ProtaGene
Analytical Excellence

Innovation Spotlight

Risk Mitigation of Your Host Cell Protein Control Strategy

Identify Gaps in Host Cell Protein ELISA Detection Using Immunoaffinity Chromatography Mass Spectrometry (IAC-MS) to Evaluate Possible Risks for Patients and Products

Innovation Spotlight

Host cell proteins (HCPs) are impurities present in all therapeutics derived from biological sources and comprise a significant portion of process-related impurities within biotechnology production. Due to product safety and efficacy risks, a drug product's overall quantity of residual HCPs as well as the presence of high-risk HCPs is a critical quality attribute (CQA) that must be characterized and controlled throughout the manufacturing process.

Overview

Immunoassay, most commonly in the form of sandwich enzyme-linked immunosorbent assay (ELISA), has been and continues to be regarded as the gold-standard method for monitoring HCP clearance at the point of product release. ELISA's high sensitivity, high throughput, ease of use, and established position in the market allowed it to earn the reputation as the "workhorse" of HCP detection. However, HCP determination by ELISA does have a key shortcoming that presents a potential safety risk. Arguably, ELISA's most significant limitation in HCP detection and monitoring is that these assays rely on custom immunoreagents, which are generated using crude protein material from the host cell to raise polyclonal antibody (pAb) reagents in different animal species such as rabbit, goat, or sheep. As such, the pAb reagent is comprised of an undefined, highly diverse mixture of antibodies to a complex set of protein targets, and as a result, the assay is limited by the level and quality of coverage of HCPs by the pAbs used for detection.

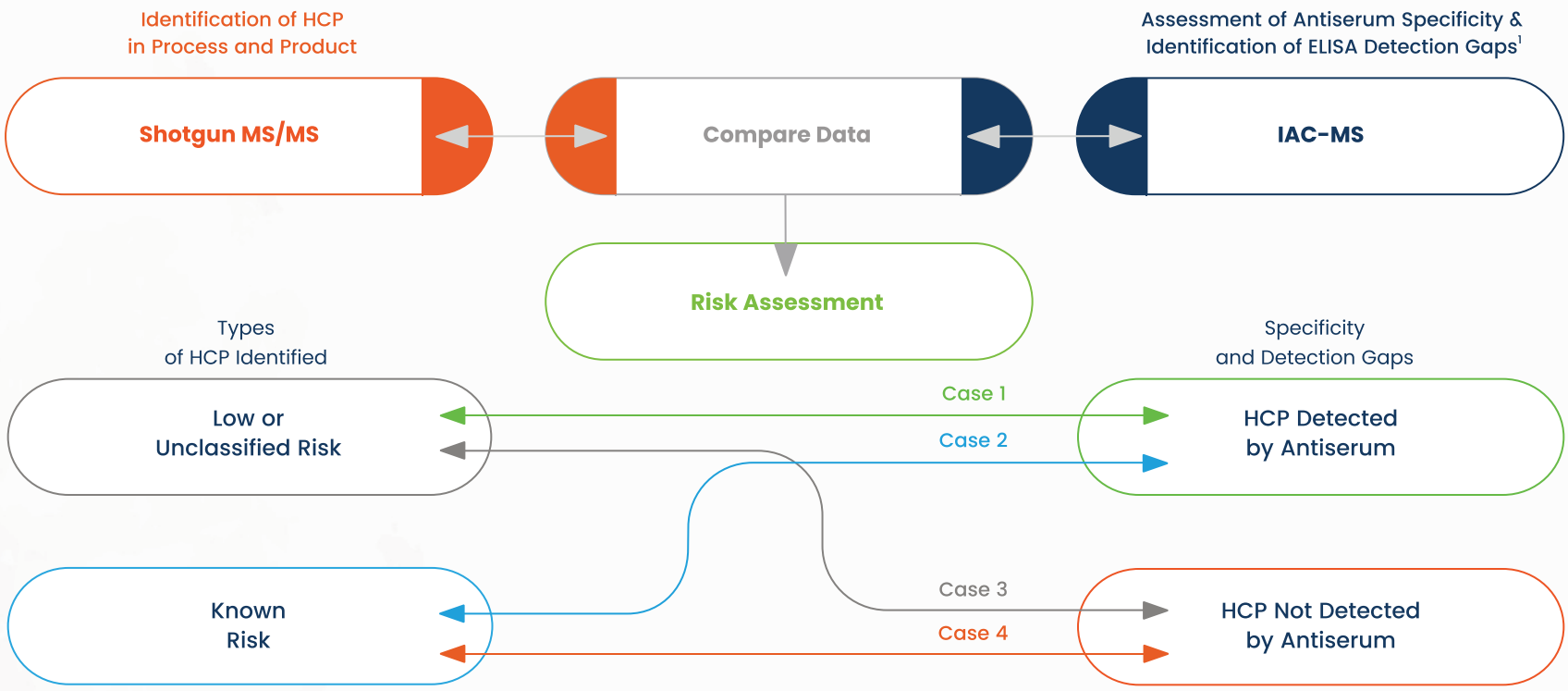
pAbs are raised against a complex HCP pool, often an upstream processing (USP) sample, in which some species may be over- or under-represented. Additionally, immunoreagents generated using a downstream process (DSP) sample can be lacking in HCPs that may appear in a downstream purification process due to variability or as the result of a DSP change. Because not all HCPs are equally represented in any one sample and individual HCPs have varying degrees of immunogenicity, the use of immunoreagents typically leads to gaps in HCP detection by ELISA methods. These gaps can lead to safety risks and diminished product quality. For example, even at low residual levels some HCPs are toxic and certain lipases can lead to significant surfactant degradation within the drug product. Fortunately, gaps can be unambiguously identified by immunoaffinity chromatography coupled to mass spectrometry IAC-MS. [1]

