# Biologics Testing Highlights Need for Analytical Skills

**Feliza Mirasol,** "Biologics Testing Highlights Need for Analytical Skills," Pharmaceutical Technology 46 (1) 38–40 (2022).

https://www.pharmtech.com/view/biologics-testing-highlights-need-for-analytical-skills



There is need for training personnel in various analytical skill sets for biologic drug substance testing.



The complexity of biologic drug substances requires specialized analytics and the training to do the analyses. It is not enough to know how to run the assays, but also how to interpret the data to give meaningful clinical value to the analyses. To gain insight into methods with which biologics drug substance testing results must be analyzed and interpreted as well better understand the challenges inherit in dealing with complex biological molecules, *Pharmaceutical Technology* spoke with Khanh Ngo Courtney, senior director of Biologics at Element, and Mahesh Bhalgat, chief operating officer of Syngene International.

## The need for skill sets

**PharmTech:** Is there currently a lack in lab personnel with the specific skill sets to interpret data from biologic drug substance testing? If so, what skills training is in most dire need?

**Courtney (Element):** More and more, we are seeing that the characterization and routine testing of biological therapeutics, particularly of advanced therapeutics such as gene and cell therapies, require advanced analytical techniques combined with a thorough understanding of biology, which requires specialized lab personnel. For example, enzyme-linked immunosorbent assay (ELISA) is becoming a less effective way to quantify host cell protein (HCP) clearance due to emerging data showing that antibodies generated against the HCPs do not provide sufficient host cell proteins coverage. Hence, a more comprehensive analysis of HCPs is required. Liquid chromatography–tandem mass spectrometry (LC–MS/MS) is emerging as a needed analytical method to quantify and characterize HCP clearance. Finding talent who could develop and perform a robust, chemistry, manufacturing, and controls (CMC)-appropriate, quantitative LC–MS/MS method for HCPs is challenging.

Similarly, biological therapeutics require an *in-vitro* cellular assay to show potency during characterization and for release and stability testing. The design and development of the appropriate cellular potency methods, including the method for read-out, require personnel who understand the mechanism of action of the therapeutic, and how to exploit the cell biology toolbox to show *in-vitro* efficacy of the drug in a robust and CMC-appropriate manner. The appropriate skill-set required to perform this type of work is not easy to find in one individual.

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**Bhalgat (Syngene):** Biological drug substance testing is rapidly evolving, and new platforms and technologies are being used for testing purpose to suit the needs of the assays and drug mechanisms. While many labs and lab personnel are technically sound, there is still a lack of understanding and decision-making on the use of analysis models for biological product characterization and for the mechanism of action (MoA) studies. We find the greatest shortage in laboratory personnel is scientists trained on the statistical designs, analysis, and validation of the qualitative assays.

Additionally, there is a lacuna in getting professionals with biologics analytical training, especially in the structural and functional characterization areas. Apart from the physicochemical area, there is a need to strengthen the knowledge base on analytical ion exchange chromatography and N-glycan analysis for glycosylated biologics.

## Interpreting/analyzing the data

**PharmTech:** What have been some of the major challenges in interpreting and analyzing data generated from drug substance testing of biologic APIs?

**Bhalgat (Syngene):** Drugs, including APIs and biologics, must be manufactured in compliance with appropriate quality standards. Some of the major opportunities associated with analysis and interpretation of data from testing of biologics include product- and process-related impurity identification and analysis, which is always challenging due to the specialized nature of testing; assay changes during stability studies due to noncovalent interaction-driven oligomer formation; and HCP analysis of in-process samples.

The major challenge lies in interpreting data for its significance when it lacks large data sets generated over a sustained period. Since monitoring and analyzing the trend of the data is important for biologics and other drugs, we apply quality-by-design (QBD) approaches for ensuring data interpretation and comparison needs are met.

