Table 16: Host Cell Proteins: Identification and Monitoring

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Scope:

Host Cell Proteins (HCPs) are process-related impurities that can pose safety risks to patients, or impact efficacy or stability of recombinant biological products. HCP-related topics, including quantitation, coverage, analytical methodologies, identification, and comparability, have been the focus of regulators and industry for years. With more recent developments in new technologies, such as affinity enrichment for 2D SDS-PAGE or LC-MS/MS for HCP coverage assessment, automated capillary Western blot system for HCP quantification and coverage assessment, as well as multi-attribute methods for HCP monitoring, this roundtable session will discuss new technologies for the identification, characterization and testing of HCPs, phase-appropriate approaches and regulatory expectations for adoption of new technologies.

Questions for Discussion:

1. Recent new technologies
    a. Affinity enrichment for HCP coverage. Can this lead to overestimation of coverage because of non-specific interactions? If so, how is this accounted for? Development of LC-MS/MS for HCP coverage assessment instead of traditional SDS-PAGE. Is this method suitable to accurately determine HCP coverage?
    b. Capillary Western blot system offers advantages of speed and simplicity over conventional Westerns. Is this being used for characterization and/or identification of HCPs or for coverage /quantification of HCPs?
    c. Have there been any new applications of LC-MS, such as multi-attribute methods (MAM) in the QC setting for HCP quantitation? What is the sensitivity in comparison with the traditional gold standard ELISA method?
2. Industry experience
    a. Have any new technologies including the ones discussed above been successfully implemented for release testing? What are some pros and cons of doing so?
    b. Any new findings about problematic HCPs such as lipases and any need to target monitor problematic HCPs? How about the use of new technologies for identification and/or monitoring of problematic HCPs, e.g., lipases?
    c. What are the challenges in introduction of new technologies into QC and what is the phase appropriate approach?
3. Regulatory expectations for implementation
    a. What is the regulatory experience to date on the submitted HCP data package applying the new technologies?

b. What are the regulatory expectations for bridging the new technologies with the gold standard?

Discussion Notes:

Question 1: Are companies using affinity enrichment for HCP coverage?

- All participants reported using ELISA methods for HCP quantitation. The newer technologies are being used for characterization purposes.
- Affinity enrichment is not being used routinely for coverage assessment. Some companies are outsourcing this method.
- The use of a mock cell harvest vs. a product specific harvest sample for antibody affinity extraction (AAE) was discussed. The method must be optimized for use with product specific harvest samples as DS can interfere with detection (masking). AAE/Mass spec can be used to mitigate this as it can exclude most of the DS from the sample, although host cell proteins can interact with product and prevent removal from the sample.
- Recommendation is to use mass spectrometry to assess the coverage as supplement to gel electrophoresis. AAE M/S tells us what the ELISA picks up. This can inform Process Development if we know the characteristics of the protein
- Overestimation of coverage can be avoided by subtraction of redundant peptides (same protein with different modifications).
- It is unavoidable to have some proteins carry over during AAE, due to protein-protein interaction rather than direct immunoprecipitation.

Are companies using Capillary Western blot (Peggy Sue and similar)?

- Advantages are speed and small sample size, amendable to automation
- This is not being used for release testing by any of the participants

Are companies using Multi-attribute methods?

- This is in use by some participants as characterization method but not for quantitation
- This technology also has issue of potential masking by product
- Useful if looking for specific HCPs (e.g., USP has a 6-His tagged plbl-2 standard)

Question 2: Industry experience

- All participants are using ELISAs for quantitation of HCPs, with some companies supplementing with mass spec for identification.
- No “magic number” from FDA with regard to coverage expectations (“word on the street” is 70% is sufficient)
- Coverage of all classes of protein (4 quadrants, low and high pI, low MW and high MW) is more critical than %
- FDA has historically expected an in-house, product or process specific detection reagent, but some participants have observed that these may not be more sensitive than commercially available reagents after protein A capture. Note that Cygnus 550 vs. 550-1 reagents are 97% similar in

coverage by M/S but react differently in ELISA due to affinity differences. It is process specific which one gives better coverage.

- Use of 2D DIGE, consensus is that AAE/LC MS/MS may be better as coverage and identity of HCP are confirmed

Question 3: Any new findings about problematic HCPs such as lipases and any need to target monitor problematic HCPs? How about the use of new technologies for identification and/or monitoring of problematic HCPs, e.g., lipases?

- Can detect lipase in HCP ELISA assays with protein-specific reagents at high pg/mL levels that we can't detect by LC/MS
- There is a need to have pg sensitivity as some enzymes are active at that concentration
- If there is a particular protein of concern, can use these methods to ensure removal (more for process mapping than release method) e.g,, hydrolytic degradation of polysorbate, where there is a need to understand the process and the HCP
- Can consider a list of top 1-20 problematic HCP to ensure that these are cleared
- Biophorum report on HCP was discussed, this list of "high risk" proteins are those with a known impact on product quality (sulfhydryl reductase A, lipases, proteases). It was suggested the term "problematic" be used instead to make clear that the conclusion was not safety related. Biophorum is now working on risk assessment
- With regard to newer technologies, "Use ELISA to understand the process capability but other methods to understand the population."