Analytical Services

Guide to Analytical Testing of Biopharmaceuticals

BioReliance® by SAFC

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Contents

Introduction

The development of a biopharmaceutical product is a costly, complex, and exacting endeavor. Purity, safety, and efficacy must be monitored and confirmed on a continuous basis in order to justify the continuation of the product development process and to meet the requirements of governmental regulatory agencies that must approve and license the product for distribution within its target market.

BioReliance continues to be a key resource for leaders in the biopharmaceutical industry on the basis of our:

- Sixty-year tradition of customer service and technological excellence
- Client-centered team of experienced, knowledgeable, and responsive professionals
- Modern analytical and biosafety testing facilities on two continents
- Corporate culture that welcomes the opportunity to enable our clients to meet the challenges of an ever-changing regulatory and commercial landscape worldwide

BioReliance's Analytical Service Capabilities

BioReliance's analytical teams, located in Stirling, Scotland and Rockville, Maryland are prepared to support you in your efforts to bring innovative and beneficial biopharmaceutical products to market. Our laboratories are equipped with a range of validated analytical instrumentation, all installed and maintained in compliance with current Good Manufacturing Practice (cGMP) and Good Laboratory Practice (GLP) requirements. As a global, full-service biosafety testing company, BioReliance has been providing analytical support to biopharmaceutical and pharmaceutical clients for over a decade. Our analytical chemistry staff has considerable experience with a wide variety of products and lot release, stability and characterization services (see Table 1).

General areas of expertise include:

- Method development, validation, and transfer
- Reference standard characterization
- Comparability and lot release testing
- Testing of product stability with respect to normal storage conditions, purposeful degradation and exposure to light

Table 1: Analytical Methods Available from BioReliance

Chromatographic & Electrophoretic Analysis:
Affinity chromatography
Capillary electrophoresis
High-performance liquid chromatography (HPLC)
Hydrophobic interaction chromatography (HIC)
Ion exchange chromatography (IEX)
Isoelectric focusing (IEF) analysis
Reverse-phase chromatography (RPC)
Sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) electrophoresis
Size exclusion chromatography (SEC)
Western blot analysis

Biological and Toxicological Analysis:
Abnormal toxicity
Biological activity/potency measurement (*in vivo* and *in vitro*)
Endotoxin testing
Extractable volume measurement
Host cell DNA analysis
Host cell protein analysis
Infectivity titer determination
Phenol red content analysis
Polymerase chain reaction (PCR) analysis of DNA contaminants
Polysorbate 80 analysis
Process component contaminant analysis
Restriction enzyme DNA mapping
Residual solvent content analysis
Sterility testing
Trace analysis

Physicochemical Characterization:
Amino acid analysis
Carbohydrate mapping
Deamidation analysis
Extinction coefficient determination
Hydrogen ion concentration (pH)
Mass spectrometry (MS) +
Moisture analysis of lyophilized material (Karl Fischer method)
Monosaccharide analysis
N-terminal amino acid sequencing
Osmolarity measurement
Peptide mapping
Physical appearance
Protein concentration determination using ultraviolet (UV) or bicinchoninic acid (BCA) methodology
Reconstitution time

Particulate Analysis:
Circular dichroism analysis
Fourier-transform infrared (FTIR) analysis
Light scattering analysis - conventional
Light scattering - SEC
Scanning densitometry
Size exclusion chromatography/multiangle laser light scattering (SEC- MALLS) particulate analysis +
UV scanning analysis

+Testing performed by a BioReliance-affiliated analytical laboratory.

Project Design and Analytical Support Teams

BioReliance's analytical chemistry staff will work closely with you to design a customized testing program for your product according to its stage of development (see Tables 2 – 7). We can develop new methods which are transferred to you, or conversely, you can transfer your established testing methods to BioReliance for deployment in our laboratories. In either case, detailed assay protocols and related information need to be provided, with the recipient laboratory performing validated procedures with respect to precision, accuracy, linearity, specificity, sensitivity and robustness. Method transfers and validations are documented with a final report.

