Analytical Methods for Biologics

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The Scientific Backbone of Biomanufacturing

by Cheryl Scott

Biomolecular analysis falls into three categories: chemical/biochemical, immunological, and biological assays. Biochemical/chemical assays account for between two-thirds and three-fourths of those performed in product characterization, and they can take 400–800 person-hours to develop and validate. Bioassays require more time and make up about 15% of the total number performed. Overall assay development and validation can cost around $1.5 million — about $150,000 for three immunological assays, around a million for maybe a dozen chemical/biochemical assays, and nearly half a million for three bioassays.

Production process development incorporates a large amount of analytical laboratory work, from cell line engineering and characterization to the formulation of culture media. Typical downstream process characterization and/or validation studies might measure membrane and resin lifetimes; in-process hold times, buffer hold times; protein load limits for columns; pH and conductivity specifications for buffers; extractables and leachables from resins and other product-contact surfaces; virus removal/inactivation; impurity removal; and small molecule clearance.

Preformulation efforts provide a scientific sketch of a drug in development. Later work fills in the details with more detailed characterization. A typical preformulation study examines the general structure and molecular weight of a molecule along with its solubility and conformation at various pH values; behavior at air–water interfaces and during freezing–thawing; compatibility with organic solvents; degradation pathways, absorbance spectra, melting point, hydrophobicity, isoelectric point, and general aggregation tendencies; and responses to time, light, temperature, and oxygen. Some of that information may come from analytical work done in product or process development.

Commonly used analytical methods include isoelectric focusing (for determining pI and changes in charge), electrophoresis and high-pressure liquid chromatography (for size, conformation, and aggregation states), biological assays (for functionality), ultrafiltration (for solubility), and differential scanning calorimetry (for melting temperature).

Other analytical methods that can come into play include polyacrylamide gel electrophoresis (PAGE) and ion-exchange chromatography (IEC) to study charge; circular dichroism, size-exclusion chromatography (SEC), capillary zone electrophoresis, and fluorescence spectroscopy to study molecular conformation; SEC, sodium-dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), laser-light scattering, capillary electrophoresis, and matrix-assisted laser-desorption ionization time-of-flight (MALDI-TOF) mass spectrometry to study molecular size; micellar electrokinetic chromatography and hydrophobic interaction and reversed-phase chromatographies (HIC and RPC) to study hydrophobicity; and cell-based, enzymatic, and enzyme-linked immunosorbant assays (ELISAs) to study biological activity.

As you'll see from the presentations in this session (both those profiled here and those remaining), analytical science is forever improving to offer new solutions to scientists working in the biotechnology field.

Mary E.M. Cromwell

Scientist and Senior Group Leader at Genentech (USA)

18 years in the industry

Assessing Aggregation: A Comparison of Techniques

During biotherapeutics development, there is a significant focus on the characterization of aggregates. Accurate assessment of both quantitative and

qualitative aspects is desired. However, the analytical methods used come with varying limitations that may affect observations. This presentation describes in a case study the relative strengths and weaknesses of several commonly used techniques for assessing aggregation.

Who will be most interested in the subject matter of your talk? Formulation scientists, analytical scientists, regulatory scientists, and directors

What do you expect them to "take away" with them? Attendees will gain an understanding of the advantages and limitations of several techniques used for assessing protein aggregates. Additionally, I plan to provide the audience with questions that they should be asking when presented with data and conclusions regarding aggregates.

Can you list the methods you'll be highlighting in your talk — in order from most to least expensive? Sodium-dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), size-exclusion chromatography (SEC), capillary electrophoresis nongel sieving (CE-SDS), multiangle light scattering (MALS), field-flow fractionation (FFF), dynamic light scattering (DLS), and analytical ultracentrifugation (AUC)

Which are most broadly applicable (e.g., for more than studying aggregation), and which are most specialized for this purpose? Each of these techniques may be used to more broadly analyze size heterogeneity (fragmentation and aggregation). The HPLC used for SEC and the CE used for CE-SDS may be used for separation based on other interactions. CE-SDS and SDS-PAGE are often used to analyze impurities unrelated to the product of interest.

