BIOPHARMACEUTICAL PROCESS EVALUATED FOR VIRAL CLEARANCE

The purpose of Viral Clearance evaluation is to assess the capability of a manufacturing production process to inactivate and/or remove potential viral contaminants. Experience and knowledge in selecting the most relevant target viruses, appropriate purification steps, and viral clearing agents are critical in the design and implementation of a cost- effective and high-quality viral clearance study.

BACKGROUND

The advent of the biopharmaceutical age has opened new avenues of disease treatment and prevention. However, these biopharmaceutical products, such as monoclonal antibodies, recombinant proteins, vaccines, blood derivatives and animal products carry an inherent risk of transmitting infectious viruses due to the source material used, manufacturing processes, and routes of administration. Over the years, instances of viral contamination of biopharmaceutical products have occurred. In order to control viral contamination of biological products, the following complementary programs are now set up by bioprocessors as a standard:

- Selecting and testing the starting materials (cell banks, raw materials etc.)
- Testing of the product at various stages of production
- Incorporating viral inactivation and removal steps into the production processes and testing the manufacturing process for viral removal or inactivation capacity (i.e., viral clearance studies)

Commonly-used methods of detecting viruses include infectivity assay [50% tissue culture infectious dose (TCID50) and plaque assays], molecular probe methods [polymerase chain reaction (PCR), hybridization, Western blot etc.], electron microscopy, antibody production in animals, reverse transcriptase test and immunoassay for viral- specific proteins, etc. However, direct testing alone is not sufficient to ensure viral safety due to the inherent limitations in all virus detection methods. For example, unknown viruses, certain virus variants, and viruses that were thought not to be potentially pathogenic, cannot be detected. In addition, low concentrations of virus may escape detection due to the sensitivity limit of the assays.

Because of these testing limitations, an evaluation of the production process for its ability to inactivate and/or remove a wide variety of known or unknown contaminating viruses is necessary to ensure viral safety of biopharmaceutical products.

REGULATORY GUIDELINES

Regulatory expectations on viral safety for biopharmaceutical products have evolved over the past several decades. Original concerns focused on a relatively small number of known viruses associated with the production cell lines. Today, the concerns are much broader, encompassing unknown and uncharacterized agents. These increasingly stringent standards are intended to decrease the risk of transmitting viruses.

ICH Q5A, Viral Safety Evaluation of Biotechnology Products Derived from Cell lines of Human or Animal origin 1, (1997) specifically requires that a manufacturer of biological products for human use demonstrate the capability of the manufacturing process to remove or inactivate known contaminants.

The FDA's Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use 2 (1997) and Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals 3 (1993) elaborate similar principles as the ICH Q5A guideline and provide further description on viral safety evaluation methods. Although this document focuses on a specific group of biopharmaceutical products, it provides a good general guideline for bioprocessors to consult when evaluating the viral safety of their products in development.

The 2008 EMEA – CHMP guidelines on viral validation,, Guideline on Virus Safety Evaluation of Biotechnological Investigational Medicinal Products 4, 1996 EMEA - CPMP guidelines, Note for Guidance on Virus Validation Studies: The Design, Contribution and Interpretation of Studies Validating the Inactivation and Removal of Viruses 5,, and CPMP's Note for Guidance on Plasma Derived Medicinal Products 6 provide detailed recommendations for the manufacturers of biopharmaceutical products to follow when performing viral validations. These recommendations also set specific values for virus clearance levels that had to be attained.

With implementation of the EU Clinical Trials Directive 2001/20/EC 7, all EU Member States now require submission of an Investigational Medicinal Product Dossier (IMPD) starting at phase 1. The virus safety evaluation is part of the IMPD's quality requirement for biotech products. The International Standard ISO 22442-3: Medical devices utilizing animal tissues and their derivatives – Part 3: Validation of the elimination and/or inactivation of viruses and transmissible spongiform encephalopathy (TSE) agents 8 describes in detail the requirements on viral clearance for the medical devices.

All of these regulatory guidelines emphasize that each viral validation study should be reviewed on a case-by-case basis and that log reduction factors obtained should be viewed under experimental limitations and product-specific risk factors.

