Roundtable Session 2 – Table 16 - Host Cell Protein Assays- Are We Still Relying on Conventional Quantification by Enzyme-Linked Immunosorbent Assays (ELISAs)?

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Abstract:

By the time you read this abstract, the new United States Pharmacopeia (USP) General Chapter <1132.1> "Residual Host Cell Protein Measurement in Biopharmaceuticals by Mass Spectrometry" will be available on the USP website. Host cell protein is classified as a critical quality attribute for potential immunogenicity effects and potential impact to product quality due to effects on protein degradation, polysorbate degradation, and interference with biological activity. Mass spectrometry methods for targeted identification and quantitation of HCPs deliver enhanced process and product knowledge and control compared to conventional quantification by ELISA alone. Is the industry ready for the next chapter in host cell protein detection, characterization, and quantification after decades of relying on immunoassays?

Host Cell Protein contaminants in mAb manufacturing

Discussion Questions:

1. Leading the way with HCP quantitation by mass spectrometry only without ELISAs? When is it appropriate to report mass spectrometry or ELISA results?

    - All industry participants use ELISA as primary release assay for HCP.
    -Majority of industry participants also collect MS data as characterization for HCP.

2. Host cell protein as part of Quality Risk Management- When are orthogonal HCP methods being applied in the product lifecycle? What kind feedback from the regulatory agencies has been received?

    -Some industry members had leveraged prior knowledge and platform to remove HCP as CoA test. Recent requests from EMA have resulted in release testing.

    - Majority of industry participants leverage HCP MS data as a characterization tool. This data is typically not shared with Health Authorities.

3. What key technical barriers and regulatory interactions need to be addressed in order to reduce risk and move forward with confidence?

    -Intensification of both process and formulation was discussed as a key contributor to increases in HCP levels, clearance, and stability challenges.

-Alignment on quantitation performance metrics across methodology (ie ELISA and MS) is needed. This includes method specific definitions for accuracy, recovery and quantitation units (ie ppm)

4. The USP chapter highlights three approaches used for HCP mass spectrometry quantitation: A) Relative to Product Protein, B) Relative to Spiked-in-Proteins, and C) Relative to Spiked-in Peptides. What are the pros and cons of each approach and what is most commonly used in the laboratories of our peers?

-Majority of the discussion for MS focused on semi or relative quantitation techniques.

-Overall discussion didn't cover details of USP <1132.1>

5. What other orthogonal HCP technology platforms are being explored outside of mass spectrometry and ELISAs? How are AI and machine learning being included as part of the HCP platform?

- Focus of discussion was on the complimentary nature of ELISA and MS specifically for HCP coverage assessments.

- No discussion on the role of AI or machine learning on HCP analysis.

- Multiple CROs are offering custom ELISA and MS workflows for HCP characterization and quantitation.

General Discussion Notes:

20 roundtable participants total with majority from industry along with CRO and academic participants.

Are people using MS as part of product development for HCP?

Majority of participants using 2 not using routinely

Industry participants shared current practices acknowledging process continues to evolve.

- Reagent generation and characterization is done with MS. Used to support primary assay (MSD not ELISA)
- MS to supplement ELISA.
- Sharing on non-mAb experience with majority having a primary capture purification step.
- HCP comparability data critical to support process changes.2D Gels used to ensure consistent coverage is achieved for ELISA reagents.

Question to group on if anyone transitioned to MS as primary?

-No one uses MS as primary HCP assay.

General discussion on coverage approach and use of 2D Gels

Question on experience with gel size changes and size of spots leveraged for coverage assessment?

- Decrease in gel size will impact resolution of the gel.
- 20x24 to 7x7 may not be able get resolution
- Smaller gels used to screen
- Increased use of MS for coverage screening with recommendation from EU Health Authority.
- Coverage workflows not fully established for small/start up industry participants. A few companies use MS as Investigation

Discussion on specification targets for HCP.

-Early phase ELISA specs rang from 50-100ppm

-Late phase/Commercial >=10ppm

-Alignment on definition of ppm.

ng/mg (ug/g) = ppm

- Companies get pressure to tighten the HCP spec as you move toward commercial when ELISA results are >10ng/mg

Quantitation- What does this mean across companies?

-Quantitation against an external standard was common practice for majority of participants.

- Consensus was these approaches are semi quantitative or relative quantitation.

- Limited discussion on absolute quantitation.

- Accuracy needs to be clearly defined for MS methods.

- MS Spikes are ~10 20ppm

-Quantitation in general should be put in context of reference standard used and scope of that coverage.

Characterization approaches for HCP.

-Some industry participants shared goal of keeping HCP ELISA in development space and not QC. Recent HA feedback is challenging this approach.

-Leverage prior knowledge and platform to not test.

-Limited experience for non-mammalian host/products.

-General agreement from industry on need to characterize cell lysate and trace through the process and scaleup.

-Intensification is occurring across cell line development and final product formulation.

-HCP impact is critical to proactively address as part of comparability.

-Confirming HCPs present and their coverage by ELISA reagents is critical.

-Scenarios do occur where you have HCP not covered by ELISA but is present in drug substance (DS)

    - One approach is this scenario is to create protein specific quantitation method is common practice.

- Product and formulation stability is key driver for holistic HCP characterization by both ELISA and MS.

-Clinical relevance is important for HCP but may require studies where multiple doses have been administered

-Understanding Tox and early clinical qualification experience for HCPs is important.

- Alignment that companies need to focus on delivering best and most consistent product quality