To ensure the success of your project, BioReliance's analytical staff is supported by many others, including personnel from our program management, quality assurance and regulatory affairs teams.

Regulatory Affairs

An in-depth understanding of regulatory requirements for different countries and product types is crucial in preparing a submission for regulatory authorities. Our regulatory affairs team keeps up-to-date with current regulations and can advise you on regulatory strategies related to analytical testing methods for development and licensing of your product anywhere in the world.

Quality Assurance

BioReliance's quality assurance team is an integral part of analytical testing, ensuring that all of our studies are carried out in accordance with regulatory guidelines, by inspecting our laboratory systems, auditing raw data, and approving final reports. All of our documents are generated, monitored, and reviewed according to our document control procedures, which include (a) Installation Qualification (IQ), Operational Qualification (OQ), and Performance Qualification (PQ) packages for instruments, and (b) Standard Operating Procedures (SOPs) for the operation and maintenance of instruments; the receipt of samples; management of chain of custody; stability programs related to testing chambers, allocations, pull points; and for training and health and safety activities.

Our quality assurance procedures are controlled by BRIQS (BioReliance Integrated Quality System), BioReliance's customized and globally-deployed quality system that helps us manage, track, and detect trends in deviations, corrective and preventive actions (CAPAs), audits, regulatory affairs commitments and compliance, and other critical regulatory needs.

Program Management

Your primary point of contact during analytical testing is your BioReliance Project Manager, who will keep you informed about the receipt of samples, project testing completion dates, and provide results of analyses, and other important information about your project from start to finish. In addition, Rockville-based analytical studies are supported by a secure internet-based system that allows you to track the status of your samples in real-time and be advised of testing completion dates.

Analytical Testing According to Clinical Stage of Development

The type of analytical tests performed on a product is dictated by its stage of clinical development (Table 2). Prior to entering clinical trials, methods are developed, transferred, and the product is characterized. Proper development of these tests are critical, as many of the characterization methods used during preclinical trials become part of the lot release and stability testing regimen.

Phase I Clinical Trials

By Phase I clinical trials, it is determined which analytical methods are acceptable for testing of a product's identity, purity, potency, concentration, inter-batch consistency, and stability. In addition, the justification for releasing product production lots for use in clinical trials is established, and a reference standard is developed that will be used to verify the drug product's integrity. Validation of methods typically begins in Phase I and continues during Phase II clinical trials.

Phase II Clinical Trials

Product formulation development, in which solutions with various physical properties and chemical components are developed and tested in varying concentrations and in the presence of degradation factors (e.g. heat, light, humidity, agitation) to assess stability and solubility, normally occurs during Phase II clinical trials. For lyophilized products, a lyophilization profiling study is used to develop optimal conditions for freezing and sublimation. Parallel to formulation development are refinements or development of methods to analyze the formulated product.

Phase III Clinical Trials and Commercial Product Testing

By Phase III and beyond, full method validation has occurred to verify that the methods selected will perform as expected under routine laboratory conditions used for lot release and stability testing. Ongoing stability and lot release testing continues to ensure product safety and efficacy. Comparability studies are required if any changes are made to manufacturing processes.

Table 2: Analytical Testing During Clinical Development

	Preclinical Trials	Phase I Clinical Trials	Phase II Clinical Trials	Phase III Clinical Trials	Commercial Product Licensing
Product characterization	■	■	■		
Reference standard characterization		■	■		
Method validation according to ICH guidelines		■	■		
Batch assessment of product consistency		■	■	■	
Lot release testing		■	■	■	
Stability testing		■	■	■	
Validation of stability indicating methods			■		
Assessment of stability under conditions of forced degradation			■	■	
Re-validation of methods according to ICH guidelines					■
Assessment of comparability before and after manufacturing process changes					■

Product Stability Testing

Stability testing demonstrates how the quality of a drug substance or drug product varies with time under the influence of a variety of different environmental factors, such as temperature, humidity, light and container/closure interactions. Data derived from a stability study is used to recommend storage conditions, re-test intervals and shelf lives.

