Whitepaper

Appropriate Methods When Developing Novel Biologics

Avomeen Life Sciences
800-930-5450
scientist@avomeen.com

AVOMEEN
LIFE SCIENCES

Appropriate Methods When Developing Novel Biologics

Biologics, especially protein therapeutics, are an intriguing and fast-growing market for biopharmaceutical companies of all sizes. Utilizing modern molecular biology techniques, proteins can be expressed and/or modified to have a certain targeted effect in the human body, such as binding an antigen or receptor, or inhibiting an enzyme, leading to novel approaches in disease diagnosis and treatment. Because biologic drugs act so precisely, they can be used to treat specific conditions without causing as many side effects as traditional small molecule drugs. Although the number of FDA-approved protein therapeutics is relatively small, several of these biologics are on the market effectively treating diseases ranging from cancer to autoimmune disorders.

Biopharmaceutical manufacturers who have identified candidates for novel protein therapeutics must be prepared to conduct a battery of chemical and biological tests to file a successful Biologics License Application (BLA). Biologics have complex structures and are more susceptible to variation during manufacturing than chemically-synthesized drugs, and even a slight process change can dramatically impact the safety, quality, and efficacy of the final product. Failing to accurately characterize a biologic, identify impurities, and study degradation pathways can lead to costly setbacks in the drug development process.

If your company is planning to make the leap into biologics, you should familiarize yourself with the essential analytical approaches you will need to ensure your products will be safe and effective in a clinical setting.

Analytical Approaches in Biologics

Below is a brief overview of a few approaches scientists commonly use when analyzing biologics. It is important to note that these methods are not typically used singly; researchers use orthogonal approaches to gather data from complementary methods, which then provide a full understanding of the product quality characteristics.

HPLC and UPLC

HPLC is a workhorse tool throughout the biopharmaceutical industry. For analysis of proteins, scientists often must employ variants of the more common HPLC approaches such as reverse-phase HPLC. These include:

- Size-exclusion chromatography – Molecules are separated and characterized by mass distribution.
- Ion-exchange – Molecules are separated based on charge.
- Hydrophobic interaction chromatography – Proteins are separated in a reverse salt gradient based upon their surface hydrophobic domains.

Newer UPLC (Ultra Performance Liquid Chromatography utilizing even smaller particles for higher efficiency) can be used in place of HPLC in many cases. It is faster and is now replacing traditional HPLC in many applications.

Electrophoresis

Electrophoresis is a technique used to separate macromolecules based on their size and electrical charge. Polyacrylamide gel electrophoresis (PAGE) is often used for the quantitative analysis of protein and nucleic acids. A newer approach, capillary electrophoresis, is often combined with tandem mass spectrometry to characterize a protein's primary structure and amino acid sequence. Capillary electrophoresis separations are typically based on size or charge, and are rapidly replacing PAGE and ion-exchange HPLC based methods,

due to their quantitation, automation and high throughput.

LC-MS

Liquid-chromatography mass-spectrometry (LC-MS) is another powerful technique used to separate and identify compounds from a mixture. Once compounds are separated by chromatography (typically RP-HPLC), they are ionized and their respective masses determined.

MALDI-TOF MS

Matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF MS) was developed to allow for MS analysis of large biomolecules. First, a sample is ionized, and its molecules become charged. The sample is then pulled into the mass analyzer section of the instrument, and the molecules are separated based on the time it takes them to pass through a time-of-flight tube, which correlates directly to their respective mass.

ELISA

Enzyme-linked immunosorbent assays (ELISAs) are used to detect highly specific regions of individual molecules allowing unique and sensitive quantitation of individual substances in a complex mixture. These assays use polystyrene plates to which antibodies or other proteins are bound, acting a targets for the species of interest. Non-bound materials can then be washed away, allowing for the highly specific measurement of the desired species.

Enzyme Assays

Enzyme biologics catalyze specific reactions to achieve their therapeutic or diagnostic effect, and in vitro enzyme assays are employed to measure the specific activity of the therapeutic or diagnostic protein species. Paired with techniques listed above, enzyme assays can be quite powerful, allowing for the determination the product's identity and purity as well as its potency.

