Table 4: Best Practices for Detecting and Accurately Reporting Host Cell Proteins

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Scope:

Host cell proteins (HCPs) are residual protein impurities that can be co-purified with the desired biotherapeutic and remain present in the final drug product. Given the potential impact HCPs pose to safety, the amount present must be monitored to demonstrate acceptable, low levels have been achieved in the final drug product. Typically, enzyme-linked immunosorbent assays (ELISAs) are utilized as a release assay to monitor HCP levels in biotherapeutics. To supplement HCP control strategies, LC MS/MS has been increasingly leveraged as it provides valuable orthogonal information to ELISA based approaches. Although LC MS/MS is now commonly utilized for HCP characterization, a standardized approach to this type of analysis does not exist and practices can vary widely. This roundtable will primarily be a discussion regarding the variety of techniques and workflows for the identification and quantitation of HCPs. Topics of discussion will include sample preparation, instrumentation, data acquisition, quantitation, and software. Time permitting, the roundtable discussion may include strategies for HCP characterization and outlining best practices for staying up to date with current methodologies.

Questions for Discussion:

1. What sample preparation techniques does you lab employ? Do you perform an enrichment step? If so, how have you benchmarked that the enrichment step is not biasing the results?
2. What instrumentation platform and acquisition strategy do you utilize? Do you utilize multiple acquisitions as part of your workflow?
3. What type of quantitation do you perform (LFQ, labeled, SIL peptides)? Does it vary depending on the goal of the experiment?
4. What software package/s does your lab use?

Discussion Notes:

Q re sample preparation: Do you prefer enrichment? How do you benchmark sample enrichment step:

No enrichment step when working with coagulation factors and other non-mAbs, enrichment is not that easy; HILIC method would be used for recombinant proteins. Challenge of target depletion: some HCPs would be lost.

Comment on cell based therapies: native method doesn't work, it's not easy to deplete abundant interferences.

Sigma universal proteomics standard can be used to spike and test your method sensitivity. People are using it and consider it beneficial (8 different concentrations, about 48 proteins). Useful to define limit of detection and quantitation.

Q re sample digestion. Using two approaches, denaturing and native. What do people use?

Depending on your therapeutic. For mAbs, native digest is helpful, great method to get rid of the target protein, but for other proteins it is not working as well.

When compare two approaches, native and traditional, need to combine both, not comfortable to do just one of them.

Q re the use of nanospray vs conventional spray

Cell therapies use nano. Recombinant protein developers use standard and capillary flow 15 ul/min flow rate; not nano. If nano is not necessary, better to avoid due to poor robustness. But Bruker claims to use nanoflow with good results. Capillary flow is good. New Thermo instruments – more sensitivity with decreased flow rate, definitely prefer low flow for sensitivity and reduced consumption of material.

Q re dynamic range limitation with ion trap type of technology

People find that the dynamic range is around 4. Exclusion lists are implemented: major peptides from the target molecule can be excluded; in practice this doesn't improve the method a lot; the usual approach is just perform digestion and run DDA on QExective

Another approach: standard chromatography; 5 injections per sample looking at different m/z space; combining the data for analysis.

Many people are using Orbitrap type of instruments successfully.

QE requires more maintenance, but it's sample dependent, depends on how much material goes in, typically we overload the system. Native digest is better, less mass is injected. Maintenance suggestion: clean transfer tube once a week; vent the system twice a year for deep cleaning.

Q re "pain points"

On one hand, it's problematic to ID low abundant proteins. On the other hand, for instance for comparability, don't want to go into more detail than historic data. Pain point: don't want to identify new HCPs with better instrumentation for commercial molecules. Process improvements, Tech Transfer, started doing the work and used older MS equipment, but now have newer more sensitive equipment. Will face questions from agencies, what to do if we see new things.

Pfizer was prescribing to look at things known to have adverse effects or any potential safety concern.

Another pain point: different workflows for different tasks.

Q re software: What software are people using?

Many are using Protein Metrics. Also Proteome Discoverer is used. Protein metrics is good but has some limitations. Different software is often used for different purposes/workflows. Proteome Discoverer, and Protein Metrics, Max Quant, Pinpoint, Skyline (evaluated by Sciex, vendor agnostic and good, although not GMP), other open source software. Comment re open source software: in development is OK, not in commercial space (Quality/GMP).

Those who used contract labs with different software capabilities find that different software, systems, etc, produced similar results. Others find that it depends on the software and how it is used. No standardization.

Important consideration, more critical than what software is used is your sample prep and chromatography.

Q re the type of quantitation people use

Does the quantitation strategy vary depending on what the goal is?

Yes, it depends. For example, used relative quantitation with top three peptides, labeled peptides are good but didn't have a case where it was needed.

Is it important to quantify to absolute levels because of the risks for patients? On the other hand why do we need to quantify HCPs absolute quant rather than relative? For example lipases, one would want to do absolute quant. From practical standpoint – just rank-order HCPs and label free, stabilized isotop labeled peptides.

Cell therapy = need to know for some specific proteins need absolute levels for safety. List of targeted peptides need for safety concerns or understanding activity, e.g. secreted cytokines. How do you measure (Nicole asked) Rich - in cell therapy it is all by ELISA. But if you have all the ELISA's in QC? MSD platform help with multiplexing, but why can't we use mass spec for this? Rich. Why not use in Quality? Can multiplex and quantify.