Advanced Characterization of HCP Polyclonal Antibodies

Combining Antibody Affinity Extraction and Mass Spectrometry

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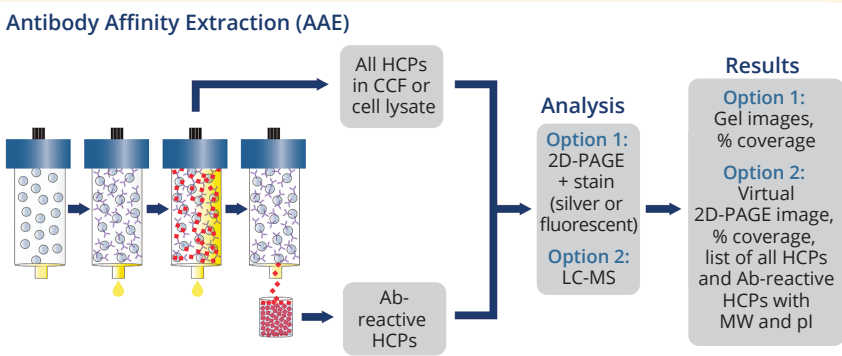
Host cell proteins (HCPs) represent a major group of process-related impurities in biological drugs produced using cell-culture technology. Many HCPs are benign; but some are immunogenic, some may interact with a drug substance (DS), and others (e.g., proteases and lipases) can reduce effective product dosage (by acting directly on the drug or interfering with its formulation buffer and stability).

To monitor the effectiveness and consistency of downstream purification processes, biomanufacturers rely on HCP enzyme-linked immunosorbent assays (ELISAs). But even ELISAs have analytical limitations, necessitating orthogonal confirmation of their detection ability to detect HCPs. Biomanufacturers must ensure that a selected HCP ELISA method is fit for its intended use. Qualifying an ELISA involves determining its range and demonstrating sufficient dilutional linearity, accuracy, and precision. A factor that distinguishes HCP ELISAs is their requirement of HCP-antibody coverage assessment to ensure broad reactivity to an array of HCPs in a manufacturing process. A well-developed and qualified HCP ELISA will provide analysts and regulators with confidence that a purification process performs consistently across batches and reduces HCPs to safe levels. Regulator agencies require use of orthogonal methods to demonstrate antibody coverage to individual, process-specific HCPs to support use of a particular HCP immunoassay.

Figure 1: Antibody (Ab) coverage analysis using the antibody affinity extraction (AAE) method, with options for analysis by two-dimensional polyacrylamide gel electrophoresis (2D PAGE) or mass spectrometry (MS); CCF = clarified culture fluid, HCP = host cell protein, LC = liquid chromatography, MW = molecular weight, pI = isoelectric point

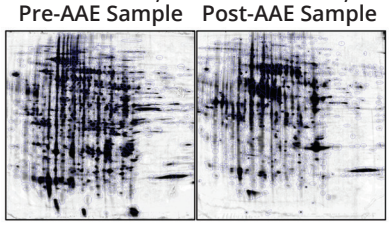


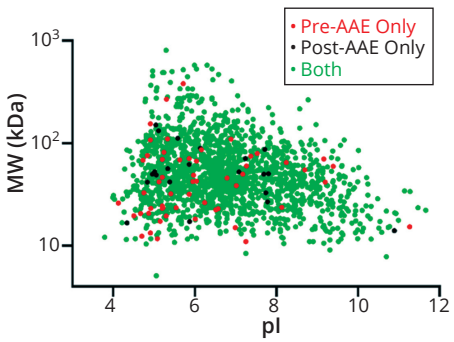
Antibody Coverage Assessment by AAE Analysis

Antibody Affinity Extraction (AAE) immunoaffinity chromatography from Cygnus Technologies is an advanced orthogonal method designed to assess polyclonal-antibody coverage for an array of HCPs that are present in a given biomanufacturing process and for downstream-process–specific HCPs that could copurify with DSs. This method overcomes analytical deficiencies of antiquated two-dimensional western blot (2D WB) and two-dimensional differential in-blot electrophoresis (2D DIBE) methods, which still are used to assess antibody coverage to total HCP content. The AAE method's most important advantage over 2D WB is that it mimics an ELISA's biophysical environment for interaction between antibodies and HCP antigens. Thus, the AAE approach is more predictive of HCP-antibody performance in a corresponding HCP ELISA.

To perform the AAE process, HCP polyclonal antibodies (pAbs) from a corresponding HCP ELISA are immobilized covalently onto a chromatography support. Next, a column packed with that affinity resin is conditioned to prevent significant antibody leaching and to minimize nonspecific binding. A native, undenatured sample containing all HCPs from a given process is passed over the column for binding (Figure 1). The column is washed to ensure removal of HCPs that remain because of nonspecific HCP binding or nonspecific protein–protein interactions. Then, immunoreactive HCPs are eluted with acid. This process is repeated for four cycles to enrich low-abundance, immunoreactive proteins to the point of detection for silver-stain and mass spectrometry (MS) methods. The final "post-AAE" sample represents all immunoreactive HCPs in the processed material. Post-

