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Critical Steps to Consider During Customized Host Cell Protein (HCP) ELISA Development

by Stefan Sommerschuh and Dr. Pia Paarmann

The manufacturing of recombinant drugs requires comprehensive monitoring of process-related impurities, such as HCPs.[1] These proteins may pose a significant health risk due to their potential to either directly induce immunogenic reactions or to serve as an adjuvant in patients. They may further alter the drug's mode of action by either modifying the drug itself and/or relevant excipients. HCPs may also impact drug potency, depending on their endogenous biological function. EMA and FDA approval thus relies on robust analytical data to prove acceptable HCP clearance. To ensure a safe product, different guidance documents offer recommendations for market authorization.[1-3]

The enzyme-linked immunosorbent assay (ELISA) is the gold standard method for monitoring HCP content during product development and batch release testing within clinical and commercial phases. Supporting data from various orthogonal methods, including 2D Western Blot/2D DIGE analysis and mass spectrometry, should be collected to further complete the analytical puzzle during HCP assay development.[4]

BioGenes has provided customized immunoassays and antibodies since 1992, and is skilled in finding solutions to the most challenging problems. A strong commitment to quality, service and proactive project management makes BioGenes a reliable industry partner. In this article, our experts - Stefan Sommerschuh (Head of Immunoassay Department) and Pia Paarmann (Head of 2D Analytics) - share their knowledge and answer some frequently-asked questions about the preparation and development of customized HCP ELISAs (see figure on the next page). Stefan and Pia give recommendations for the entire process, from proper mock preparation to antibody performance criteria and reagent characterization by orthogonal methods.

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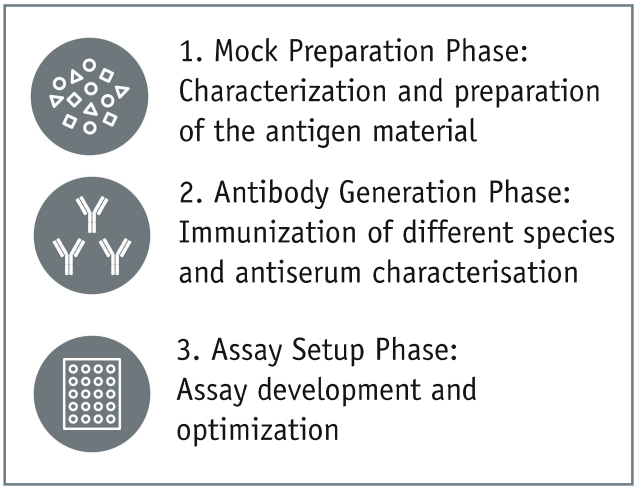

BioGenes customized ELISA development process

Mock Preparation & Characterization

Mock fermentation mimics the production process, and the resulting mock material should represent the HCP impurities of the production process as much as possible. The bottleneck of many development projects is the preparation of proper mock material as antigen for immunization.

Question: How should suitable mock material be prepared?

Answer: Our recommendation for generating a mock sample which is representative of the corresponding production process is transfecting the cell line with an empty vector, but without the product-encoding gene, then using the same cultivation conditions as those used for product fermentation. Cell viability at harvest should be comparable to ensure similar HCP expression patterns. One shall be careful to use the correct HCP antigen fraction (*i.e.*, cell lysate or supernatant), which depends on the way in which the drug is produced in cells.

Another aspect to consider is hitchhiker HCPs. The purification of the mock sample may result in the depletion of host cell proteins which are normally non-covalently bound and, accordingly, co-purified with the drug substance. This is why we recommend collecting the mock sample at an early downstream step prior to effective HCP purification. The mock sample will thus provide an HCP pattern which is as complex as possible, closely resembling the regular production process.

Question: How do you know that the mock material is suitable?

Answer: We generally recommend comprehensive characterization of the mock material, using a broad spectrum of analytical methods, such as 1D and/ or 2D electrophoresis, prior to antibody generation.
To evaluate whether the mock material successfully represents the HCP composition of a product-production process harvest sample, a comparative 2D DIGE analysis can be performed. The HCP spot patterns of the respective mock and production harvest samples are then visualized using fluorescent labelling and qualitatively compared. Provided that there is a high degree of similarity, the mock sample can serve as antigen material for HCP antibody generation.

