Roundtable Session 1 – Table 13 – Host Cell Protein Assays - Are We Still Relying on Conventional Quantification by Enzyme-Linked Immunosorbent Assays (ELISAs)?

Facilitator: Scott Kuhns, *Amgen, Inc*

Scribe: Ailish McCartan, *Bristol Myers Squibb Company*

Abstract:

Accurate detection and quantification of HCPs are critical to ensure product safety, efficacy, and regulatory compliance. For decades, enzyme-linked immunosorbent assays (ELISAs) have served as the gold standard for HCP quantification due to their sensitivity, specificity, and scalability. However, advancements in analytical technologies, such as mass spectrometry-based methods, have prompted a re-evaluation of conventional ELISA approaches. This round table seeks to explore the current landscape of HCP assay methodologies, weighing the benefits and limitations of traditional ELISAs against emerging alternatives. In addition, we will address whether the biopharmaceutical industry should continue its reliance on ELISAs or accelerate the adoption of more advanced, orthogonal techniques and what may be factors to consider before moving in that direction.

Discussion Questions:

- What are the main advantages and limitations of conventional ELISA-based HCP assays compared to newer analytical methods such as mass spectrometry?
- In what scenarios might ELISAs fail to detect critical host cell proteins, and how could alternative technologies address these gaps?
- What are the regulatory considerations and industry best practices regarding validation, comparability, and implementation of non-ELISA HCP quantification methods?
- What factors should guide a biopharmaceutical company's decision to transition from conventional ELISA assays to more advanced or more focused HCP quantification platforms?

Notes

This session had a great mix of voices across large biopharma companies, regulatory agencies, a government institute, experts from the major HCP kit suppliers, and even a student researcher.

Where We Are Today

The conversation opened with the new USP <1132.1> chapter. It puts more emphasis on MS and raises an important question: not *can* MS detect HCPs, but *how* do we validate it in a way that works for our applications and control strategies?

Most roundtable attendees agreed that companies do already invest heavily in their HCP analytics. HCPs score high on PQAAs because of their obvious influence on quality, safety and efficacy, with processes often requiring optimisation in process development, process risk

assessments, and sometimes even QC release to accommodate the HCP profile observed for various products. It was agreed by all that whatever technology we do use needs to be fully capable of reaching the high bar we need to set for HCP analysis.

Will ELISAs Always Be Enough?

There was a bit of a philosophical debate on this. ELISAs have worked well from a safety/efficacy standpoint, but the room was very aware of their limitations. People were asking: *Where do ELISAs start failing us? Where would higher-resolution approaches—like MS—fill those gaps?*

Other immunoassay platforms like Gyros GyroLab and Protein Simple Ella were mentioned, but right now they mostly live in process development. When it comes to IPC and release, ELISA still dominates for all companies polled at the table. Part of the reason is simple practicality: these other platforms are expensive, vendor-locked, and are harder to validate/transfer to support global operations.

CDMOs, in particular, tend to be a decision-making crosshair. CTLs are far less likely to have any more expensive platforms and equipment so it's hard for sponsors to switch technologies.

Operational Realities

The group was candid about the operational headaches:

- Ella and Gyros are costly and not truly plug-and-play as you need to develop your own plates and reagent sets.
- AlphaLISA, while excellent as it removes the wash steps which traditional ELISA rely so heavily on, suffers from lot-to-lot variability of the beads that makes ‘controlling’ your control strategy difficult. The beads required are also extremely expensive.
- While SPR/Biacore has been utilised in some QC environments, the technology and expertise required isn’t really viable for QC long-term.
- Promega’s Lumit platform is fast and high-throughput, and again removes the wash requirements, but experience of high assay variability was noted at the table.
- Automation for any of these alternative platforms can also be a resource and cost drain, with companies struggling to maintain the technical expertise to use/maintain/troubleshoot such instruments.

Mass Spec considerations and discussion

Everyone agreed that MS-based HCP analysis is being talked about more, but it’s still not ready to replace ELISA. Even creating a multiplexed assay for HCP maxes out around 16 proteins, which doesn’t work when you may need to understand 40+ proteins.