**Courtney (Element):** The heterogeneity of the biological molecule impacts how methods are developed, data are generated, and interpreted. Large molecules are tens, if not hundreds, of thousands of small molecules chemically interacting with one another to form tertiary structures, which translate to function. Yet, not all the molecules in a solution of the drug substance are going to be exactly the same—they vary in the amount of post-translational modifications such as glycosylation, phosphorylation, and other chemical modifications as well as endured damages, such as amino acid deamidation, reduction, and oxidation. All of these chemical modifications together impact the structure of the biological molecule, its function, and how it interacts with other molecules in the cell and how it behaves in analytical methodologies.

**PharmTech:** What methods or approaches have thus far been successful in generating clinically meaningful data and analyses of that data?

**Courtney (Element):** Meaningful data in the clinic require that the appropriate biomarkers are selected, and the methods for detecting and quantifying said biomarkers are robust and sensitive. The Meso Scale Discovery (MSD) and Quanterix immunological technologies are common methodologies and have been successful in the analysis of clinical samples. LC–MS/MS, if available, remains a powerful tool for the analysis of clinical data for its sensitivity and robustness in detecting and quantifying not only large molecules, but small-molecule biomarkers.

**Bhalgat (Syngene):** In clinical research, obtaining meaningful data is built on several factors. Best practices that have been successful in generating clinically meaningful data and have aided the interpretation/analysis of the generated data include drug-specific pharmacokinetic (PK), pharmacodynamic (PD), and immunogenicity assays, rather than generic/off-the-shelf kit assays or methods. Validated methods are also required in assuring assay performance, as per expectations for the set dosage, administration route, and intended clinical interventions. In addition, a careful review of the clinical trial protocol early in assay development, including a two-way dialogue between the clinician and the bioanalytical scientist, helps in setting up fit-for-purpose methods for the relevant trial. Finally, paying special attention to the performance of the method for a particular trial subject population, their age, disease state, ethnicity, etc., helps in generating reliable data.

## Meeting industry's needs

**PharmTech:** Where is there the most unmet need in term of analytical solutions for complex and challenging biologic drug substance testing?

**Bhalgat (Syngene):** The bioanalysis of biologics requires the ability to use many different approaches, so there are many unmet needs. For instance, there is a need for high throughput automation and practical biostatistics for complex and challenging biologic drug substance testing. Meanwhile, immunoassays for biologics are labor-intensive and people-dependent. The move to much more automated technology for conducting sensitive and specific immunoassays is a clear opportunity. There is also a need for the availability of assays for biophysical characterization in the presence of various process matrices, host-specific, HCP assay, and non-destructive assay to understand the tertiary structure of drugs at the atomic level (for example, cryo-electron microscopy or single-molecule fluorescence techniques are yet not available for industrial use). Additionally, due to lack of proper guidelines, and there are differences in regulation across different geographies resulting in the development of varying approaches to testing products.

**Courtney (Element):** Highly complex biological drug substances, such as genetic information in the form of viral or nanoparticle encapsulated therapeutics (e.g., lentivirus, adeno-associated virus [AAV], nanoparticles), are extremely difficult analytically due to the heterogeneity in the quality of the drug substance. One example is the analytical challenge of separating and quantifying fill-amounts of viral particles (i.e., empty vs. partial vs. full). When AAV or lentiviral APIs are produced, the drug substance solution will comprise partially of viral particles containing the correct number of copies of genetic information, particles with too much material, particles with too few, and particles containing no material at all. Having the analytical capability and technology to obtain resolution between these different molecular compositions of the drug substance is still an unmet need. Current methods all have their own pitfalls. Analytical ultracentrifugation is expensive, not robust, and requires a significant amount of sample. Transmission electron microscopy is time-consuming and lacks quantitative power. High-performance liquid chromatography has poor resolution. Finding the right analytical solution for the intended purpose is unmet need for many quality-defining methods for advanced therapeutic molecules at the moment. **PT**