HCP ELISA and HCP Antibody Coverage Analysis Methods

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Host cell proteins (HCPs) constitute a major group of process-related impurities in biological drugs produced using cell culture technology. HCPs are produced inadvertently during the expression of recombinant biopharmaceuticals as secreted from host cells from aberrant cellular trafficking due to cell stress and cell lysis over the course of bioprocess manufacturing. Many HCPs are benign, but some are immunogenic; some may interact with the drug substance, and others, like proteases and lipases, can reduce effective product dosage through direct action on the drug or its stability by interfering with formulation buffer. Since HCPs can pose a risk to patients and affect the efficacy and stability of the drug, they constitute a significant component of a biopharmaceutical drug developer's overall risk-management strategy (1, 2).

A robust and broadly reactive HCP enzyme-linked immunosorbent assay (ELISA) is a critical tool for monitoring purification process consistency as well as final drug substance purity and is the gold standard method for process monitoring and product release testing for HCPs (**Figure 1**). It is critical to ensure that the selected HCP ELISA method is fit for its intended use. This is important from a regulatory perspective as well as for process development and manufacturing (1, 2).

Regulatory agencies around the world have put measures in place to ensure the HCP ELISA used by a sponsor is fit for the purpose of monitoring purification process consistency and product lot release (1-3). A well-developed and qualified HCP ELISA will ensure that HCPs have been reduced to safe levels and that the purification process is consistent from batch to batch. It is advised to employ orthogonal methods to demonstrate antibody coverage to individual process-specific HCPs to support use of a particular HCP immunoassay. HCP coverage evaluations help assess the ability of the antibodies to recognize a wide range of HCPs in the calibration standard and those present in in-process and drug substance samples. Equally important is to qualify

Figure 1. Cygnus HCP ELISA diagram

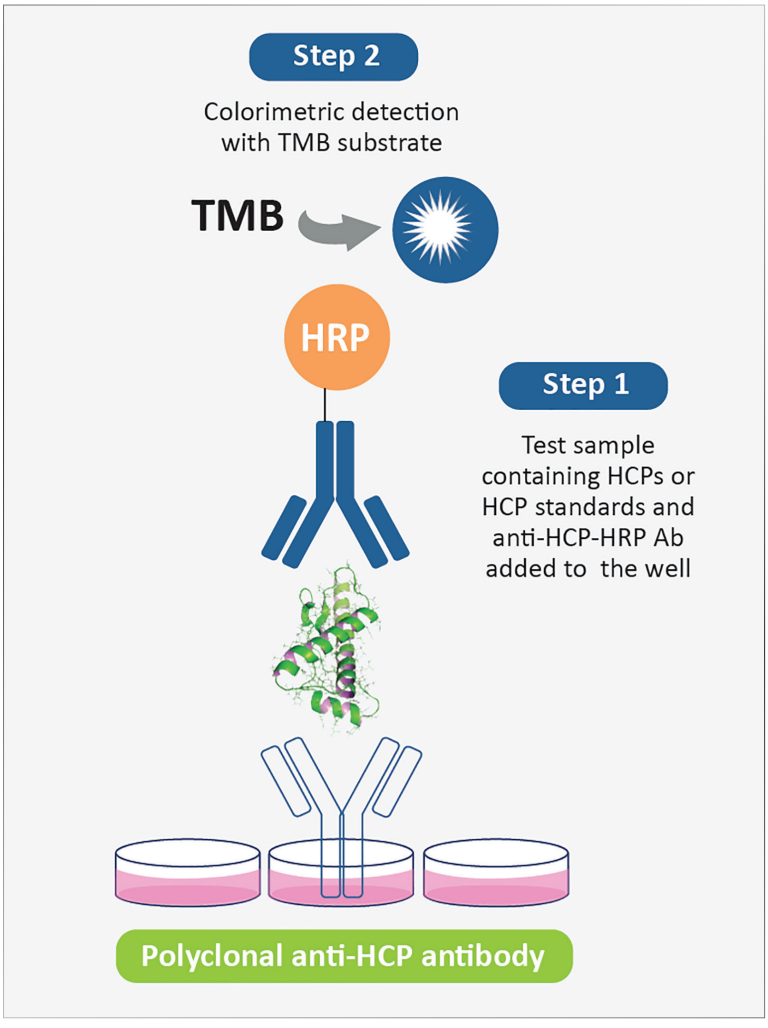
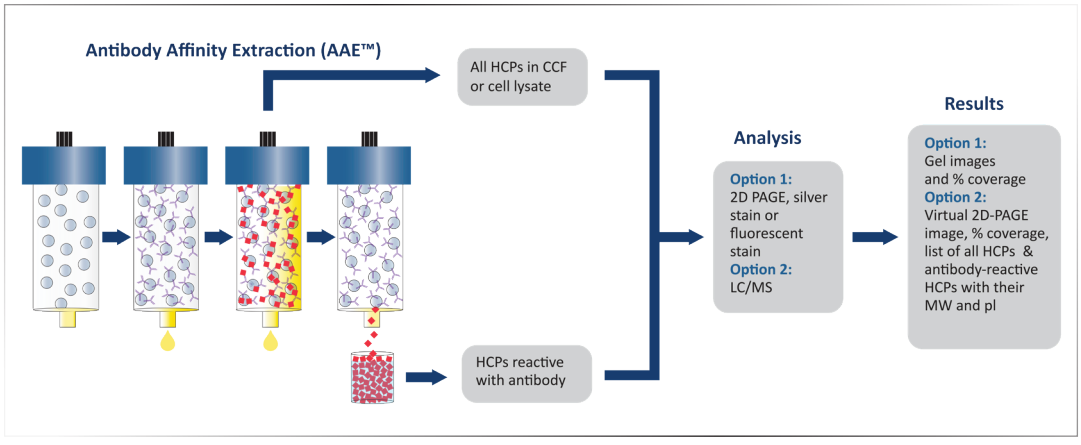