Figure 2: Host cell protein (HCP) polyclonal antibody (pAb) coverage analysis using the antibody affinity extraction (AAE) method; a Chinese hamster ovary (CHO) cell-culture harvest sample was loaded on an AAE column with a pAb from a F550-1 CHO HCP enzyme-linked immunosorbent assay (ELISA) kit, 3G. Pre- and post-AAE samples were analyzed by two-dimensional polyacrylamide gel electrophoresis (2D PAGE) and silver-stain detection (LEFT) or by Cygnus's AAE with mass spectrometry (AAE-MS) method. The latter approach enabled plotting of virtual 2D-PAGE images (RIGHT) based on molecular weight (MW) and isoelectric point (pI) data for individual HCPs found in the pre-AAE and post-AAE samples. The table (BOTTOM) compares 2D PAGE and MS determination of pAb coverage.





Detection Method	Pre-AAE HCPs	Post-AAE HCPs	pAb Coverage
2D PAGE	1176	1005	86%
MS	1694	1639	97%

AAE samples can be separated by two-dimensional polyacrylamide gel electrophoresis (2D PAGE) and analyzed by comparison with a silver stain of a starting, unextracted "pre-AAE" sample that contains all HCPs present in upstream harvest.

This approach enables assessment of whether an HCP antibody is broadly reactive — with immunoreactive proteins found in all four gel quadrants, representing species of low molecular weight (LMW), high molecular weight (HMW), low isoelectric point (LpI), and high isoelectric point (HpI). The approach also can estimate antibody-coverage percentage, but it does not identify specific HCPs (Figure 2).

Generating Further Insights

Combining AAE immunoaffinity chromatography with MS — Cygnus's proprietary AAE-MS method — provides a powerful means for HCP-antibody coverage analysis. In addition to assessing coverage percentage, AAE-MS analysis identifies HCPs that are present in harvest material and antibody-reactive. It also yields protein MW and pI data (Figure 2). Importantly, the AAE-MS method is currently the only approach that can assess HCP-antibody coverage reliably when the only available sample is a product (DS) containing harvest material and not clarified culture fluid derived from a null cell line.

The AAE-MS approach enables analysts to shift the conversation about HCP-antibody coverage assessment from a simple question — "What is the coverage percentage?" — to a more insightful one: "What specific HCPs does your HCP ELISA quantify?" And whereas in the past we could not confidently determine, e.g., whether a Chinese hamster ovary (CHO) HCP antibody was reactive to a certain HCP, now we can obtain definitive MS-based answers about the identity of reactive HCPs. Such information is especially important for assessment of problematic process-specific HCPs to ensure that a corresponding HCP ELISA detects and quantifies them (Table 1).

Early application of tools such as the AAE-MS method helps analysts to identify HCPs that could diminish product stability, drug efficacy, and even patient safety. The better that an organization understands the HCP profile for a particular process, the higher will be the associated product's chance of success in clinical trials and, ultimately, the market. To date, more than 300 biologics-manufacturing projects have used AAE-based assessment of process-specific HCP profiles and respective HCP-antibody coverage. Data obtained from those studies have been submitted to regulators to support drug developers' investigational new drug (IND) or biologics license application (BLA) submissions.

Table 1: Representative problematic Chinese hamster ovary (CHO) host cell proteins (HCPs), with isoelectric point (pI) and molecular weight (MW) data; aside from cathepsin E (*), all listed HCPs were identified in a harvest (pre-AAE) sample and were covered by the HCP antibody in a F550-1 CHO HCP enzyme-linked immunosorbent assay (ELISA) kit, 3G (post-AAE sample). Cathepsin E was not identified in the harvest sample.

Problematic Species	pI	MW (Da)
Cathepsin B (CTSB)	5.73	35,646.9
Cathepsin D	6.54	44,110.9
Cathepsin E*	4.61	42,726.4
Glutathione S transferase P	7.64	23,638.2
Glyceraldehyde 3 phosphate dehydrogenase	8.49	35,747.9
G-protein–coupled receptor 56	9.06	77,370.5
Heat shock cognate 71-kDa protein	5.23	70,804.9
Heat shock protein 90	4.94	83,166.1
Lipoprotein lipase	7.94	52,900.3
Lysosomal protective protein	5.64	56,110.7
Matrix metalloproteinase 19	7.71	58,942.0
Metalloproteinase inhibitor 1	8.84	22,401.0
Monocyte chemo-attractant protein 1 (C–C motif chemokine)	9.32	15,858.4
Peptidyl-prolyl cis-trans isomerase	9.59	23,634.4
Peroxiredoxin 1	8.22	22,262.6
Phosphoglycerate kinase 1	8.02	44,562.5
Phospholipase A2 (Group XV lysosomal)	6.16	87,100.0
Phospholipase-B–like 2	5.63	61,824.4
Procollagen C endopeptidase enhancer 1	8.16	50,446.5
Procollagen lysine 2 oxoglutarate 5 dioxygenase 1	6.46	83,550.2
Procollagen-lysine 5-dioxygenase (PLOD3)	6.57	83,327.9
Protein disulfide isomerase	5.98	56,796.4
Pyruvate kinase	6.88	57,893.8
Serine protease HTRA1	6.62	34,404.5

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