GENERAL STEPS OF A VIRAL CLEARANCE STUDY

Generally, a viral clearance study, from initial planning to final report issuance, is comprised of the following steps:

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- Risk assessment of the product regarding viral safety
- Selection of target viruses to be tested for viral clearance
- Selection of process steps to be evaluated for virus elimination
- Down-scaling the selected manufacturing steps
- Preliminary studies of cellular toxicity and viral interference
- Viral-spiking process runs
- Collection and titration of the process samples
- Data generation and calculation of viral clearance factors
- Issuance of the final reports

RISK ASSESSMENT OF PRODUCT

Assessing the viral safety risks associated with a specific product requires the consideration of several factors, including type of starting materials, reagents used in the purification, manufacturing process, phase of clinical development, product indication and specific patient population. This assessment is used to determine appropriate target testing viruses and the goals for the viral clearance study. As an example, for products derived from murine cell lines at IND submission stage or for phase 1 clinical trial, it is usually sufficient to examine only the clearance of murine retrovirus. However, a comprehensive panel of at least four viruses would be necessary before phase 2 and 3 materials are made.

SELECTION OF VIRUSES FOR THE STUDY

Based on viral safety risk assessment of the product, a panel of “relevant” and “model” viruses should be chosen for the viral clearance study. “Relevant” viruses refer to the viruses that have been identified as contaminants or potential contaminants of the starting or intermediate materials. When the potential viral contaminant cannot be readily propagated or assayed in the laboratory, a “model” virus, i.e., a virus with properties similar to the agent in question can be used. In addition, the model viruses represent a broad spectrum of different physicochemical properties of viruses, so that the safety of the product from contamination by adventitious viruses is ensured.

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Other biological and laboratory aspects of virus, such as ease of achieving high titer; availability of a sensitive and robust assay system; and safety of staff when using high-titered virus should be considered in the selection of viruses for use in viral clearance studies.

A manufacturing process for biopharmaceuticals should incorporate at least two distinct robust virus clearance steps, with at least one step effective on non-enveloped viruses. Robust steps are those able to clear a wide range of viruses and are not influenced by process variables (pH, protein concentrations, buffers, temperatures etc.)

Other considerations for selection of process steps for viral clearance evaluation include avoiding the selection of steps with the same mechanism of clearance and ease of scaling- down of the steps.

DOWN-SCALING THE MANUFACTURING STEPS

Scaling-down the process steps to be evaluated is a prerequisite to performing the actual spiking experiments, as it would be impractical to use the actual production scale for the viral clearance study due to the volumes of virus needed. Also, obviously, it would be inappropriate to introduce high-level infectious virus into the cGMP manufacturing facility.

In order for studies performed on a laboratory scale to be extrapolated to the manufacturing scale, the validity of the scale-down must be proven. For an inactivation step, the pH, protein concentration, buffer, and temperature must be considered. For a chromatography step, the resin, bed height, linear flow rate, contact time, buffer, pH, ion strength, protein concentration, elution profile, and temperature must be considered. The overall level of purification (product purity and yield) is critical for any process scale- down.

In addition to process scale-down, other issues such as column sanitization and reuse are important for a satisfactory viral clearance study. It needs to be demonstrated that when the chromatography columns are expected to be reused, the viral contaminants bound to the resin will be inactivated between production cycles and the viral elimination capacity will remain consistent throughout the life cycle of the columns.

PRELIMINARY STUDIES FOR CYTOTOXICITY AND VIRAL INTERFERENCE

In order to obtain accurate viral clearance data for a process step, the effect of the test samples (process intermediates) on the viral assaying system must be evaluated.

Generally, clearance factor could be underestimated if production components are cytotoxic or viral interfering.

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As most viral infectivity assays utilize mammalian cell lines as the host, the toxicity of the process materials on the host (indicator) cells is evaluated. Serial dilutions of the test sample are incubated with the indicator cells. Those dilutions that result in a change in cell morphology will be regarded as cytotoxic and excluded from the spiking experiment. The lowest dilution at which no cytotoxicity is observed is regarded as the non-cytotoxic dilution.

In addition to cytotoxicity, more subtle problems, such as samples interfering with the ability of the virus to establish infection in the host cells, may occur with the process samples. In many cases, samples that show no signs of cytotoxicity can show significant viral interference. Serial dilutions of the test sample are mixed with a range of virus dilutions and inoculated onto the host cells. The infectivity titer obtained from test sample treated titrations is compared to the titer from cell medium-made titrations. The lowest dilution at which no interference is observed is regarded as the non-interfering dilution.