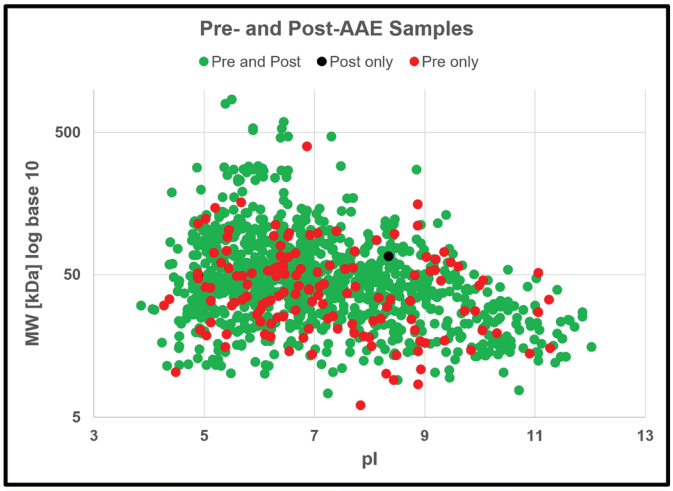
Figure 2. Antibody coverage by AAE with 2D PAGE or MS analysis options.



the assay for dilution linearity, accuracy, and precision (3).

For lack of better coverage analysis methods, 2D Western Blot (2D WB) has been traditionally performed from large format two-dimensional polyacrylamide gel electrophoresis (2D PAGE) gels with protein transfer to membranes for 2D WB comparison to silver stain. Because of the recognized sensitivity limitations of 2D WB, the conventional acceptance criteria are that >50% of the total HCP should be reactive and that the antibody must recognize HCP in all four quadrants of a 2D PAGE gel. While using 2D WB, coverage can only be estimated on upstream harvest samples where the concentration of most HCPs is still within the sensitivity limitations of various staining methods. In addition, 2D PAGE loading capacity, destruction of native epitopes by harsh sample treatment, and potential steric hindrance of HCP antibody binding epitopes lead to poor sensitivity and specificity of 2D WB. Due to these reasons, 2D WB significantly underestimates true antibody coverage to upstream HCPs. More importantly, 2D WB does not predict how that antibody will quantitatively react to the most important HCPs which are those that co-purify with the drug substance.

Figure 3. Virtual two-dimensional gel of harvest sample: molecular weight versus isoelectric point.



