Table 5: Host Cell Proteins: Sample Preparation, Methods, Instruments, Software, and Validation

Facilitor: Michelle English, *Protein Metrics, Inc.*

Scribe: Jacky Smith, *Pfizer, Inc.*

Scope:
Late stage biotherapeutics manufacturing campaigns often require significant process intensification efforts to achieve the clinical and commercial strategy. Host cell proteins (HCPs) are residual protein impurities expressed along with the desired therapeutic protein. Their levels must be minimized during cell culture and purification process development to result in safe and efficacious products. An enzyme-linked immunosorbent assay (ELISA) is utilized to monitor HCPs at each step of the purification including final drug substance. Standard ELISA provides broad coverage and reliable quantitation of HCPs; however, it lacks specificity and quantitation for particular HCPs due to incomplete generation of anti-HCP antibodies. Recently, the application of liquid chromatography-tandem mass spectrometry (LC-MS/MS) has emerged as an orthogonal approach to ELISA, providing qualitative and quantitative information on individual HCPs with similar sensitivity.

Questions for Discussion:
1. MS Basics- What instruments are used? (QToF, Orbi or Triple-Quad) What methods seem best? (digestion method, instrument method, replicates, etc) When are you testing for HCPs (DS vs. in-process samples, clone selection, manufacturing batches, process validation, final DS, or commercial lots)? How do you measure system suitability? (instrument, sample prep, "good" run criteria) Have you done performance validation to show consistency/reproducibility?
2. Identification and Quantitation- What is your limit of detection/quantitation vs reporting (is it sufficient?) Different quantitation methods (label-free vs. labeling techniques) Software packages for data analysis.
3. What is the future of HCP analysis? Are we doing too little or too much? Are shared/harmonized libraries of HCPs needed for common cell lines? What is your relationship to bioprocess groups and is it changing? (Service provider vs partner?) Is there a QC or real-time monitoring role within the next 5-10 years? Other changes?

Discussion Notes:
1. MS Basics
What instruments are used? (QToF, Orbi or Triple-Quad)
-Lumos, G2Si, and Thermo HFX mass spectrometers
What methods seem best? (digestion method, instrument method, replicates, etc)
-Only a few organizations are doing replicates (due to instrument time restrictions). Standards – protein spike or duplicate prep of NIST many are following the Lilly paper. All of industry at the table were using the Waters Xselect LC column.
When are you testing for HCPs (DS vs. in-process samples, clone selection, manufacturing batches, process validation, final DS, or commercial lots)?
-**Most people do not check at all stages.**
How do you measure system suitability (instrument, sample prep, "good" run criteria)?
-**Some use the NIST mAb for system suitability, some use older projects that have known HCPs.**
Have you done performance validation to show consistency/reproducibility?
-**Only a few organizations are doing replicates (due to instrument time restrictions) Standards – protein spike or duplicate prep of NIST and many are following the Eli Lilly paper.**
2. Identification and Quantitation
What is your limit of detection/quantitation vs reporting (is it sufficient?)

-Some of the folks do a semi-quantitative analysis first and then do MRM for a few interesting HCPs of interest. They said it depends on how clarified the material is. Some use a method to remove mAb to enrich the HCPs. Some are using ProA depletion as well. Spike in heavy labeled peptides after digestion to have an absolute concentration for ng/mg. Some are using the mole ratio of HCPs instead of mass ratio. Needs to be <10 ng/mg.

Different quantitation **methods (label-free vs. labeling techniques)**

-Most are doing the high 3 method, or all peptides, but some are spiking 4 or 5 different HCPs. Most are doing label free analyses.

Software packages for data analysis

-Most use proteome discoverer coupled to mascot and Protein Metrics.

3. What is the future of HCP analysis?

-A lot of discussion about attempts to correlate MS and ELISA.

Are we doing too little or too much?

-Most would like govt/central info to provide a list of known bad acting proteins.

Are shared/harmonized libraries of HCPs needed for common cell lines?

-Difficult to educate bio-process groups about assay variability and meaning of LC/MS/MS data.

What is your relationship to bioprocess groups and is it changing? (Service provider vs partner?)

-Difficult to educate bio-process groups about assay variability and meaning of LC/MS/MS data. Some do partner with them closely.

Is there a QC or real-time monitoring role within the next 5-10 years?

-Most were not sure.

Other changes?

Biggest Hurdles?

- Reproducibility and Data Quality, sample run time, and data analysis.