The cytotoxicity and viral interference studies are typically performed prior to the spiking experiments. The results are used to determine the sample dilution ratio in order to remove any cytotoxic and viral interfering effect of the collected process samples.

PERFORMING VIRAL-SPIKED PROCESS RUNS

After the preliminary studies are completed, the viral spiking experiment can be performed on down-scaled process steps. The starting material for each step is spiked with high-titered virus stock and processed. The spiked load, process intermediates and product fraction (or post-treatment sample for inactivation studies) samples are collected, diluted with medium (to remove cytotoxicity and/or viral interference) and assayed.

Worst-case conditions are used when process parameters are variable in order to illustrate the extremes of the viral clearance capacity.

For virus inactivation studies, the kinetics of inactivation need to be examined by comparing the pre-, intermediate and post-treatment samples. For virus removal studies, the distribution of virus should be studied by assaying the process intermediates in addition to the spiked load materials and the product fraction.

COLLECTION AND TITRATION OF THE PROCESS SAMPLES

Upon collection and dilution (quench), the process samples are typically titrated immediately. If this is not possible and it is necessary to freeze samples before titration, a freeze/thaw virus control should be added.

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Serial dilutions of the process samples are inoculated onto host cells and incubated under appropriate conditions for a defined period of time. At the end of incubation, each well is scored for viral presence, typically by observation of viral-induced cytopathic effect (CPE). The virus titer of each sample is then calculated by methods such as the Spearman-Karber method.

Viral titers are normally expressed with 95% confidence limits. The accuracy and reliability of the viral assaying methods are emphasized by all regulatory guidelines. Accuracy, reproducibility, repeatability, linearity, limit of detection, and limit of quantitation need to be demonstrated by assay validation.

The limit of detection of the infectivity assay is directly related to the volume of inoculum. Therefore, when virus concentration is low, assaying a larger volume of a test sample increases the sensitivity of the assay and, in turn, may increase the viral reduction factor able to be demonstrated. The large volume sampling is particularly useful for achieving a good reduction factor when the test samples have a high level of cytotoxicity, as the input viral load is limited by the titer of the challenge virus stock.

CALCULATION OF VIRAL CLEARANCE FACTORS

The viral load of the spiked starting material is compared to that from the post-processing material to calculate the viral reduction factor for the process step. The titer of each sample is multiplied by the volume of the sample and any post-collection volume manipulation (such as dilution or pH neutralization). The 95% confidence interval for the reduction is calculated based on the 95% confidence limits for the input and output samples.

The log viral reduction factor for each process step can be added together to obtain the viral clearance value for the production process as a whole, however, it must be determined that the steps evaluated are independent, i.e., they utilize different mechanisms to eliminate the virus.

ISSUING THE FINAL REPORTS

After the viral clearance data is generated, Microbac's Microbiotest Division will issue an unaudited preliminary results summary to the client within 2-3 business days. Then the final report will be written and sent to the Quality Assurance unit for audit. The entire data package will accompany the final report.

When interpreting viral clearance results, emphasis is often given by regulatory groups to the robustness of the process steps rather than the actual clearance factor. CPMP guidelines require at least one robust step for recombinant murine products, and at least two robust steps for blood products 4-8. The ICH and FDA "Points to Consider" documents emphasize the robustness of the overall production procedure and require that for murine retrovirus, the level of clearance should be substantially in excess (³ 104-106) of the potential viral load 1,2,3.

After completion of the QA review, the final report will be issued to the sponsor.

VIRAL CLEARANCE SERVICES AT MICROBAC

Microbac is a contract microbiology and virology testing laboratory serving biopharmaceutical and consumer product companies in the United States and abroad. We are focused and specialized in viral clearance; and with a team of highly-experienced staff members, worldwide regulatory experience, exceptional client services and competitive pricing, we can help you design and execute high-quality and cost-effective viral clearance studies to meet your goals.

Authored by S. Steve Zhou, Ph.D. Microbac Laboratories, Inc., Microbiotest Division

For more information, please contact: microbac_info@microbac.com