Stability protocols describe the product to be tested, the sampling process, the duration of the study, the number of samples required, replicates per time interval, storage conditions and methods of analysis. A stability indicating profile should be selected that assures that changes in the identity, purity and potency of the product will be detected. Assay methods need to be sufficiently specific to differentiate between the analyte and possible degradation products. The stability studies should be conducted on material that is representative of the quality of material to be made on a commercial scale, and stored in a container closure system identical or similar to the packaging proposed for storage and distribution. For multi-dose products, it may be necessary to test the stability of reconstituted material.

Stability Testing Frequency

When a shelf life of one year or less is established for a product, real-time stability studies need to be conducted monthly for the first three months and at three-month intervals thereafter. For a product with a shelf life greater than one year, such studies should be conducted every three months during the first year of storage, every six months during the second year, and on a yearly basis thereafter, until the product's expiration date.

Stability Storage Conditions

A regulatory-compliant stability program may require the testing of several storage conditions, such as variations in temperature or humidity (see Table 3). This is typical for biological products, as their sensitivity to temperature changes usually requires precise storage temperatures.

Table 3: A Typical Stability Testing Protocol for a Recombinant Protein Product

Timepoints (months)	Ambient	-20° C	5° C	25° C, 60% Relative Humidity	40° C, 75% Relative Humidity
0	A- F	A-E	A-E	A-E	A-E
1		A-E	A-E	A-E	A-E
2		A-E	A-E	A-E	A-E
3		A-E	A-E	A-E	A-E
6		A-E	A-E	A-E	A-E
9		A-E	A-E	A-E	A-E
12		A-F	A-F	A-F	
18		A-E	A-E		
24		A-F	A-F		
36		A-F	A-F		

A = Appearance, pH, protein concentration, particulate testing
B = Electrophoretic analysis
C = HPLC analysis
D = Peptide mapping
E = Functional assay, such as ELISA or bioassay
F = Bioburden, sterility, potency testing

Accelerated Degradation Studies

Stress testing determines the intrinsic stability of a drug product by identifying degradation pathways, degradation products and validates analytical procedures with respect to their ability to assess product stability. Stress testing represents severe conditions that may be encountered during product distribution. These studies should address the effects of temperatures in 10° C increments above the typical storage conditions and, where appropriate, humidity, oxidation, photolysis, and hydrolysis across a wide range of pH values.

Summary Tables of Recommended Analytical Tests

The following tables list suggested analytical methods depending upon the type of product. These lists may not be exhaustive, as particular characteristics of a product might dictate the requirement of additional assays.

Table 4: Analytical Testing of Recombinant Protein-, Antibody-, and Blood-Based Products

Type of Test	Analysis	Product Characterization	Lot Release	Stability Testing
Abnormal toxicity (general safety)	Safety	●	●	
Appearance	Sample quality, integrity	●	●	●
Biological activity/potency	Sample quality, integrity	●	●	●
Carbohydrate mapping	Identity, sample integrity	●		
Colorimetric assay (BCA, Bradford)	Protein concentration	●	●	●
Contaminants from process (e.g. antibiotics, serum components)	Purity	●	●	
Endotoxin	Purity	●	●	
Extractable volume	Sample quality		●	
Host cell DNA (quantity and size)	Purity	●	●	
Host cell protein (quantity)	Purity	●	●	
HPLC	Identity, purity, sample integrity	●	●	●
Karl Fischer titration (lyophilized samples)	Moisture content, Sample integrity	●	●	●
Monosaccharide analysis	Identity, sample integrity	●	●	
Osmolality	Sample integrity	●	●	●
Peptide mapping	Identity, sample integrity	●	●	●
pH (liquid samples)	Sample quality	●	●	●
SDS PAGE	Identity, purity, sample integrity	●	●	●
Sterility	Purity	●	●	●
Ultraviolet absorbance measurement	Protein concentration	●	●	●
Western blot	Identity, sample integrity	●	●	●

