BEBPA 2020 – Key topics and take-home messages

October 2020

Due to Covid-19 related travel restrictions BEBPA's 8th annual Host Cell Protein Symposium October 26-28 was held as a virtual conference.

Fortunately, still 130 participants, consisting of HCP experts from biopharma, service- and technology providers and regulatory agencies, attended the event.

The BEBPA organizing committee had assembled a great programme with high level scientific talks, audience poll questions, and a workshop for LC-MS workflow for HCP analysis. The well-prepared chairpersons facilitated high-level discussions despite the virtual meeting format.

Honestly, we had our concerns beforehand about the event being virtual. However, the content of the symposium and the lively discussion from participants was a success - although we miss the social interaction in the coffee breaks. Well done BEBPA!

For those of you curious to know what was discussed, my colleague Rikke Lund and I have made a short summary of the key topics and take-home messages from the meeting:

Main Topics discussed

- ELISA Assay and Reagent Development
- Regulatory Trends
- Management of Identified HCP's
- Gene Therapy
- Workshop on LC-MS Workflows for HCP analysis

ELISA Assay and Reagent Development

Several presentations discussed methods to evaluate ELISA antibodies and how to obtain high HCP Coverage.

One example was Pia Paarmann from BioGenes, who presented "Case Study - Comparison of Orthogonal Methods for Reliable HCP Coverage Determination", with an overview of different HCP Coverage methods including: 2D Western Blots, Immuno-Affinity-Capture (IAC) combined with 2D DIGE or LC-MS, and the ELISA-MS method introduced by Alphalyse at BEBPA 2019.

The presentation included a discussion of advantages and disadvantages of the methods. I.e. the IAC LC-MS and the ELISA-MS methods have the advantages that they are performed under native binding conditions (not denaturing like 2D PAGE) and they provide the identity of each host cell protein covered.

Regulatory Trends

Alexey Khrenov from FDA gave a talk on "Control of Host Cell Proteins Throughout Product Lifecycle".

According to him, the two most frequently asked HCP questions (which have no answers) are:

1. What percentage HCP coverage is acceptable?
    - The percentage itself is not reflective of the assay capabilities
    - Calculated number depends significantly on quality of analysis method

2. What HCP specification limit is acceptable?
    - HCP risk assessment in each individual product depend on patient indications and dosing
    - The HCP limit is highly dependent on the assay
    - Actual concentration of HCP is unknown (since ELISA number is immunologically weighted)

Diane McCarthy, US Pharmacopeia, presented "USP Standards to Support Host Cell Protein Analysis", and the establishment of a Host Cell Protein Standards Expert Panel. The purpose of this panel is to write a new general chapter on best practices for identification, characterization, and quantitation of Host Cell Protein (HCP) impurities in biological products using mass spectrometry (MS).

USP will soon release a list of the first protein and peptide standards for quantification and evaluation of MS-based HCP-analyses.

Management of Identified HCPs

On day two, Rikke Lund from Alphalyse presented a study titled "HCP Profiling in Commercial mAbs and Biosimilars". It included MS-based HCP analysis of 30 different purified mAbs and detailed data on 16 commercial mAbs.

Two types of HCP analyses were performed:
- A quantitative analysis with thorough protein denaturation, with an LOD of 1-5 ppm.
- A sensitive identity analysis with native-digest and HCP enrichment by mAb precipitation, with an LOD of 0.1-0.5ppm. Generally, the study showed that mAbs only contain very low levels of HCP, but even commercial monoclonal antibodies on the market contain HCPs of potential concern.

Gene Therapy

A rapidly developing class of therapeutics with new analytical challenges was presented and discussed in this session. The main challenges are short timelines for patient-specific therapies (days) as well as small batch size (e.g. 25ml). Both limit the options for HCP-analysis.

Workshop: LC-MS Workflows for HCP Analysis

The objective of the workshop on day 3 was to discuss and propose the best practices for HCP analysis by LC-MS. An expert panel consisting of key companies in the field (Sanofi, BioAnalysis LLC, Pfizer, Alphalyse, Novartis, NIBRT, University of Nebraska, Novartis, Covance) presented their typical workflows, including sample preparation, LC-MS instrumentation, software, as well as types of samples and projects. MS-vendors (Bruker, Waters, Sciex) also presented their MS instruments for HCP analysis.

The presentations enabled Session Co-Chair Martha Stables to provide an overview of the commonly used methods and parameters:

- Many different sample types are processed, but CHO and *E. coli* are most common.
- HCP-analysis is used both in upstream optimization of the cell culture conditions, as well as in downstream process optimization, and drug substance analysis.
- The most challenging samples are early process samples in complex matrices. However, downstream samples with Tween, PEG and high salt also cause problems.

- For sample preparation, the majority uses in-solution digest combining LysC and trypsin. Generally, HCP enrichment is avoided, but it is useful for mAb analysis.
- The CSH-column is widely used and combined with all flow types: nano, micro and analytical in a 1D setup. For MS the setup is mostly untargeted with limit of detection as low as 0.1ppm. A variety of instruments is used.
- For database search Uniprot is preferred - and used in combination with many different search engines.

Overall, the virtual BEBPA 2020 HCP symposium was well-organized and gave us a lot more knowledge about HCP impurity control, and inspiration for new projects and improvements.

We will certainly participate at the next virtual BEBPA HCP Conference in May 2021.

Yours Sincerely

Rikke Lund and Ejvind Mørtz, Alphalyse