viral clearance evaluation should take into consideration the expectations for safety at the initial stages of clinical studies, while accommodating aspects that will be needed to handle changes to the subsequent production process.[7]

Viral clearance expectations relative to clinical phase and target population

Testing expectations can be different from product to product, depending on such factors as proposed disease indication and number of patients in each phase of the trial. Products for which the intended population is normal, healthy individuals (such as a vaccine for an infectious disease), will have higher expectations for viral clearance than one for which the target population has a life-threatening disease for which no effective treatment is available. By the same token, it may be appropriate under some circumstances to exceed testing requirements for one phase of clinical development in order to ensure that the requirements for a later phase will be met. Testing requirements may also be exceeded to prepare for changes in process development. For example, Phase I clinical material may have been prepared by a process with a minimally acceptable endogenous retrovirus clearance, but a process change will be made before later clinical production. It is advantageous to determine whether the new process will meet the viral clearance requirements before the revised process is finalized.

Strategies in anticipation of manufacturing process changes

Cost-effective strategies can be implemented if material for Phase One clinical testing is produced using process steps that may be changed before later stage clinical lots are produced. For example, if a change in chromatographic resin, buffer, load or flow rate will be implemented for Phase Two material production, it may not be necessary to test the viral clearance capability of the original process step. This would be the case if a careful assessment determines that adequate retrovirus clearance for Phase One can be achieved without evaluating the process step that is anticipated to be changed.

Maximizing viral clearance evaluation reduction factors

It is also possible to design clearance studies so that each demonstrates the maximum level of clearance by selected process steps. This design becomes important when the overall purification process may have a low endogenous retrovirus clearance, and some process steps will not be tested because they are expected to change. In such a situation, enhanced viral clearance estimates can be achieved in several ways. One strategy is to use higher titer virus spikes than usual in evaluating a process step, while making sure not to exceed the capability of the clearance step. Another way is to use large volume viral assays that may demonstrate viral clearance 10 to 100-fold higher than the standard viral assay. In this approach, a larger volume of material is tested for the presence of virus than in standard assays. If no virus is detected following the viral clearance step, a higher effective clearance will have been demonstrated.

Pitfalls in strategic planning for viral safety

There are many pitfalls to viral safety testing and viral clearance studies. Following are some common problems:

- Improperly employing two or more viral clearance steps that use the same clearance mechanism. It is necessary to use orthogonal mechanisms for viral clearance to include each effective process step in the viral clearance total. This use can result in the untimely discovery that the production process may provide inadequate viral clearance, especially in studies with retroviruses where a targeted level of clearance is desired.

- Not assessing changes in endogenous retrovirus levels after alterations in culture methods. Changes in culture methods or media components can alter the clearance of virus in some cases. In addition, it is advisable to monitor the level of endogenous retrovirus particle levels when culture conditions are changed because these changes can alter the level of endogenous retrovirus present in the harvest. Monitoring is especially important when low levels of virus are detected in initial testing and a low target for clearance is set. If the altered culture conditions increase endogenous retrovirus particle levels, the clearance target may need to be raised.

- Inadequate controls as required by rigorous scientific design of the study, especially under product-related special circumstances. For example, controls are required for virus inactivation that may occur during storage before testing for virus remaining after a process step, or for toxicity to virus detector cells. Absence of such controls may lead to erroneous conclusions about the effectiveness of the process to clear virus.

- Using different process conditions, buffers, loads, or chromatographic parameters such as column height, or flow rates, in clearance studies than those in the actual production process. Assumptions are sometimes made that small differences in the foregoing parameters will not affect viral clearance. Unless these assumptions are tested, significant effects on viral clearance may be unrecognized. For example, slight changes in column buffer ionic strength can alter the ability to reduce virus. Slight changes in pH can alter retrovirus inactivation kinetics.

- Inadequate process control, such as flow rates, pH, or ionic strength. It is critical to demonstrate tight process control during the viral clearance studies so that critical parameters are within specifications. If flow rates, pH or other parameters do not meet specifications, viral clearance in these studies may not reflect what occurs in the actual process. Discovering such discrepancies when the IND or BLA is being finalized may

require expensive re-testing and/or delays in a filing. Not discovering them can be even more of a problem if they are found during the review by regulatory authorities.

■ Not ensuring that virus to be used in spiking experiments is prepared appropriately. Some viruses have a tendency to form aggregates. Such aggregated virus preparations may provide an exaggerated high filtration clearance rate because the filters may retain more aggregated virus than non-aggregated virus preparations.

■ Not re-assessing the process for viral clearance after process changes. Changes are frequently made to the production process during development to improve yields and purity, or for a variety of strategic reasons. All such changes should be assessed for their impact on viral clearance.[7]

■ Changes in regulatory expectations and requirements during development from Phase One through BLA submission.

