The Complete e-Book of Biosafety Testing

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Comprehensive ***Biologic Raw Materials Testing,*** including development and validation for unique methods.

Mitigating Risk and Reducing Regulatory Scrutiny of Biologics Raw Materials

Andrew D. Schaefer and Terry Schuck

The emergence and significant clinical success of cell-based therapies and the multifaceted clinical utility of targeted antibodies have led the pharmaceutical industry to shift an increasing share of its focus toward the biologics market. As new therapies begin to receive approval from regulatory agencies, biomanufacturers must ensure product efficacy, process efficiency, and cost effectiveness. The high manufacturing costs of requisite bioprocesses and an ever-changing regulatory landscape have created new challenges in managing biologics raw materials

Ensuring product efficacy with process efficiency requires significant forethought and planning. The economies-of-scale benefits associated with conventional small-molecule manufacturing are not applicable. As a result, an obvious conclusion is to maximize efficiency by using manufactured prepackaged reagents. That has led to an ever-growing consumption of proprietary media and sera, recombinant enzymes, and trademarked resins needed as raw materials for a number of bioprocesses and purifications. Because of proprietary restrictions, manufacturers of such reagents can provide only redacted information about the composition of many of such complex mixtures. As cell therapies move toward phase 3 testing and marketed release, compliance risks associated with accepting a manufacturer's certificate of analysis for quality release might no longer be a relevant option.

With no established test procedures and very little compendia-based guidance, there is an obligation to establish suitable test methods for quality control (QC) release of such raw materials. Because information about the base composition of a proprietary formulation often is unknown to users, test methods must be developed to gain a better understanding of the attributes of the materials used in processes and establish quality controls around those attributes.

Eurofins Lancaster Laboratories is well versed in evaluating the identity, purity, potency, and quality of a diverse range of materials used in a wide array of bioprocesses. Platform methods can be used to establish a suite of QC tests in any phase of production quickly and cost effectively. Custom assays and residual methods can be developed to pinpoint critical material attributes that require monitoring. That combination of rapid-turnaround platform QC tests with robust custom methods enables clients to meet or preempt regulatory agency scrutiny promptly.

Our 20 years of biochemistry experience, dedicated teams of scientists, and state-of-the art instrumentation heighten our comprehensive laboratory capacity to proficiently advance projects of any size, phase, or complexity. Using established good manufacturing practice (GMP) platform methods as well as customized methods developed to fit unique client needs enables us to mitigate compliance and business risk. Eurofins Lancaster Laboratories understands the needs of the industry and is prepared and equipped to meet those needs.

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The Importance of Proper Cryopreservation of Cells for Cell Bank Preparation

Svetlana Mogilyanskiy

Quality of cryopreservation depends on many factors, with the main ones being the cell line itself, freeze media, the freeze profile used, and method of freezing selected. It also should be noted that sometimes change in the size of a bank (for example from a 20-vial bank to a 500-vial bank) can result in higher vial-to-vial variability due to temperature fluctuations, handling nuances, and differences in timing.

Certain cell lines freeze well in general, regardless of the point of a growth curve at which they freeze or the freeze media and freeze profile used. These cells have high viability when thawed (≥90%), with viability staying high through the period of recovery (first few days after cells are thawed). These cell lines usually have minimal vial-to-vial variability regardless of bank size. However, not all cell lines are created equal. If thawing of cells after cryopreservation results in poor viability and recovery, alternating freeze profiles sometimes can improve quality of a bank.

Controlled-rate freezers typically are used for producing cell banks in a good manufacturing practice (GMP) environment. Freezing cells is controlled by two probes: one to monitor temperature of a chamber in a controlled-rate freezer and one to monitor a sample (vial containing cell). A controlled-rate freezer usually comes with the manufacturer's recommended freeze profile, which typically works for most cells lines, but freeze parameters (speed and timing of each freezing phase) can be modified to improve cryopreservation for a particular cell line of interest. Sometimes simple freeze of 1 °C per minute ("Mr Frosty") provides better results than a sophisticated freeze profile with multiple cycles, including accommodation for the heat of fusion. Sometimes clients have a particular freeze profile they want us to use. We'll evaluate the profile doing a "mock freeze" before cell bank initiation. Sometimes we'll notice "red flags" that need to be discussed with the client. For example, we had a case in which a probe inserted into a vial showed that heat of fusion was bringing a sample dangerously close to 0 °C. We modified the freeze profile and successfully froze the bank.