Antibody Generation

The polyclonal antibodies of the HCP ELISA determine the sensitivity and selectivity of the respective assay. They are essential reagents, and should therefore be properly examined.

Question: How can the analytical range of an ELISA be improved?

Answer: HCPs are a complex mixture of proteins, showing a broad range of molecular size, physicochemical properties and immunogenic potential. A limitation of an ELISA is that antibodies do not usually cover the entire HCP spectrum of the production process. In particular, low molecular weight (LMW) HCPs are often weakly immunogenic and/or low abundant, so LMW HCP-specific antibodies might be underrepresented in the final reagent. Consequently, the detection of these HCP species may be insufficient.
To achieve broader HCP coverage, we recommend preparing a suitable LMW HCP

fraction (*e.g.*, by ultrafiltration of the total mock material) and separately immunizing the LMW HCP and total HCP fractions in a parallel approach.

Question: Why does BioGenes use a dual animal approach?

Answer: Antibody properties and their suitability for HCP detection in a given process may differ significantly between animal species. Antibody generation can take several months. BioGenes recommends immunizing two different animal species (*e.g.*, rabbit and goat) right from the start to be able to compare antibody performance head-to-head by multiple methods (*e.g.*, Western blotting and Sandwich ELISA setup).

Reagent Characterization & Assay Development

The HCP ELISA should be able to recognize a broad spectrum of HCPs in process-derived samples with proper assay performance.

Question: How does BioGenes assess antibody suitability for reliable HCP detection in process samples?

Answer: To evaluate the suitability of a given set of HCP-specific antibodies, the respective ELISA data and the corresponding antibody performance criteria should be taken into account. Generally, ELISA should detect HCPs down to low ppm-range based on a sensitive HCP standard curve. When real process sample measurement occurs during assay development, the HCP ELISA should adequately reflect the reduction of the HCP impurity content alongside consecutive purification steps. Dilution linearity (dilution-corrected HCP concentrations remain comparable over successive dilution steps) should be evaluated, in particular for highly purified samples (*e.g.*, final bulk drug substance).
Finally, HCP coverage is an important parameter when evaluating antibody suitability for monitoring HCP clearance in a given production process. Coverage analysis should therefore be performed, ideally by using a set of orthogonal methods.

Question: Why should HCP antibody coverage analysis be conducted using orthogonal methods?

Answer: Every analytical method comes with technical limitations: orthogonal approaches help to compensate for this.
The two-dimensional Western blot enables a direct comparison of a given HCP spot pattern with the corresponding signal pattern from immunostaining by using the ELISA antibodies. Fluorescent protein labelling allows for the visualization of both patterns on the same membrane and precise determination of HCP antibody coverage by individual spot counting. Western blotting does not require physiological buffer conditions for the sample of interest, thereby enabling coverage analysis of some very special sample types (such as a solubilized inclusion body). However, the denaturing assay conditions and further technical restrictions regarding the dynamic range of detection when analyzing several hundred proteins on one single membrane usually limit the informative value of a 2D Western blot.

Potential method-related underestimation of HCP antibody performance is best addressed through orthogonal methods. We have established immunoaffinity chromatography (IAC) in combination with 2D DIGE to further support antibody coverage analysis. The latter method allows for protein-antibody interaction under nearly native assay conditions. The dynamic range of detection is usually higher when compared to Western blot-based approach. Low abundant protein species can be enriched on the capture column, and there are fewer technical restrictions related to high levels of the drug substance in real process samples. However, one problem with the IAC method is the potential co-detection of indirect binders.

Question: Why should mass spectrometry (MS) be considered?

Answer: Mass spectrometry is clearly a powerful supportive method for rounding up the analytical package and further digging into the details. The identification of single HCPs may be very helpful in the context of risk assessment. As with the other methods, MS technology also presents technical challenges, such as detection limits, suitable protein databases and proper assay controls. We feel that customers should make use of any available technique to characterize their process and reagents, thus finding good solutions to their individual project-specific questions.

References

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Key points

- Allow sufficient development time for an HCP ELISA
- Make sure to use proper mock material for antibody generation
- Use total HCP and LMW HCP fractions for immunization in a dual animal approach to tackle immunogenicity issues
- Use orthogonal analytical methods for comprehensive reagent characterization

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