How much viral clearance is sufficient?

No one answer to the question: "how much clearance is enough?" is appropriate for all products because several factors must be considered.

Because many cell lines used for production of biotechnology-derived products contain endogenous retroviruses, adequate clearance is expected for Phase One clinical testing under an IND. In general, clearance of retroviruses should be on the order of 10^4 to 10^6-fold greater than the potential retrovirus load that could be present in an unpurified harvest volume. As can be seen by the range, this target is not firm and is dependent on several factors. One often-overlooked consideration is that initially targeting a greater clearance than what may be expected can have a good strategic payoff. Subsequent process changes may result in reduction in viral clearance, and the extra initial clearance provides a buffer.

As mentioned previously, several factors influence the level of retroviral clearance expected under an IND. These factors include drug indication and target population, as well as the number of patients in the trial.

The level of retroviral clearance expected for Phase Three may increase if a small number of patients are used in the earlier phases. By the same token, whereas adequate clearance of endogenous retrovirus is usually the only viral clearance required under the IND, clearance assessment for other adventitious model viruses may be required if very large numbers of patients are used in Phase Three. If a significant increase in the number of patients is anticipated in the later clinical studies, it would be prudent to discuss viral clearance study plans with regulators before embarking on the plan.

No specific target for clearance of adventitious model viruses is specified in regulatory guidance documents. The goal of demonstrating clearance of these viruses is to assess how robust the process is for reducing potential adventitious viruses that could enter the process undetected. Therefore, the goal is to demonstrate that the process is capable of clearing, to some extent, a broad spectrum of viruses as represented by the three or four different types used. A process shown to be capable of removing several $\log_{10}$ of various types of viruses, especially small non-enveloped viruses, is considered robust enough to eliminate viruses that may have gone undetected during testing of the raw materials or cell harvests. In general, it is desirable to demonstrate some degree of clearance of each type of virus tested, including enveloped viruses and both large and small non-enveloped viruses.

Clearance studies for adventitious model viruses are usually not expected until the license application is submitted, or the pre-approval inspection (PAI) is scheduled and the purification process is fixed (except as noted above). However, it is wise to do adventitious viral clearance studies early enough so that there will be time to fix problems that may occur, or if inadequate clearance is observed.

Summary

Viral safety and viral clearance evaluation are high profile areas for the overall consideration of product safety. Regulatory authorities are keenly focused on viral safety and expect high quality data to support it. Familiarity with the process and regulatory requirements, as well as expertise in the key areas of viral clearance, are essential for good strategic planning and high quality data.

The examples provided in this article are not intended to be all-inclusive. They are used to demonstrate how an understanding of the requirements, and their underlying scientific rationale, can help avoid problems and improve viral safety. With attention to the key principles and potential pitfalls of viral clearance studies, strategic planning can often result in a significant savings in time, effort and money.

References

1. Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin. International conference on Harmonization Guidance Document (ICH Q5A). Available at www.ifpma.org/ich5q.html and Federal Register, 1997, Vol 63, No. 185, 51074.

2. Derivation and characterization of cell substrates used for production of biotechnological/biological products. . International conference on Harmonization Guidance Document (ICH Q5D). Available at www.ifpma.org/ich5q.html and Federal Register, 1997, Vol 62, No. 185, 24311.

3. FDA guidance document. Points to consider in the manufacture and testing of monoclonal antibody products for human use. Available at www.fda.gov/cber/guidelines.htm and Federal Register, February 1997, Vol 62, No. 40, 9196.

4. EMEA Guidance document. Virus validation studies: the design, contribution and interpretation of studies validating the inactivation and removal of viruses. Available at www.emea.eu.int/pdfs/human/bwp/026895en.pdf. March 1996.

5. Minor, PD. Mammalian cells and their contaminants, in Brown F, Lubiniecki, AS (Eds): Viral safety and evaluation of viral clearance from biopharmaceutical products. *Dev. Biol. Stand.*, Basel Karger,1996, Vol 88: 25-29.

6. Dinowitz, M., Sernatinger, J. and Wiebe, M. Regulatory expectation for data on viral testing and process evaluation for phase I studies and product licensing in the United States, Europe and Japan. *Dev. Biol. Stand.*, Basel Karger,1996, Vol 88: 148-151.

7. Dinowitz, M. Viral safety and clearance evaluation – implications for process change and comparability, in Brown, F, Lubiniecki AS, Murano, G (Eds): *Biologics 2000 – Comparability of Biotechnology Products. Dev. Biol.* Basel, Karger, 2002, vol109, 121-126.