It is recommended to run freezing studies that mimic preparation of a cell bank to evaluate whether viability and recovery will be as expected. If thawing cells results in poor viability, inadequate recovery, or significant variability between vials, the attempt to address the issue should be made.

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Need to Test Viral Products for Adventitious Viruses?

Katherine Marotte

Biopharmaceutical products for gene therapy applications are manufactured in living systems such as animal or human cells or tissues. As such, they are at risk of contamination from adventitious viruses (foreign viruses introduced into a product accidentally during a manufacturing process). Many viral assays used to detect adventitious viruses use live cell cultures, which often can present challenges in products that contain viruses. Eurofins Lancaster Laboratories, Inc. has experience working with those products and overcoming the difficulties that accompany them.

Oncolytic viruses are designed to kill tumor cells while sparing normal cells. They may be able to infect and kill the indicator cells used for assays. The virus may need to be neutralized with an antibody before testing for the presence of adventitious viruses, and achieving complete neutralization may be challenging depending on the concentration of the virus. Viral vectors for gene therapy are engineered to produce a therapeutic product using a viral particle that cannot replicate and produce new viruses. Most viral vectors exhibit some degree of toxicity in the indicator cells used in assays, so those products may need to be diluted before testing. Viral vectors also can generate a false-positive result in cell-based assays. The vector may be able to initiate an infection in certain cell types. Although the infection may not be productive, it can produce signs that can be mistaken for a true viral infection. Additional testing will be needed to determine whether the result is due to the vector or a true infection.

Eurofins has extensive expertise in handling challenging but novel viral products in cell-based viral assays. Please contact Eurofins for further information regarding your oncolytic or gene therapy product testing.

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Viral Clearance Studies

Doug Rea

Viral clearance studies are a necessary component of any regulatory submission for clinical trials or commercial product approval for a biopharmaceutical product. These studies are performed to evaluate the capability of a purification process to remove or inactivate viruses that could contaminate starting material. They are complex studies and require substantial financial and personnel resources to perform. Typically, they are performed at a contract testing company rather than in-house, which adds to their complexity. From both financial and timeline standpoints, conducting a viral clearance study right the first time is important.

Some fundamental questions must be asked when planning a study, including which steps of a process should be tested with which viruses and what the spiking percentage should be. However,

addressing additional questions early in a planning process also will ensure that there are no surprises during the execution of a study.

How soon do I need to begin planning? As early as possible! Decisions made during development of a purification process can affect the design and results of a viral clearance study. If you have been considering the needs for your viral clearance study all along, you will be in a good position when it is time to conduct it.

Who is responsible for what? Will you be performing the study, or will a contract laboratory be performing it? Who is performing the scale-down purification validation? If a contract laboratory will be performing either part, it will need detailed information about the process steps. The contract laboratory also will need time to develop a study design and prepare documentation.

What materials are needed? Samples and solutions often are pulled from a pilot or GMP purification that can occur months before a clearance study. Not only is it necessary to ensure that there is enough sample to load the unit operations to a target goal, but also you must ensure that the amounts collected are enough to provide sample volume adequate for the assay. You also must take into account viral assay sampling losses and sampling for toxicity and interference testing. In addition, it is prudent to collect extra samples for possible repeats.

What supplies will be needed? If your process needs special items such as filters or housings, don't assume that a testing laboratory has them. You must check whether that is the case.

