Live Webinar Q&A Sheet:

Quality control of purified proteins to improve research data reproducibility: improving the time-efficiency and quality of your results

Dr. André Matagne

The recorded webinar may be viewed from the SEC-MALS webinars page. These questions were submitted by live viewers. Additional information on SEC-MALS may be found on the Wyatt web Library under Webinars, Application Notes, Featured Publications and Bibliography, as well as on the corresponding Product page and Solutions page of our web site.

The responses to the questions below were prepared with the assistance of the webinar speaker, Dr. André Matagne of the University of Liège, Belgium, to whom Wyatt extends sincere appreciation for his support. Please contact info@wyatt.com with any additional questions.

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Questions & Answers

Q. *What have been the main challenges faced by researchers and laboratories in adopting the new guidelines for recombinant protein quality control, especially concerning minimal information requirements and quality tests?*

A. We see a lot of concerns of researchers not knowing about techniques and they are worried about how much sample it would require, but as you saw in the talk it requires less than 200 µg of protein to perform the minimum QC tests, based on the five first-line methods (i.e. SDS-PAGE, UV-Vis spectrophotometry, mass spectrometry, SEC-MALS and DLS) described in the presentation.

Q. *How was the impact of the new quality control guidelines on experimental reliability assessed over the past year? Could you provide specific examples where the implementation of these guidelines has notably improved research outcomes?*

A. One of the easiest ways to assess this is to compare research outcomes before and after the implementation of new quality control guidelines to identify any improvements. One example of implementation and improvement is in this recent nature publication: Quality control of protein reagents for the improvement of research data reproducibility | Nature Communications

Q. *Can you elaborate on the importance of the specific quality tests recommended in the guidelines, such as spectral and thermal denaturation signatures, and how these tests contribute to determining the quality of recombinant proteins?*

A. Knowing if the protein is folded correctly or not is critical because of its link to activity. As discussed in the webinar, DSC or DSF are two techniques commonly used to measure protein thermal unfolding. The resulting curves (often a simple sigmoid, corresponding to a simple two-state transition between the native and unfolded molecules) represent the thermal denaturation signatures specific to the protein being analyzed. Any alteration (e.g. unfolding, chemical modification, etc.), whether complete or partial, of protein molecules in solution is likely to induces changes in the shape of these unfolding curves. Similarly, the optical properties of proteins, such as fluorescence and circular dichroism (CD), directly reflects their structural properties. Therefore, any modification in protein structure will inevitably result in significant changes in their fluorescence and CD spectra (i.e. spectral signatures).

Q. *How does improving the quality control of recombinant proteins directly benefit various stakeholders in the life sciences, such as researchers, journal editors, and funding agencies? Can you discuss any feedback received from these groups regarding the guidelines?*

A. The confidence it provides and how it protects the reputation of our field.

Q. *What are the next steps in the evolution of quality control standards for biological reagents, particularly recombinant proteins? Are there plans to update or expand the guidelines?*

A. For right now we are just focusing on proteins, but the need does exist for other molecules that we are seeing frequently like nucleic acids.

Q. *Thank you for the great presentation. You did not mention the measurement of contaminating bacterial endotoxin in the protein samples which can have significant effects on the outcome and reproducibility of in vitro and in vivo experiments?*

A. While our focus was solely on testing the product, we acknowledge the importance of addressing minor contaminants such as host cell proteins. I agree that these process contaminants are critical but outside the scope of this work. To validate a protein sample as endotoxin-free for use in in vitro cell-based assays, we typically send samples to external specialized labs, such as Lonza Testing Services, that focus on these types of tests. Their expertise ensures adherence to best practices in endotoxin testing.

Q. *What are your thoughts or experience on obtaining QC results from commercial protein provider? Have you encountered a company that was not willing to share sufficient information and if so, how did you handle the situation?*

A. Information on the final commercial product is sometimes incomplete. Additionally, quality may vary from batch to batch, and we always perform a minimal set of quality control tests on the protein samples we purchased.

Q. *Do you think it would be realistic to perform all these tests in an industrial setting? For every batch of protein? Or are these guidelines meant more for the laboratory setting and once the process is finetuned and ready for upscale, then the tests do not need to be performed for every batch?*

A. Actually, I think industrial or commercial labs already do this more frequently than in academia because of the regulatory implications on their work. As shown in the talk the amount of sample required for these tests is a small requirement.

Q. *Has there been any investigation into which properties are the most important for maintaining function in different downstream applications? What are the attributes in downstream that are most critical to check immediately?*

A. This really depends on your sample and what is critical for it to function properly. If you require glycosylation then this should be checked, concentration is always important, and aggregation will be important to check throughout the entire process. Most protein samples will benefit from optimization of their formulation, both in terms of stability and activity. This process is typically lengthy and tedious, but we've automated it for high throughput at our Robotein® facility.

Q. *For those of us producing recombinant proteins but unfamiliar with some of the QC tests and methodology is there possibility of training/collaboration with you or one of your partners?*

A. Please initiate contact with our networks (P4EU and ARBRE) to start communication. Also, please be aware that the Robotein® installation in Liège is accessible via the Instruct-ERIC network.