Table 5: Analytical Testing of Synthetic Peptide-Based Products

Type of Test	Analysis	Product Characterization	Lot Release	Stability Testing
Abnormal toxicity (general safety)	Safety	●	●	
Appearance	Sample quality, integrity	●	●	●
Biological activity/potency	Sample quality, integrity	●	●	●
Capillary electrophoresis	Identity, sample integrity	●	●	●
Colorimetric assay (BCA, Bradford)	Protein concentration	●	●	●
Endotoxin	Purity	●	●	
Extractable volume	Sample quality		●	
HPLC	Identity, purity, sample integrity	●	●	●
Karl Fischer titration (lyophilized samples)	Moisture content, Sample integrity	●	●	●
Mass spectroscopy	Identity, sample integrity	●	●	●
Osmolality	Sample integrity	●	●	●
pH (liquid samples)	Sample quality	●	●	●
Sterility	Purity	●	●	●
Ultraviolet absorbance measurement	Protein concentration	●	●	●

Table 6: Analytical Testing of Viral Vaccine Products

Type of Test	Analysis	Product Characterization	Lot Release	Stability Testing
Appearance	Sample quality, integrity	●	●	●
Colorimetric assay (BCA, Bradford)	Protein concentration	●	●	●
Endotoxin	Purity	●	●	
Extractable volume	Sample quality		●	
Host cell DNA (quantity and size)	Purity	●	●	
Host cell protein (quantity)	Purity	●	●	
HPLC (cation or anion exchange)+*	Identity, particle integrity, concentration	●	●	
HPLC (RPC protein map)+	Identity, purity, sample integrity	●	●	
Infectivity titer*	Biological activity, concentration	●	●	●
Light scattering	Identity, purity, sample integrity	●	●	
Osmolality	Sample integrity	●	●	●
PCR with number of primer pairs*	Identification	●	●	
pH (liquid samples)	Sample quality	●	●	●
Process contaminants (e.g. BSA, antibiotics)	Purity	●	●	
Restriction enzyme mapping*	Genetic integrity	●	●	
SDS PAGE	Identity, purity, sample integrity	●		
Sterility	Purity	●	●	●
Western blot	Identity, sample integrity	●		

+ Method is dependent upon vector

* Only applicable to intact viral vectors

Table 7: Analytical Testing of Gene Therapy Viral Vectors

Type of Test	Analysis	Product Characterization	Lot Release	Stability Testing
Appearance	Sample quality, integrity	●	●	●
Colorimetric assay (BCA, Bradford)	Protein concentration	●	●	●
Endotoxin	Purity	●	●	
Extractable volume	Sample quality		●	
Host cell DNA (quantity and size)	Purity	●	●	
Host cell protein (quantity)	Purity	●	●	
HPLC	Identity, particle integrity	●	●	
Infectivity titer*	Biological activity, concentration	●	●	●
Light scattering	Identity, purity, sample integrity	●		
PCR with number of primer pairs*	Identification	●	●	
pH (liquid samples)	Sample quality	●	●	●
Process contaminants (e.g. BSA, antibiotics)	Purity	●	●	
Restriction enzyme mapping*	Genetic integrity	●	●	
SDS PAGE	Identity, purity, sample integrity	●		
Sterility	Purity	●	●	●
Western blot	Identity, sample integrity	●		

* Only applicable to intact vectors

Regulatory references related to analytical testing methods

1. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), Quality Guidelines, www.ich.org
2. Code of Federal Regulations, Title 21, www.access.gpo.gov/cgi-bin/cfrassemble.cgi?title=200521
3. U. S. Pharmacopeia, www.usp.org
4. The European Medicines Agency (EMEA), www.emea.eurpora.eu

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