MS is great at detection but quantification is still the sticking point. Enrichment of sample pools through AAE helps with reducing interference from the product , but it challenges acccurate quantification because enriched samples can often inherently skew the HCP population.

Regarding reagent characterization, in a presentation on day 1 of WCBP, Cygnus shared AAE numbers, >90% coverage while 2D gel analysis for the same reagents showed lower coverage

(85%?). This raised the question of if the coverage expectations may evolve with newer assessment technologies. Regulators don't give a target coverage number, so companies have created their own norms, in general, most at table agreed that the below were broadly acceptable

- **50–70%** for older more traditional 2D + Western blots
- **70–80%** for AAE + 2D Silver stain
- **80%+** for AAE + MS

Most people assess coverage with upstream samples like HCCF. Downstream coverage isn't usually meaningful because key high-risk proteins may have been cleared already for that run, plus the drug substance itself overwhelms the readout unless some extra purification step is introduced.

There was some discussion about how MS data would be used in a control strategy for HCP, specifically how to most accurately quantify and what to base the quantification on. Current technology is best executed by measuring the HCP peptides against spiked peptides and then translating the total quantity to a concentration relative to the product so that conventional units of PPM or ng/mg can still be reported. The identities of the measured peptides would be available for trending or other orthogonal purposes presumably.

Immunisation Strategies and Reagent Variability

Fractionation-based animal immunisation came up, i.e. purposefully inoculating the animal in question with lysate fractions skewed towards HMW or LMW HCP species to force creation of a wide-ranging antibody pool; this was deemed very interesting theoretically, but impractical as recreating the fractionation process in a repeatable manner for each new critical reagent lot supply would be almost impossible.

Most groups outsource their immunisation work. Cygnus explained how their antigen design helps pull in low-MW, low-pI proteins that often get lost in coverage studies.

Affinity purification of generated antibodies is standard everywhere and essential if a team does plan to use a platform like Ella.

Robustness Studies

The question was raised about where in the lifecycle are robustness studies being performed for HCP ELISA assays. Most present said that they are still figuring out how to reconcile historical robustness data with new ICH expectations from the revision of ICH Q2(R2). Regulators do expect robustness data to be present in an MAA filing, or at least to see a defensible explanation as to why it's missing. This is particularly relevant if the company is using the same test method in multiple locations/test sites.

Lipases, Proteases, and Polysorbates

Lipase activity has been a HCP hot topic for years, and some attendees mentioned that they have seen a more recent increase in polysorbate degradation questions coming from regulators. To help answer these queries, many companies rely on surrogate quality attributes like particles or HMW.

High-concentration processes are putting more stress on HCP control, as increased HCP levels go hand-in-hand with increased protein concentration and process titres.

Commercial Kits vs. Process-Specific Antibodies

QC teams are still deeply tied to ELISAs. Feedback from Cygnus was that ~90% of issues related to %CV failures from their customers are coming from plate washing issues alone, so the move to more complex technology and assays does need to be weighed against the realities of a QC environment.

Regulators seem open to using commercial kits for licensed products if they're shown to be fit-for-purpose. Health Canada is evaluating this more actively. Reagent supply stability continues to drive the need for process-specific antibodies and assays.

A new USP CHO HCP standard is available and is being tested by Cygnus (no reports from companies present of them evaluating it). It was created from a pool ~20 batches of lysate, which raised questions about how variable the resulting antibodies might be.

Non-CHO Systems

A few examples were shared:

- One company working with an *E. coli* host struggled with a persistent HCP; they had to adapt their analytical control strategy to include a assay to demonstrate clearance.
- Cygnus has developed a specific BL21(DE3) *E. coli* kit to address bacterial carrier protein issues.
- To the awareness of those present, all current approved CGT products use kit-based HCP assays, partly because viral vector processes introduce large variability in HCP pools, making the creation of process-specific methods very problematic.