When/why did you get involved in the industry? What interested you the most? I got involved in the biotechnology industry because it provided a meaningful application for my interest in the structural and functional relationships of proteins.

Michele Fiscella

Senior Scientist in the Clinical Immunoassay Department at Human Genome Sciences (USA)

~8 years at HGS

Qualification, Validation, and Execution of the Immunogenicity Assay for a Biopharmaceutical Product

Biopharmaceutical products can induce immune responses leading to clinical consequences, which vary from loss of efficacy to serious adverse events. Therefore, it is important to develop immunogenicity assays that can provide accurate assessments of antibody responses. In this presentation we provide an example of the life cycle of an immunogenicity assay for an HGS product.

Who will be most interested in the subject matter of your talk? Bioanalytical scientists

What do you expect them to "take away" with them? General concepts of immunogenicity assays; how to design and execute assay qualification/validation; and how to implement the assay in the real life scenario of a clinical trial

Which presentation(s) are you most looking forward to attending? Anything related to biological assays

Was risk assessment part of the validation process for this assay? Yes

Is it a cell-based assay? No

How long have you been in the industry? I have been with HGS in different capacities for about eight years.

When/why did you get involved in the industry? What interested you the most? Eight years ago, I wanted to experience the industry setting and looked forward to focusing on bringing drugs to the clinic.

Stephan O. Krause

Manager of QC Technical Services and Compendial Liaison at Bayer Schering Pharma (USA)

8+ years in the industry

Regulatory Expectations for Analytical Method Validations (AMVs) and Their Extensions

Strategic processes for establishing, monitoring, and controlling the lifecycle of an analytical test method are provided. Analytical method transfer, component equivalency, and comparability protocols are discussed in light of risk-based strategies for validation extensions. To ensure proper risk-based validations, risks must first be identified, then assessed for their (potential) impact on patients and the company, and finally manifested as protocol acceptance criteria. Suggestions are provided on how to handle failures for those AMVs that did not pass protocol acceptance criteria. Time permitting, practical tips will be offered for lowering the predictable risk to patient and company by reducing the observed process variability.

Who will be most interested in the subject matter of your talk? Method development and validation scientists, process development and validation engineers and scientists, QC and QA management

What do you expect them to "take away" with them? Understanding the impact of stakeholders' needs

Do you think the risk-management paradigm has provided an improved framework for handling analytical method validation — or has it complicated matters? It has had no affect on my opinion yet because it has not been really properly addressed.

Does it represent a major change in thinking about such things — or simply recast what people have been doing all along? It will require a major change in thinking.

What do you think is the most common mistake made in risk assessment of analytical methods? Not understanding the risk and impact to stakeholders

When/why did you get involved in the industry? What interested you the most? The money was good. There is also good job security for good people. And I prefer dealing with commercial products and quality issues.

Patrick Liu

Director of Bioanalytical Sciences at Tanox (USA)

10+ years in the industry

Bioanalytical Approaches for Immunological Characterization and Biological Activity Assessment of Antibody Therapeutics

Development of antibody-based therapeutics is gaining increasing interest as numerous antibody drugs are showing great medical benefit. Appropriate bioanalytical strategy is one of the critical elements to developing a successful antibody therapeutic. Immunological properties and biological activity are the most important parameters to be monitored at each stage during development. They are also measured in patients for evaluating clinical pharmacokinetics. This presentation presents bioanalytical technologies for assessing the molecular integrity and biological activity of a humanized monoclonal antibody.

