Antibody Affinity Extraction Enables Identification of Host Cell Proteins by Mass Spectrometry

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Introduction

Host cell proteins (HCP) constitute a major group of impurities for biologic drugs produced using cell culture technology. Even at nanogram per milligram concentrations of HCP to drug substance (DS), HCPs can elicit undesired immune response, interfere with drug safety and efficacy, or impact DS stability.

A broadly-reactive HCP ELISA should be used during the purification processes to ensure removal of HCPs and to demonstrate process consistency and final DS purity. Regulatory authorities are requesting biopharmaceutical companies employ orthogonal methods to demonstrate antibody coverage to individual HCPs and provide a comprehensive assay qualification package to ensure the HCP ELISA used by a sponsor is fit for this purpose.

Antibody Affinity Extraction™ (AAE™), a novel method developed by Cygnus Technologies, is used to determine antibody coverage and reactivity to those HCPs that co-purify with DS. AAE is more predictive of the anti-HCP antibody performance in the HCP ELISA and facilitates identification of individual downstream HCPs.

While ELISA is the gold standard for monitoring HCP levels, it does not provide information about what HCPs are present in the DS. Identification of HCPs by mass spectrometry (MS) is a powerful orthogonal method to ELISA. However, one of the limitations of MS is that IgG drug substances often mask HCPs by a factor of 10^4 - 10^6. To improve MS sensitivity, AAE can be used as a sample preparation method to enrich HCPs and eliminate most of the DS in a sample.

Enrichment of HCPs by AAE and detection by MS is a powerful orthogonal method to ELISA. Integration of orthogonal methods for comprehensive HCP analysis provides data throughout DS purification to inform process development teams of how to modify their purification processes to improve DS purity as well as ensures comprehensive quality control data for regulatory agencies.

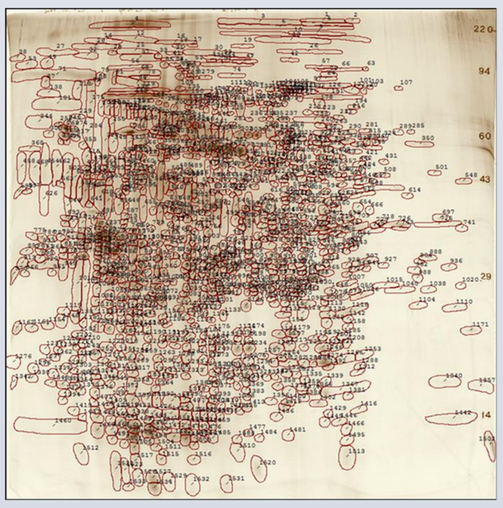
Objectives

1. To illustrate the superiority of AAE to traditional 2D Western blot for HCP antibody coverage analysis
2. To demonstrate the power of integrated AAE and MS for HCP identification in process samples and final drug substances

Antibody Affinity Extraction™ (AAE™)

Antibody Affinity Extraction is a method devised by Cygnus Technologies to overcome the technical challenges and limitations of other orthogonal methods in assessing the coverage of a polyclonal antibody to total host cell protein (HCP). In this method the polyclonal antibody is covalently immobilized on a chromatography support. The column is then conditioned to prevent significant leaching of the antibody and to greatly minimize any non-specific binding. The HCP sample in its native, un-denatured state is passed over the column for binding and then eluted with acid. The HCP sample is again cycled over the column by binding and elution until a no additional HCP is bound. All HCP elution fractions are pooled, buffer exchanged, and concentrated back to the original sample volume. The final sample is then separated by 2D SDS-PAGE and analyzed by either a comparison to a silver stain of starting, unextracted sample or by Differential Gel Electrophoresis (DIGE) using Cy3 and Cy5 to label the extracted and starting, unextracted samples.

Coverage Analysis by AAE™ with Silver Stain Detection

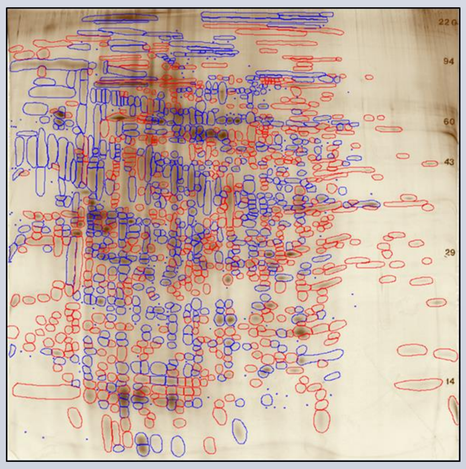


Null Harvest

AAE Eluate Fraction

The goat anti-CHO HCP antibodies reacted to 827 of the 1138 total spots for a total coverage of **73%.**

