

## Orthogonal Testing

Interviewer: Hello, I am here today with a group of experts who work in the biologics analysis group in our labs. Welcome, Dr. Steve Becht, Dr. Bob White and Dr. Jeff Staecker. They will provide an overview of the needs for orthogonal testing in biomolecule characterization and testing. The first question is for Bob. Why should one use orthogonal testing for biomolecule analysis?

Bob: The complexity, size and unique characteristics associated with different biomolecules necessitate an orthogonal testing approach. Complementary orthogonal methods overcome the limitations of certain stand-alone technologies and provide highest assurances of drug quality. This is critical to support biomolecule drug development such as protein drugs, where “process is the product” in certain cases.

Due to the unique characteristics of each biomolecule, a menu with a wide range of tests is evaluated and applied. Multiple methods are frequently used to measure the same attribute because each analysis provides information in a different light.

Interviewer: Jeff, could you give an example of orthogonal testing?

Jeff: For example, RP-HPLC, SEC-HPLC, IEX-HPLC, SDS-PAGE, and IEF can all measure relative purity. While relative purity may be the analytical result for each assay, the relative purity values themselves may vary greatly depending on the attribute of a specific biomolecule being measured, such as aggregation, tertiary structure, post-translational modification, etc.

Interviewer: And Steve, how is the test menu selected for orthogonal testing?

Steve: Establishing a multi-faceted menu of tests ([view our cGMP test menu](#)) for analyzing a biomolecule early in the development process maximizes the probability of later having appropriate assays available for particular needs. Thorough evaluation of data obtained from development, qualification and validation studies identifies the analytical tests required for the characterization and quality-control testing of these biomolecules. The resulting testing menu can be used to monitor and control product identity, purity, potency and batch consistency during ongoing release and stability studies over the lifetime of the product.

Interviewer: So Jeff, how can orthogonal testing address a clinical issue?

Jeff: For example, issues with immunogenicity are more prevalent with biopharmaceuticals, and molecular structure can have a significant impact on immunogenicity. Modern methodology, such as mass spectrometry ([see a PPD poster on mass spectrometry](#)), SEC-MALLS and cIEF provide insight into the understanding of protein structure. In particular, SEC-MALLS is well-suited to the detection of biomolecular aggregates. Incorporating SEC-MALLS analysis into formulation development can minimize the risk of encountering immunogenicity issues caused by

protein aggregation. In later stages of drug development, SEC-UV, preferably, or SEC-MALLS, where needed, can be used as a quality-control tool for clinical product evaluation, stability and commercial release testing.

Interviewer: And finally, Bob, what is PPD's experience with orthogonal testing?

Bob: The PPD cGMP lab in Middleton, Wis. works with a diverse group of clients ranging from virtual companies with a single drug under development to large pharma companies with extensive pipelines. Our current experience includes a broad range of analytical instrumentation and procedures that provide a comprehensive analytical menu ([see test menu](#)) with alternative approaches to address ever-changing analytical needs. This allows PPD to partner with clients in developing an effective program, addressing technical and business needs to the extent and details appropriate to the project. Early PPD participation in the development team brings our diverse experience into the process at the stage that provides the largest long-term benefit for the drug development process.

Interviewer: Great, thanks to all three of you for taking time to provide this introduction to the needs for orthogonal testing for biomolecule characterization and testing.

Bob, Steve, Jeff: You're welcome

Interviewer: Thank you all for listening to our podcasts on biomolecule analytics. For further information about this topic, please visit our cGMP Web pages or fill out the [contact form](#). Thank you again for listening!