Raw Material Testing

Chemical analyses for biologics should start with the raw materials used in the manufacturing process. All raw materials should be tested for identity, purity, and stability. Manufacturers must develop and validate GMP-compliant methods for quality control.

Residual Process Impurity Testing

Following a risk assessment, residual amounts of raw materials used in the process that could affect patient safety should be quantified and evaluated for clearance to safe levels during the downstream purification process.

Biologics Characterization

Characterization testing for biologics is an important step in developing protein therapeutics. This testing is also challenging because of the diverse materials used in bioprocesses and the complex structures of proteins. Scientists typically conduct a wide range of characterization assays, including tests for:

- Purity – Purity is defined by the FDA as "relative freedom from extraneous matter in the finished product, whether or not harmful to the recipient or deleterious to the product." Factors that can affect a product's purity include moisture, heat, external contaminants, and product and process related impurities the manufacturing process itself.
- Potency – For biologic products, testing for potency means confirming that the biological activity of the therapeutic is producing a defined result at a defined dosage. Researchers must

develop new potency assays for novel biotherapeutics. These methods are typically cell based assays, where receptor binding and subsequent signal transduction is demonstrated.

- Peptide Mapping – Peptide mapping is an identification technique used to characterize the amino acid structure of proteins. It is also used as a quality control measure for lot-to-lot consistency.
- Host Cell Protein Detection – Host cell proteins are process related impurities derived from the cell line generating the product that can co-purify with a product during the downstream process, potentially altering the activity and safety of the therapeutic. Of particular concern is the potential immunogenicity of these impurities. ELISAs are a common approach to quantify a broad range of respective residual host cell proteins.
- Glycosylation – Post translational modifications adding a carbohydrate to a target protein or lipid is the process of glycosylation. Some proteins will not undergo protein folding properly unless they are glycosylated. Glycosyation can also increase the stability of proteins through the linkage of carbohydrates to certain residues of the protein. Glycosylation affects not only their physicochemical properties and thermal stability, but also their circulating half-life and reactivity with their binding site.

The most extensive characterization testing usually takes place during the development phase of the therapeutic, but additional testing may be required if the manufacturing process changes.

In most cases, researchers will need to develop and validate new methods to characterize novel biologics. The International Council for Harmonization (ICH) has developed a set of guidelines (Q6B) that can be used as a starting point when designing a characterization method panel.

Impurities Characterization

Impurities testing is typically considered part of the characterization process, but it is broken out in its own section here to emphasize its importance in therapeutic development. It is essential for researchers to identify impurities because if they go undetected, they can interact with the therapeutic protein and have a negative effect on the stability, safety, and efficacy of the product.

Impurities typically come from one of two general sources:

Product Related Impurities

Product related impurities can come from many sources, either during fermentation or cell culture, or during downstream processing. For example, changes to the protein amino-acid sequence, or variant species that arise during processing that do not have the same potency as the biologic product (i.e. misfolded, aggregated, clipped, deamidated, etc).

Process Impurities

Process impurities are defined essentially by everything that comes into contact with the product. These may include buffers, chaotropes,

refolding agents, surfactants, or additives used to promote growth in cell cultures, as well as specific materials that are known to leach off during processing (i.e. protein-A). Researchers must develop methods to quantitate these impurities so that they can demonstrate clearance to regulatory agencies.

The other class of process related impurities is linked to the specific host cell expression system (i.e. *E. coli*, CHO, etc.). The two most common impurities to monitor are residual host cell protein (by ELISA) and host cell DNA (by qPCR). The expectation from a regulatory perspective is that these impurities are reduced to levels <100ppm.

It can be difficult to completely remove all impurities from a biologic, but manufacturers are responsible for ensuring that the level of impurities in the final product remains safe and consistent. If manufacturers determine there is an unsafe amount of impurities, they must implement purification steps to their development process to reduce those impurities to an acceptable level.