Who will be most interested in the subject matter of your talk? Bioanalytical scientists in the area of biologics development

What do you expect them to "take away" with them? Strategies and approaches for molecular integrity assessment of therapeutic antibodies

Which presentation(s) are you most looking forward to attending? Most of them

Can you list the methods you'll be highlighting in your talk — in order from most to least expensive? Immunoassays, cell-based bioassays, and chromatographic methods

How are they more broadly applicable? understanding of drug substance and biologic impurity, appropriate pharmacokinetic analysis

When/why did you get involved in the industry? What interested you the most? Pharmaceutical development

J.W.M. Mulders

Executive Director of the Department of Analytical Chemistry for Development at NV Organon (The Netherlands)

16 years in the industry

Regulatory Approval for the Use of Isoelectric Focusing (IEF) As an Alternate Method for the Determination of the In Vivo Bioactivity of Recombinant Follicle Stimulating Hormone (RecFSH, Follitropin Beta)

This presentation focuses on the relationship between physicochemical properties and in vivo bioactivity (potency) of recFSH, validation of the IEF method for in vivo bioactivity prediction, and the regulatory process to arrive at approval for the use of IEF as an alternative method for potency analysis.

Who will be most interested in the subject matter of your talk? People working in regulatory affairs and analytical development

What do you expect them to "take away" with them? Development of a non–animal-based alternative for a bioassay in which using animals is a long and troublesome process; however, the experience of going through such a process — certainly if it is successfully completed (regulatory approval) — is worth every step.

What method(s) does IEF replace in your case study? IEF replaces the potency test that has been an in-vivo bioassay using rats.

What are its relative benefits — e.g., cost, accuracy, time? Although the test saves cost and time, its most important benefit is that animals are no longer needed.

How long have you been in the industry? Since 1990

When/why did you get involved in the industry? What interested you the most? To be able to work on medicines that help improve the health of people in general; and because the industry offers great opportunities for personal and career development

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Arakawa T, et al. **Aggregation Analysis of Therapeutic Proteins, Part 1: General Aspects and Techniques for Assessment.** *BioProcess International* 4(10) 2006: 22–31.

Belew B. **Vendor Voice: Particle Counting Basics — Using Airborne-Particle Counters for ISO Certified Cleanrooms.** *BioProcess International* 4(8) 2006: 48–53.

Burke JF, Mann CJ. **Vendor Voice: Rapid Isolation of Monoclonal Antibody Producing Cell Lines — Selection of Stable, High-Secreting Clones.** *BioProcess International* 4(5, supplement) 2006: 48–51.

Champion K, et al. **Defining Your Product Profile and Maintaining Control Over It.** *BioProcess International* 3(8–10) 2005: 52–58, 50–56, 50–56.

Clark KJR, Furey J. **Suitability of Selected Single-Use Process Monitoring and Control Technology.** *BioProcess International* 4(6, supplement) 2006: 16–20.

Garidel P, Schott H. **Fourier-Transform Midinfrared Spectroscopy for Analysis and Screening of Liquid Protein Formulations.** *BioProcess International* 4(5,6) 2006: 40–46, 48–55.

Gombold J, et al. **Lot Release and Characterization Testing of Live-Virus Based Vaccines and Gene Therapy Products.** *BioProcess International* 4(4–5) 2006: 46–56, 56–65.

Jefferis R. **Criteria for Selection of IgG Isotype and Glycoform of Antibody Therapeutics.** *BioProcess International* 4(9) 2006: 40–44.

Kay M. **Vendor Voice: Adaptive Automation Accelerates Process Development.** *BioProcess International* 4(4) 2006: 70–78.

Kayser K, et al. **Cell Line Engineering Methods for Improving Productivity.** *BioProcess International* 4(5, supplement) 2006: 6–13.

Kim HY. **Improved Expression Vector Activity Using Insulators and Scaffold/Matrix-Attachment Regions for Enhancing Recombinant Protein Production.** *BioProcess International* 4(5, supplement) 2006: 24–31.

Kline JB, et al. **Vendor Voice: Whole-Genome Evolution Technology — Improving Protein and Antibody Yields in Scalable Mammalian-Cell–Based Manufacturing.** *BioProcess International* 4(5, supplement) 2006: 42–47.

