Making the Right Choices for Reliable HCP Monitoring by ELISA

The clearance of biopharmaceutical drugs from host cell protein (HCP) impurities during manufacturing remains a constant challenge, necessitating the reliable monitoring of their removal from the final drug product. The ELISA is the broadly accepted gold standard for HCP monitoring. Important steps for customised HCP ELISA development will be discussed

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Biopharmaceutical drugs make up a large portion of global pharmaceutical sales, with eight of the top ten global drug blockbusters in 2019 being recombinant biopharmaceuticals (1). To ensure high levels of patient safety during clinical trials and upon drug product release, a multitude of regulations have been authored by different regulatory bodies, such as the FDA and the EMA (2-3). The market authorisation application requires a profound overall assessment of potential risks and benefits, the critical quality attributes (CQA), which are included in the Common Technical Document. Host cell proteins (HCPs) are one such CQA (4). They form a complex mixture of proteins with different physiochemical and immunological properties, and are released by the production cell line during biological product manufacturing (5).

The HCP formation by complex cellular production systems is influenced by a multitude of biotic and abiotic factors, as summarised before, making it hard to predict the HCP pattern of individual manufacturing processes (6-7). Defined as process-related drug impurities, HCP can negatively influence the quality, safety, and efficacy of a biological drug product (8). In particular, ICH Guidelines Q6B, Q8(R2), and Q11 define such impurities and address the need for precise monitoring and the reduction of HCP during stepwise downstream processing (DSP), all the way down to low amounts (9). Although no precise values are specified, the common agreement is to reduce the HCP burden below 100ppm in the final drug substance and below 10ng/dose (4).

Naturally, the accurate detection of HCP impurities in subsequent DSP samples down to the final drug substance depends greatly on the establishment of a reliable and robust method for HCP measurement. To achieve this, the use of multi-faceted HCP analysis methods is recommended (10). The enzyme-linked immunosorbent assay (ELISA) is still considered the gold standard for HCP measurement, having advantages like high speed, sensitivity, and high throughput.

Figure 1: Overview of drug development steps, including downstream process development and recommended use of different HCP ELISA formats for HCP monitoring

Nevertheless, to overcome intrinsic limitations of individual methods for HCP quantitation, the implementation of orthogonal methods is strongly advised (10). Undoubtably, the emergence of mass spectrometric methods for total HCP determination and individual HCP identification has broadened the overall spectrum of HCP monitoring, as outlined, for example, by Bracewell DG *et al* (4). However, the ELISA has many advantages with regard to the ease of handling, speed, and sensitivity. Therefore, the focus of this article is on key points of HCP ELISA development.

Selection of the Appropriate HCP ELISA

The HCP ELISA for HCP quantitation can be divided into three main formats: i) the generic ELISA, also referred to as commercial HCP ELISA; ii) the platform (or multi-product) HCP ELISA; iii) the process-specific HCP ELISA. While the generic HCP ELISA makes use of a broadly active antibody coverage approach, which is specific only for the selected cell line of recombinant protein production, the latter two HCP ELISA formats are based on greater specificity towards the manufacturing and processing of particular biopharmaceuticals.

The answer to the question: 'Which HCP ELISA is best to use during which phase of development of a drug candidate?' is not a clear one (see **Figure 1**). As depicted, changes in DSP development can still happen during Phases I and II of clinical trials. Completion of the latter then requires the definition of process parameter specifications for high process robustness. By contrast, the drug substance used for testing in Phase III of clinical trials has to meet identical requirements as for continuous drug production after marketing.

For this phase of drug manufacturing, all of the processes need to be validated, including the use of analytical methods. If changes to manufacturing steps are carried out at this stage, process validation has to be repeated until a sufficient consistency is achieved. This also includes the monitoring of residual HCP impurities, which can technically vary in amount and composition when changes during manufacturing or DSP are introduced. The most common recommendation is, therefore, only to rely on a broadly active generic HCP ELISA during method development.

When moving forward towards application for extended clinical trials in Phases II and III, the implementation of a process-specific HCP ELISA usually proves adequate for HCP monitoring, allowing the criteria for assay validation to be met. The use of a platform HCP ELISA can be sufficient when manufacturing and DSP conditions and the principal nature of different biopharmaceuticals only vary slightly, without having a major influence on the respective HCP pattern. Still, it needs to be considered that a generic HCP ELISA might have limited availability throughout the lifecycle of a biopharmaceutical. This risk can be significantly mitigated when choosing the development of a customised HCP ELISA which ensures proper reagent supply.

Careful Functional Assay Design

The suitability of an HCP ELISA for HCP monitoring is usually determined by performance criteria. This is the assay's sensitivity to detecting HCP trace amounts even in highly purified samples demonstrating the HCP log-reduction over the various steps of DSP, the stringent dilution linearity, and a sufficient HCP-specific antibody coverage, as highlighted in **Figure 2** (page 62). The selection of appropriate HCP mock material for polyclonal antibody (pAb) generation is critical for HCP ELISA development.

Ideally, great similarity in the HCP spectrum of both the HCP mock material and a process sample originating from recombinant drug substance production would indicate mock suitability for pAb generation. This can be analysed using 2D difference gel electrophoresis (DIGE) technology. Here, the HCP spot pattern of an early drug substance production sample is qualitatively compared to the pattern of a given mock sample. Secondly, HCP of low molecular weight (LMW) tend to be less immunogenic, and thus an underrepresentation of LMW-specific antibodies is frequently observed in standard HCP immunisation regimes.