Stability Testing

Stability testing involves evaluating a biologic over an extended period of time to ensure its quality is maintained under different storage conditions. It is a regulatory requirement that helps determine the product's shelf life.

It typically takes several years to carry out stability tests, so manufacturers usually start planning for this testing as soon as possible, once the process development is complete. Due to the long-term storage requirements, manufacturers often outsource their stability testing to independent laboratories that can keep the biologic samples in controlled storage chambers and then pull samples and analyze them over the course of the stability program.

In addition to real-time stability testing, pharmaceutical manufacturers will also conduct accelerated stability testing, which exposes samples to temperatures than their normal storage conditions in order to speed up their degradation and determine their point of failure. Besides being a regulatory requirement, accelerated stability testing can sometimes help manufacturers predict shelf life and identify degradation pathways early in the development process.

Extractable and Leachable (E&L) Studies

Extractable and leachable (E&L) testing is used to determine whether any organic or inorganic compounds could potentially leach into a drug substance or drug product from an outside source, such as the product's administration device, packaging, or manufacturing equipment, including the final container closure. Because these compounds can affect the way a drug product works, E&L studies are required, particularly for parenteral products..

The FDA and Product Quality Research Institute (PQRI) have developed guidelines for a proactive, risk-based approach to extractables and leachables testing. In addition, the United States Pharmaceopeia has published Monographs on the conduct of E&L studies. Pharmaceutical manufacturers need to conduct E&L studies on any packaging or manufacturing equipment that comes into contact with the biologic. This will help manage the risk associated with compounds potentially leaching into the biologic product and compromising its safety or alter its efficacy.

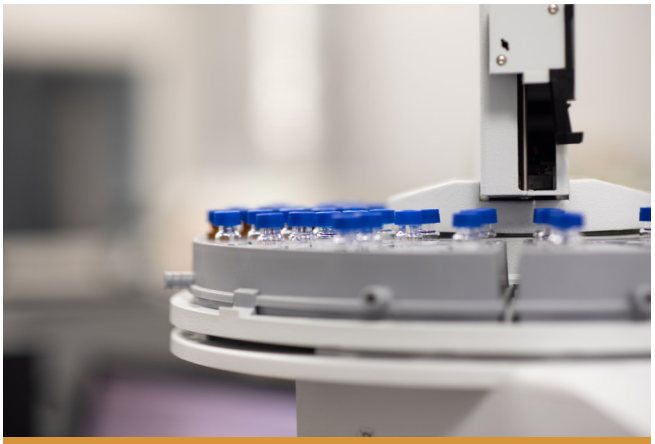
Extraction testing is the first part of E&L studies and is used to illustrate a "worst-case scenario." The goal is to identify all possible species that could contaminate the biologic under appropriately defined conditions. Researchers develop from extraction studies to help

them develop analytical methods for leachable studies.

Leachable testing generally takes place in the context of in-process hold or final product stability conditions, since the goal is to identify leachants that could contaminate a biologic under situations of normal use. Researchers will compare chromatograms from leachables studies to those from extractables studies. The chromatograms should show the same peaks, but the peaks should be smaller for the leachables studies due to the realistic storage conditions. Researchers should not see any new peaks in a leachables chromatogram that were not in a corresponding extractables chromatogram.

Working with an Independent Analytical Lab

Biologics take years to develop and approve for use in clinical settings. If your biopharmaceutical or diagnostics company decides to expand into the development of protein-based products, you cannot afford any unnecessary delays. One way to ensure your biologics development stays on track is to work with a trusted independent lab that has the equipment and skilled chemists necessary to carry out your biologics analyses.



Avomeen Life Sciences

Contact Avomeen to learn how our scientists can develop customized testing solutions for your biologics challenges. As a full-service, GMP-compliant testing laboratory, we can develop and validate new testing methods for your novel biologics and conduct all assays required by the FDA and other regulatory agencies.

Call us at 800-930-5450 or email scientist@avomeen.com to request a biologics analysis consultation.