Coverage Analysis by 2D Western Blot: Low Sensitivity and Specificity

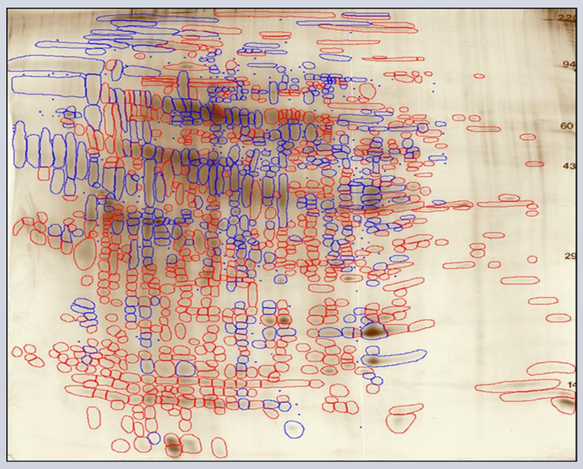


2D WB of CHO HCPs using Anti-CHO Ab

blue circles & dots were detected in both the silver stain and WB

red circles are spots detected by silver stain only

Specific WB Coverage = 717 spots of the 1293 =**55%**



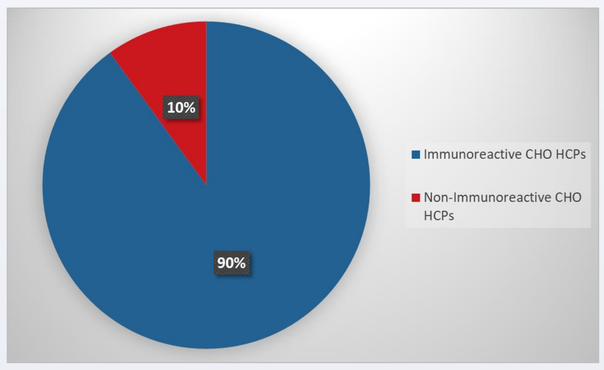
2D WB of CHO HCPs using non-immune goat IgG

blue circles & dots were detected in both the silver stain and WB

red circles are spots detected by silver stain only

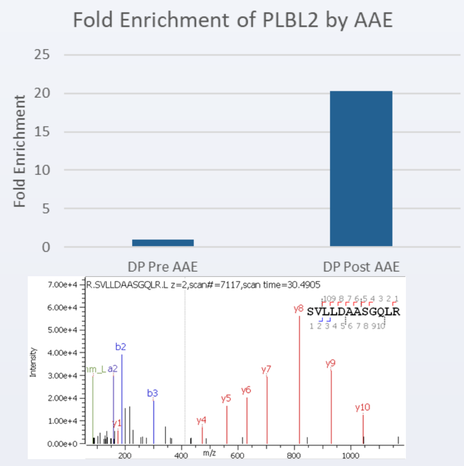
Non-Specific WB Coverage = 571 of the 1191 = **48%**

Antibody Coverage Analysis by AAE™ and Mass Spectrometry



CHO 3G Ab Reactivity of Process Sample by AAE and MS

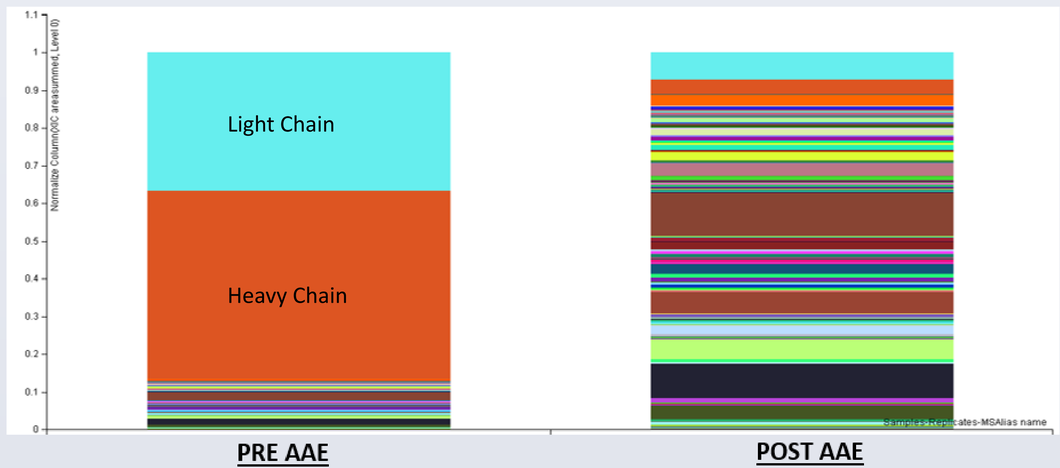
AAE was performed on the null harvest process sample. Pre- and post-AAE samples were tryptic digested and 8 µg was injected at a flow rate of 5 µL/min and detected by a Sciex 6600 QTOF in DIA mode. MS data were searched using the Uniprot CHO K1 proteome within Sciex Protein Pilot software. Antibody coverage of 90% was calculated by matching CHO HCPs identified in Pre and Post AAE samples.



