

Roundtable Session – Table 8 – MS for HCP

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Abstract:

Host cell proteins (HCPs) remain a critical quality consideration in the development and manufacture of biotherapeutics due to their potential impact on product quality, stability, efficacy, and patient safety. Advances in mass spectrometry, including data-independent acquisition (DIA), next-generation instrumentation, and increasingly sophisticated bioinformatics tools, are expanding our ability to detect, quantify, and assess low-abundance HCPs throughout the product lifecycle. At the same time, the industry is moving beyond simple identification toward understanding biological relevance, risk prioritization, and the use of analytical data to guide process development and regulatory strategies. This roundtable will provide an opportunity to discuss current best practices, emerging technologies, and future directions for MS-based HCP characterization and control.

Discussion Questions:

1. How are new mass spectrometry platforms influencing HCP workflows?

Are modern high-speed, high-sensitivity platforms providing incremental value (finding more biologically meaningful HCPs or getting more consistent coverage across replicates or do they simply increase the number of identifications)?

Notes:

- a. **Improved sensitivity:** More sensitive MS instrumentation, sometimes combined with higher sample loading, enables identification of additional protein species.
- b. **LC configurations:** Both micro-flow and nano-flow LC systems are in use. For untargeted workflows, detection sensitivity is typically 5-10 ppm, while targeted workflows can achieve below 1 ppm.
- c. **Therapeutic protein depletion:** Depleting the therapeutic protein can enhance HCP detection sensitivity. However, a key concern is that some HCPs may co-deplete with the therapeutic protein and be inadvertently removed.

2. How is DIA changing HCP analysis?

Are organizations moving from DDA to DIA for routine HCP characterization? If so, what improvements in sensitivity, reproducibility, or quantitation do you gain? Are there still challenges regarding spectral libraries, data processing, and validation that are slowing adoption?

Notes:

- a. **Broad applicability:** DIA is making a meaningful difference in HCP workflows and has been applied across multiple therapeutic modalities, from mAbs to AAVs. It is used to monitor purification processes as well as the final drug substance (DS), and it offers more robust quantification than DDA.
 - b. **Low-abundance protein challenges:** DIA can struggle with detecting low-abundance proteins. In some cases, peptides are detected at the MS2 level but lack corresponding MS1 data. Caution is warranted when interpreting new protein IDs from DIA compared with DDA, and a minimum of two peptides per protein is generally required.
 - c. **Sample preparation:** SP3 on-bead digestion is preferred for AAVs and vaccines, while native digestion is preferred for mAbs. Automated SP3 on-bead digestion coupled with DIA provides higher throughput for both sample preparation and data acquisition.
 - d. **Data analysis tools:** The library-free approach in Spectronaut is commonly used for DIA data analysis. An all-in-one data analysis solution supporting both DDA and DIA, as well as global and targeted data, would be highly desirable.
3. **Are you being asked to conduct more HCP analyses in earlier or later phases?** *(Discovery, Cell line selection, Process development or PPQ/commercial manufacturing). Are earlier HCP insights changing process development decisions or later analyses aiding manufacturing? Maybe related: How are HCPs handled together with product quality attributes / MAM/NPD?*

Notes:

- a. **Lifecycle coverage:** HCP analysis is conducted throughout various development stages, including cell line development, process development, and DS characterization.
- b. **Process optimization:** When problematic HCPs are identified during process development, the process can be optimized to remove them. Developing cell lines with problematic HCP knockouts can be time-consuming, and not all HCPs can be knocked out because some are essential for cell survival.
- c. **Naming vs. actual risk:** HCP names can trigger caution or concern, for example, proteases and lipases, but identification of a problematic HCP does not necessarily mean activity will be observed. In some cases, proteases have been identified without any evidence of actual clipping.
- d. **Cross-modality observations:** The same set of HCPs can be observed across different therapeutic modalities, including mAbs, AAVs, and vaccines.
- e. **Product-specific risk:** polysorbate-degrading enzymes (PSDEs) may be a greater concern for mAbs than for vaccines, since mAbs are administered at high

concentrations in the drug product, whereas vaccines are typically diluted prior to administration.

4. What constitutes a "high-risk" HCP in 2026?

How do you rank HCPs for investigation? Is abundance still the primary criterion, or is biological function more important? How are immunogenicity, enzymatic activity, product interaction, and patient impact incorporated into risk ranking and should industry develop a common framework for HCP risk scoring?

Notes:

- a. **"High-risk" HCP list expansion:** It would be valuable to see the current BioPhorum list of problematic HCPs expanded beyond the CHO proteome. The comprehensiveness of the existing list, as well as the actual activity or impact of the listed HCPs, remains uncertain.
- b. **Regulatory perspective:** The FDA does not explicitly identify which HCPs are truly problematic. Sponsors must demonstrate that a product's HCP profile is safe, and in most (if not all) cases, only ELISA data are included in BLA submissions.

5. Are there best practices for quantitative HCP measurements?

(Relative quant versus absolute quant; Protein standards, peptide standards, or whole-proteome standards). How are organizations defining LOQ, precision, and acceptance criteria?

Notes:

- a. **Relative quantification:** Relative quantification with spike-in proteins or peptides is widely used, and the Top 3 / Top N approach is commonly applied to estimate protein abundance. Standard proteins and peptides are not always ideal, and absolute quantification is generally considered a last resort due to the significant time required for method development. Relative quantification is typically sufficient to demonstrate HCP clearance during process development, and an absolute ppm value may not be necessary given that regulations have not defined absolute safe HCP levels.
- b. **ELISA vs. MS:** ELISA and MS readouts for HCPs cannot be directly correlated unless targeted ELISA data are compared against absolute quantification by MS. ELISA is generally faster than MS, and ensuring sufficient ELISA coverage for the specific cell line is critical. HCP-MS is typically performed only when issues arise.

6. Looking 5 years into the future, what do you think will be changing for HCP detection by MS?

Notes:

- a. **Regulatory expectations:** HCP data will continue to be expected by regulatory agencies, particularly for newer therapeutic modalities such as multi-specifics.

Whether ELISA or MS data are submitted will depend on the product and on specific questions raised by the agency. To date, few if any BLAs have contained HCP-MS data, and if ELISA results are already low, additional MS analysis may not be necessary.

- b. **Complementary techniques:** MS is unlikely to fully replace ELISA, although newer MS instruments will continue to detect more HCPs.

7. Additional topics discussed: continuous improvement & innovation.

Notes:

- a. **Sensitivity and throughput:** Continued improvements in sensitivity and throughput are anticipated, for example, through platforms such as the EvoSep-timsTOF system.
- b. **Multiplexed ELISA:** Multiplexed ELISA may be developed to improve throughput for HCP detection, although quantification is likely to remain challenging.