Lee CC, et al. **High-Throughput Screening of Cell Lines Expressing Monoclonal Antibodies: Development of an Immunoprecipitation-Based Method.** *BioProcess International* 4(5, supplement) 2006: 32–35.

Ludwig, DL. **Mammalian Expression Cassette Engineering for High-Level Protein Production.** *BioProcess International* 4(5, supplement) 2006: 14–23.

Lundblad RL, Bradshaw RA. **Addressing Product Improvement Using Chemical Modification in Biopharmaceutical Manufacture: A Case Study Using Blood Coagulation Factor VIII.** *BioProcess International* 4(8) 2006: 30–36.

Mollah AH. **Risk Analysis and Process Validation.** *BioProcess International* 2(9) 2004: 28–36.

Mollah AH. **Focus on Project Management: Application of FMEA for Process Risk Assessment.** *BioProcess International* 3(10) 2005: 12–22.

Neway J. **Vendor Voice: How the Data Ecosystem Affects Process Development and the Bottom Line.** *BioProcess International* 2(5) 2004: 82–91.

O'Flaherty B. **Vendor Voice: Automated Sanitary Sampling: Optimizing Bioreactor Product Quality and Yield.** *BioProcess International* 4(1) 2006: 60–62.

Restivo G. **Focus on Compliance: The PAT Initiative — Modernizing the Biopharmaceutical Industry.** *BioProcess International* 3(8) 2005: 24–26.

Rau TD, Balcarcel RR. **A 96-Well Plate Assay for Relative Monoclonal Antibody Titers.** *BioProcess International* 4(3) 2006: 42–48.

Robinson CJ, et al. **A Survey of Bioassays in the Biopharmaceutical Industry.** *BioProcess International* 4(6) 2006: 24–36.

Schleef M, et al. **Long-Term Stability Study and Topology Analysis of Plasmid DNA By Capillary Gel Electrophoresis.** *BioProcess International* 4(8) 2006: 38–40.

Schroeder HG. **Sterility Assurance with Filtration: Taking Bioburden, Membrane Integrity, and Process Conditions into Account.** *BioProcess International* 4(6) 2006: 38–47.

Scott C. **Chapter Three: Process Development — Turning Science Into Technology.** *BioProcess International* 4(3, supplement) 2006: 24–41.

Scott C. **Chapter Four: Formulation Development — Making the Medicine.** *BioProcess International* 4(3, supplement) 2006: 42–57.

Simmerman H, Donnelly RP. **Defining Your Product Profile and Maintaining Control Over It, Part 1: Session One of the WCBP CMC Strategy Forum, 19–20 July 2004.** *BioProcess International* 3(6) 2005: 32–42.

Singer R, Lansky DM, Hauck WW. **A Bioassay Glossary.** *BioProcess International* 4(9) 2006: 52–62.

Tholudur A, et al. **Using Design of Experiemnts To Assess *Escherichia coli* Fermentation Robustness.** *BioProcess International* 3(9) 2005: 46–48.

Tholudur A, et al. **Comparing Automated and Manual Cell Counts for Cell Culture Applications.** *BioProcess International* 4(9) 2006: 28–38.

Uettwiller I, Dubut D, Voute N. **Quantification of Protein Adsorption onto the Surface of Single-Use Flexible Containers.** *BioProcess International* 4(6, supplement) 2006: 22–26.

Winter W. **Analytical Instrument Qualification: Standardization on the 4Q Model.** *BioProcess International* 4(9) 2006: 46–50.

Wolter T, Richter A. **Assays for Controlling Host-Cell Impurities in Biopharmaceuticals.** *BioProcess International* 3(2) 2005: 40–46.

Xu Y, et al. **Mycoplasma In-Process and Lot Release Testing: To PCR or Not to PCR.** *BioProcess International* 3(11) 2005: 30–39.