PLBL2 is Immunoreactive with CHO 3G Antibody

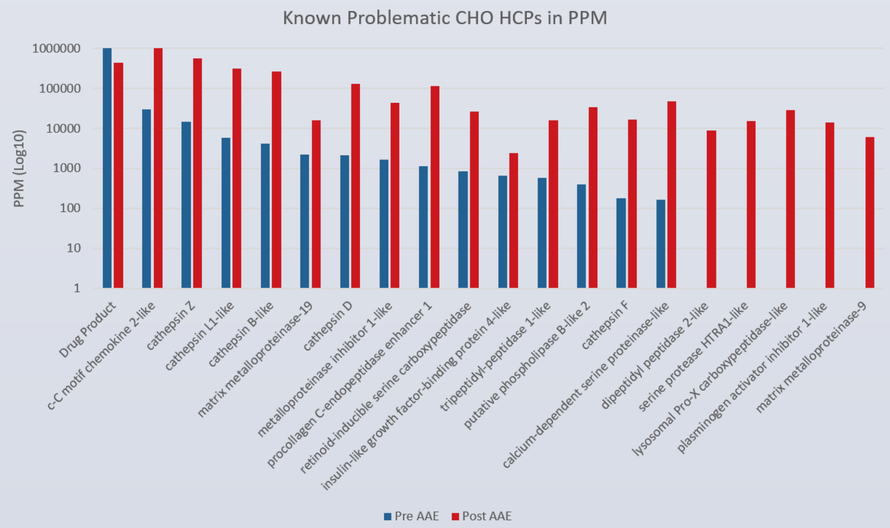
HCPs were enriched in a drug product sample by AAE and detected by a Thermo Fisher QE HF operating in DIA mode. By doing a relative comparison of the extracted ion chromatograms for this DS sample before and after AAE, we show that the CHO 3G antibody is immunoreactive with PLBL2, and enriched 20-fold.

AAE™ is Highly Effective at Enriching HCPs and Depleting DS



Proteins were identified before and after AAE by mass spectrometry and data were analyzed using the Protein Metrics Byos HCP workflow. The relative percentage of drug product and HCPs according to their extracted ion chromatograms in each sample were both normalized to one and graphed in a stacked bar chart.

AAE™ Enriches Known Problematic HCPs



An upstream CHO sample containing DS was analyzed pre- and post-AAE by MS. PPM quantification was calculated relative to the most abundant protein present in each sample. For the pre-AAE sample, known problematic CHO HCPs are quantified relative to the drug product. For the post-AAE sample, CHO HCPs are quantified relative to the most abundant protein, C-C motif chemokine 2.

Conclusions

1. AAE™ is more predictive of how the anti-HCP Ab will perform in HCP ELISA and provides sufficient sensitivity to evaluate individual HCPs that persist through purification process.
2. Due to the inherent limitations of the 2D WB method, such as (1) loading capacity, (2) destruction of native epitopes by harsh sample treatment, (3) failure to transfer some HCPs out of the gel, (4) HCPs bound to the membrane such that antibody binding is sterically inhibited, (5) difficulties in aligning PAGE gel to a WB membrane images, and (6) poor specificity, 2D WB significantly underestimates true Ab coverage to upstream HCPs. More importantly, 2D WB does not predict how that Ab will react to the most important HCPs which are those that co-purify with the drug substance.
3. AAE and MS are two complimentary orthogonal methods that should be employed to identify individual HCPs present in a drug substance and aid in the decision on whether immunoassay is fit for process monitoring and product lot release.
4. HCPs are more than a check off box on a IND or BLA submission, knowledge of what HCPs are present can inform purification strategies to reduce their level in drug substances.
5. Reducing the level of HCPs in a drug substance using targeted chromatography against identified HCPs can increase the confidence that clinical trials may be successful and positively affect drug safety and efficacy.