A counter-strategy is the fractionation of the HCP prior to the immunisation of host animals, with both fractions carried out at the same time. Furthermore, a differential pAb panel can be generated by employing two different host animal species (goat and rabbit) to be immunised, which allows for another level of selection. The HCP-specific immune response in individual animals is monitored by ELISA titre determination and Western blotting. This also includes antiserum testing for cross-reactivity against the drug substance (DS), in order to exclude false-positive HCP ELISA results during the process sample analysis. A preliminary ELISA is then set up with affinity-purified antibodies, and its performance is evaluated based on the key parameters detailed above. From this, the species that best matches the quality criteria is selected for extended immunisation and subsequent large-scale antibody purification.

Reagent Characterisation by Use of Orthogonal Methods for HCP ELISA Qualification

The assessment of the suitability of an HCP ELISA for HCP monitoring includes HCP-specific antibody coverage. This coverage analysis is performed to determine the ratio of HCP species that are successfully detected by the pAb, expressed as percentage HCP coverage. The traditional method is the 2D Western blot, using the HCP mock material and/or an early DSP sample. As mentioned above, the use of orthogonal approaches is advised. One such method involves the usage of immunoaffinity chromatography (IAC) with immobilised pAb, followed by 2D DIGE. The 2D DIGE

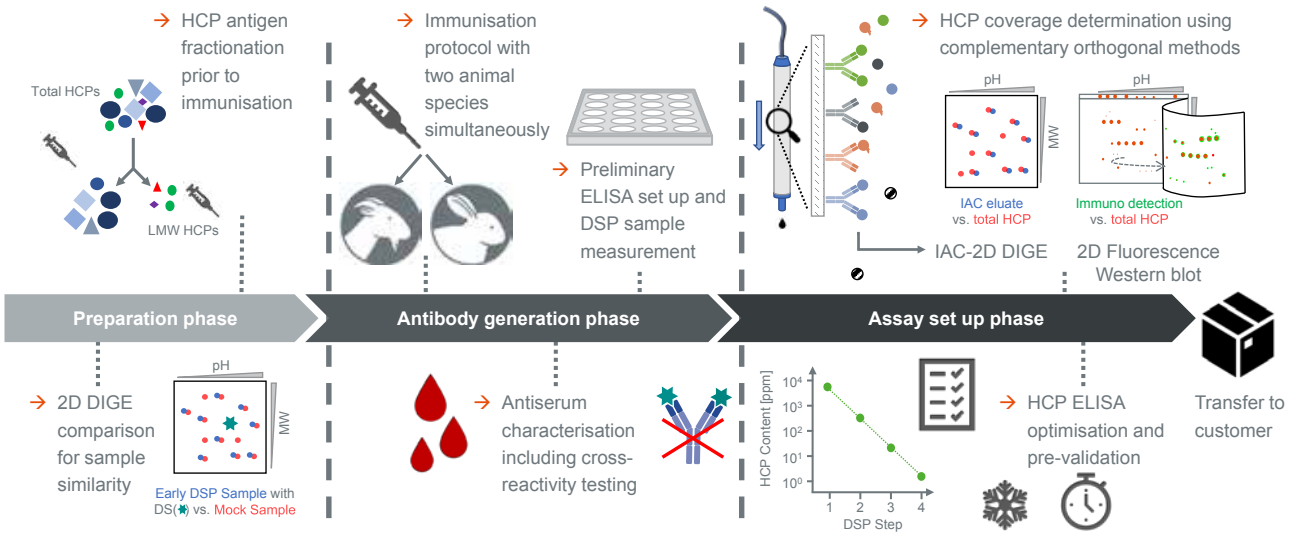

Figure 2: Schematic workflow, including critical steps marked along the different phases of specific HCP ELISA development

comparison of the IAC eluate of the pAb-bound HCP fraction with the total HCP sample allows for the estimation of HCP coverage by the antibodies under non-denaturing conditions. However, both analytical approaches come with inevitable methodological limitations.

To achieve a scientifically sound estimation of the HCP coverage, the application of both methods for best reagent characterisation is strongly recommended. As briefly touched upon above, advances in mass spectrometry may aid the HCP coverage evaluation. Recently, interesting technical combinations of immunoaffinity and mass spectrometric methods have been suggested, such as bead-antibody affinity purification or the directly linked ELISA-MS (11-12). It remains to be seen how quickly these methods will be accepted as analytical tools for complementary coverage determination in the future.

The completion of an HCP ELISA set up includes optimisation with a focus 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 here includes both the HCP mock material and a relevant process sample (such as purified DS) during method optimisation. When factoring in the average developmental time for a process-specific HCP ELISA of at least 1.5 years, manufacturers of biopharmaceuticals are advised to begin planning for the introduction of reliable HCP monitoring assays during drug development as early as possible, in order to have a functional HCP ELISA at hand when assay validation is due. This also plays a role when a switch of manufacturer for continuous drug supply is allowed. The consideration of the important steps mentioned here allows for the implementation of robust and reproducible HCP monitoring during biological drug manufacturing.

Take-Home Messages

- Ensure HCP monitoring throughout the biopharma drug production process
- Allow sufficient development time for a customised HCP ELISA assay
- Use LMW HCP fraction for immunisation to tackle immunogenicity issues
- Consider methodological limitations and increase the reliability of your HCP coverage determination by applying orthogonal methods, such as 2D Western blotting, IAC-2D DIGE, and potential supplementary mass spectrometry

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