

Challenges and Solutions in HCP ELISA Development



Excellence in Antibody and ELISA Development

How BioGenes uses Quality by Design (QbD) to overcome critical steps of HCP ELISA development for the reliable determination of process-related HCP impurities to be monitored during biopharmaceutical manufacturing

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The Need for HCP Monitoring

Regulatory authorities have made congruent recommendations on the use of different host cell protein enzyme-linked immunosorbent assay (HCP ELISA) formats to detect HCPs. Depending on the developmental phase of biologicals, two general approaches were established: the generic HCP ELISA, sometimes also referred to as a commercial HCP kit, and the process-specific or multi-product HCP ELISA. The suitability of an HCP ELISA is usually determined by performance criteria, such as the assay's sensitivity to detecting HCP trace amounts even in highly purified samples demonstrating the HCP log-reduction over the downstream process (DSP), the stringent dilution linearity, and a sufficient HCP-specific antibody coverage. The application of QbD approaches during HCP ELISA method development allows for the identification of critical steps, which BioGenes is able to address with a variety of sophisticated services and techniques (see also Fig. 1).

Careful Assay Design

Undoubtedly, the performance of an HCP ELISA mainly depends on the overall quality of the polyclonal antibodies (pAb), which are generated by the immunization of animals, with the HCP mock material serving as the antigen. One critical factor is a high similarity in the HCP spectrum of both the HCP mock material and a process sample originating from recombinant drug substance (DS) production, which indicates that the mock is suitable for pAb generation. This challenge is addressed prior to immunization by two-dimensional Difference Gel Electrophoresis (2D DIGE). Here, the HCP spot pattern of an early DSP sample is qualitatively compared to the pattern of a given mock sample. Another challenge results from

the varying immunogenicity of individual HCP species. In particular, HCPs of low molecular weight (LMW) tend to be less immunogenic, and thus an underrepresentation of LMW-specific antibodies is frequently observed in standard HCP immunization regimes. Therefore, BioGenes performs the fractionation of the HCPs prior to the immunization, which is then carried out using the total HCP material and the LMW fraction, respectively. Secondly, a differential pAb panel can be generated by employing two different host animal species (goat and rabbit) to be immunized, which allows for another level of selection. The HCP-specific immune response in individual animals is monitored by ELISA titer determination and western blotting. This also includes antiserum testing for cross-reactivity against the DS, to exclude false-positive HCP ELISA results during the process sample analysis. Furthermore, a preliminary ELISA is set up with affinity-purified antibodies and its performance is evaluated by a DSP sample measurement based on the key parameters mentioned above (sensitivity, log-reduction, dilution linearity). From this, one species which matches the quality criteria is selected for extended immunization and subsequent large-scale antibody purification.

Completion of an HCP ELISA

A coverage analysis is performed to determine the ratio of HCP species that are successfully detected by the pAb, which are then divided by the total number of HCPs detected (percentage HCP coverage). This is performed by high resolution 2D fluorescence western blotting, using the HCP mock material and/or an early DSP sample. Alternatively, BioGenes uses immunoaffinity chromatography (IAC) with immobilized pAb, followed by 2D DIGE as an orthogonal method for coverage analysis.

However, both analytical approaches come with inevitable methodological limitations. To achieve a scientifically sound estimation of the HCP coverage, BioGenes strongly recommends applying both methods for best reagent characterization. For the final QbD step, the actual HCP ELISA is set up. Optimization is focused on the titration of all reagent concentrations and incubation times, accompanied by an evaluation of assay specificity, accuracy and precision. The state-of-the-art HCP assay development strategy suggested by BioGenes includes both the HCP mock material and a relevant process sample (such as purified DS) during method optimization. Taken together, the consideration of the critical steps mentioned here allows for the implementation of robust and reproducible HCP monitoring during biological drug manufacturing.

Take home messages:

- Ensure HCP monitoring along the biologics drug production process
- Allow sufficient development time for an HCP ELISA assay
- Use LMW HCP fraction for immunization to tackle immunogenicity issues
- Perform immunization of two different species for optimal results
- Consider methodological limitations and increase the reliability of your HCP coverage determination by application of orthogonal methods such as 2D western blotting, IAC-2D DIGE and potential supplementary mass spectrometry

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Fig. 1: Quality by Design approaches to support HCP ELISA development