What are the parameters of the scaled-down process? Discuss your process with the viral clearance laboratory, especially if the laboratory will be executing your process and even if your staff is going to the viral clearance laboratory to execute that process. To calculate clearance, the viral clearance laboratory will need to know volumes used and generated during the process. Often those volumes are obvious, but not always. That is true especially for inactivation steps: They may be simple to execute, but planning can be challenging.

If viral clearance laboratory staff will be executing some or all of the unit operations, remember that the staff has not lived with the process as you have. So details of specifications, limitations, and quirks must be communicated.

What is your schedule for performing the study? It is important to share your daily schedule with your testing laboratory. The viral plaque assays used for most clearance determinations require growing indicator cells, which must be used within a fairly narrow time frame. Knowing how long unit operations will take allows a sponsor and viral clearance laboratory to develop a schedule within the resources available.

Every company has its own jargon. Remember that just because doing something a certain way is standard for you and your company, not everyone understands your way to do it. Communicating details is important to success. Even when flexibility is acceptable, a viral clearance laboratory needs to know that. The more a viral clearance laboratory knows and understands about your process, the smoother your study will run.

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Next-Generation Sequencing Revolutionizes Adventitious Virus Detection

Jeri Ann Boose and Thomas Brefort

Adventitious virus detection is an integral part of safety testing of biopharmaceutical products. Viral detection methods currently considered as industry standards generally fall into two categories: *broad-spectrum screening assays* such as cell culture (in vitro), animal (in vivo) based testing, and transmission electron microscopy (TEM); or *target-specific assays* (typically quantitative polymerase chain reaction, qPCR). Although broad-spectrum screening assays can detect a wide range of viruses, some viruses can remain undetected because of their diverse viral physiology and limitations in assay sensitivity. Target-specific qPCR methods on the other hand often are highly sensitive, but they can detect only predefined/known targets. Thus, those approaches are used more often as tools to aid the investigation after a positive result has been identified during broad screening assays.

Next-generation sequencing (NGS) technology has revolutionized genome sciences. In recent years, NGS has been adopted to detect adventitious viruses. This technology allows deep sequencing of all nucleic acids in a given sample. When coupled with powerful bioinformatics, NGS can overcome existing practical problems and provide a universal platform for not only detecting, but also identifying viruses with high sensitivity. The biopharmaceutical industry continues to make ongoing efforts to optimize this assay as well as identify best practices to fully leverage the many advantages NGS can provide to adventitious virus testing.

To that end, Eurofins Lancaster Laboratories and Eurofins Genomics (Ebersberg) have partnered and performed several successful NGS studies in which contaminating viruses were identified quickly in biopharmaceutical samples of various matrices. With a turnaround time of preliminary results as quickly as within one week,

our findings have helped clients identify quickly the root cause of contamination and implement appropriate corrective and preventative actions. It is also interesting to note that in a few cases, NGS confirmed that putative positive results were not related to viral contamination, but rather to false-positive results because of the nature of the sample/sample matrix. That prevented clients from unnecessary and costly rejection of manufactured materials.

In addition to offering NGS testing services to our clients, Eurofins Lancaster Labs and Eurofins Genomics are active members of the Advanced Virus Detection Technologies Interest Group (AVDTIG), a task force coordinated by PDA and joined by regulatory and industry experts.

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Compendial Quality and Function Testing of Fetal Bovine Serum

Heather Beyer and Andrew D. Schaefer

Fetal bovine serum (FBS), as a byproduct of the cattle industry, is a preferred animal serum for cell culture proliferation. With an abundance of protein, growth factors, enzymes, and other chemical components as well as a characteristically low or absent concentration of interfering antibodies, FBS is ideal for promoting cell health and growth.

In an effort to provide guidance and standards for use, the European Pharmacopeia (EP) and US Pharmacopeia (USP) have released general quality attributes and functionality tests of FBS. The quality attributes tests provide standards to ensure consistency in protein content, hemoglobin level, pH, osmolality, IgG, species identification, and electrophoretic profile. The functionality testing outlined by the compendia can be performed with specified cell lines, comparing cell growth in test sera-supplemented media versus reference sera-supplemented media. However, in many cases, clients will favor establishment of their own user-defined functionality assays to ensure that test sera supports appropriate growth of their own cell lines.