	Pre-AAE	Post-AAE	% Coverage
Number of HCPs	1129	993	88%

Antibody Affinity Extraction (AAE™), a type of immunoaffinity chromatography, is an advanced orthogonal method designed to assess the coverage of a polyclonal antibody to an array of HCPs present in a given process, as well as HCP antibody reactivity to downstream, process-specific HCPs that may co-purify with a drug substance. This method was developed by Cygnus Technologies in 2013 to overcome the analytical deficiencies of the traditional 2D WB and two-dimensional differential in blot electrophoresis (2D-DIBE) orthogonal methods used to assess the coverage of polyclonal antibodies to total host cell protein.

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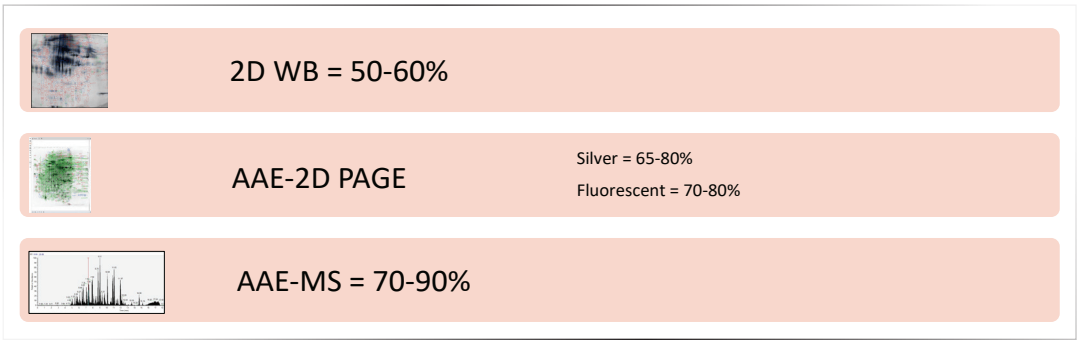
Figure 4. Coverage is dependent on evaluation method.



ANTIBODY COVERAGE ANALYSIS BY AAE™

To perform AAE, the polyclonal antibody is covalently immobilized on a chromatography support. The column is then conditioned to prevent significant leaching of the antibody and to minimize any non-specific binding. The HCP sample in its native, undenatured 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 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 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 (**Figure 2**). The coverage is assessed by the number of HCPs in the AAE elution fraction (silver stain or Cy3 labeled) compared to those seen in the 2D PAGE of the starting antigen sample (silver stain or Cy5 labeled).

Combining AAE with mass spectrometry (AAE-MS™) for HCP antibody coverage analysis is a powerful method which in addition to % coverage, identifies HCPs in the harvest material and HCPs reactive with the antibody and yields protein molecular weight and pI (isoelectric point) information (**Figure 3**). Importantly, AAE-MS is the only method that can reliably assess HCP antibody coverage when the only available sample is a product (drug substance) containing harvest material and not the clarified culture fluid (CCF) derived from a null cell line.

It is important to note that for the same anti-HCP antibody and process, coverage percentage depends on the assessment method and can significantly differ between various methods. Numerical coverage comparisons should be used with caution because of the many method variables and the art required to reproduce results, even with the same reagents in the same laboratory. Because of this variability, the results are best evaluated qualitatively (Figure 4). The main purpose of HCP antibody coverage analysis is to demonstrate that the antibody is broadly reactive to an array of HCPs that may potentially co-purify with a drug substance in a given process. To demonstrate that HCP ELISA is fit for purpose, the assay has to be qualified for dilution linearity, accuracy, and precision.

Cygnus Technologies, part of Maravai LifeSciences, offers generic HCP ELISA Kits for 23 different expression platforms, advanced orthogonal antibody coverage analysis services, HCP identification in process samples and drug substances by AAE-MS, generic assay qualification services, and expert process-specific antibody and assay development services. Cygnus' reputation for quality is recognized by the industry and global regulatory agencies, with several generic HCP ELISA Kits supporting marketed biologics. Cygnus' proprietary technology has also been utilized to develop over 100 process-specific antibodies and immunoassays for many global biopharmaceutical companies.

REFERENCES

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2. ICH Q6B "Test Procedures and Acceptance Criteria for Biotechnological/ Biological Products," 1999
3. USP Chapter 1132 "Residual Host Cell Protein Measurement in Biopharmaceuticals," 2016