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Eurofins Lancaster Laboratories, Inc. (ELLI) has established a broad array of physical, chemical, electrophoretic, and immunochemical techniques for determination of quality attributes of sera. With substantial experience and expertise in cell culture needs, ELLI can support serum functionality testing, including collaborative design and execution of user-defined testing. Eurofins Lancaster Laboratories' vast capacity and extensive capabilities can provide clients with rigorous quality attribute testing and critical functionality testing to ensure fast-growing healthy cell lines, making animal serum a practical quality controlled means for cell culture enhancement.

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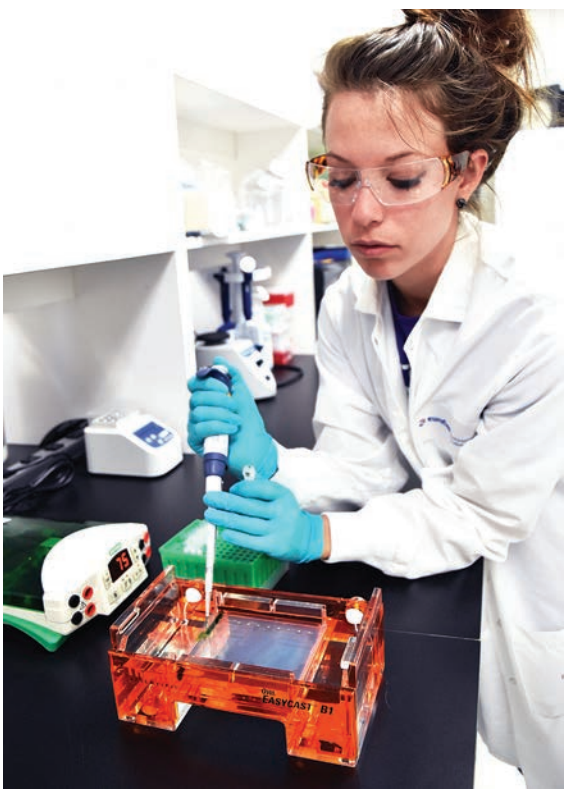
Genetic Stability Testing Ensures Product Integrity

Weihong Wang

Genetic stability testing is a key component of production cell bank characterization, and a regulatory requirement. Typical mammalian production cell lines are created by stable transfection of the expression vector into the host cell line. During subsequent cell culture, genomic events such as deletions, rearrangements and point mutations may occur and result in an altered cell phenotype and/or gene expression profile. The instability of the cell line is of great concern as it may negatively impact product integrity, posing a risk to patients. Even when product integrity is not immediately impacted, the possible reduction of productivity and the elevated risk of future events still raise concerns from an operational perspective.



Genetic stability testing includes an array of assays that are typically performed on a manufacturer's master cell banks (MCB) and representative lot(s) of end-of-production cells (EoPC). Genetic stability testing of the working cell banks (WCB) may also be performed at a manufacturer's discretion. Typical assays include, but are not limited to, those intended to confirm the integrity of the product transcript (mRNA/cDNA sequencing and Northern analysis), the genomic structure at the integration site (restriction digestion map by Southern analysis), and the ratio of insert gene copy number relative to host genome (via qPCR). Testing results from the EOPC and WCB are compared to those of the MCB to allow detection of any changes that may indicate cell line instability.

The Molecular Biology testing group at Eurofins Lancaster Laboratories has established generic methods of cDNA sequencing, restriction digestion mapping by Southern analysis, and gene copy number determination via qPCR. A generic method for transcript size determination via Northern analysis is currently under development and is expected to be available soon. These generic methods for genetic stability testing can be quickly adapted to each client's unique cell line/cell bank. Product-specific method validation can also be performed per client request to support CMC filings for later phase clinical trials and/or commercialization.

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