IAC-MS involves comparative analysis in which shotgun MS/MS analysis is used to identify the full set of HCPs present from cell culture fluid harvest and DSP samples, and most importantly, within drug substance (DS) as shown in Figure 1. In parallel, specificity of the pAbs used for ELISA are assessed as a control to provide identities of all HCP detected and, notably, reveal those not detected, to inform safety gaps. Correlation of all HCPs identified within DS with all HCPs detected by ELISA antiserum comprises the basis for risk assessment as shown in Figure 1. On this basis, potentially necessary further action can be taken to remediate risks.

An actual example of risk assessment for two different ELISA pAbs is shown in Table 1. In this case an unambiguous decision for ELISA reagent 2 could be made due to complete detection of all HCPs present in DS.

1 Host cell protein detection gap risk mitigation: quantitative IAC-MS for ELISA antibody reagent coverage determination, MAbs, August 2021

Figure 1. IAC-MS Approach and Risk Evaluation



Case	Description	Risk	Action
1	HCP with unclassified risk present in DS All of these HCP recognized by antiserum	Low	None: ELISA sufficient
2	HCP with known risk present in DS All of these HCP recognized by antiserum	Low to Medium	Optimization of DSP
3	HCP with unclassified risk present in DS Not all of these recognized by antiserum	Medium to High	Establishment of additional antiserum to close ELISA gap. Alternatively: MS analysis of all production batches
4	HCP with supposed high risk present in DS Not all of these recognized by antiserum	High	Optimization of DSP. Establishment of HCP-specific ELISA/MS assay or additional antiserum to close ELISA gap

Table 1. Example of Comparative Risk Assessment Using IAC-MS Approach Applied to Two ELISA Immunoreagents

HCP Identity from Shotgun MS of DS	HCP Level (ppm) by MS of DS	Detected by IAC-MS Using ELISA 1 Antiserum	Risk Level ELISA 1	Detected by IAC-MS Using ELISA 2 Antiserum	Risk Level ELISA 2
Carbonic Anhydrase	21	Yes	Low	Yes	Low
Protein Disulfide Isomerase	39	Yes	Low	Yes	Low
Annexin A2	13	Yes	Low	Yes	Low
Peroxiredoxin 1	15	Yes	Low	Yes	Low
Actin, Cytoplasmic	26	Yes	Low	Yes	Low
PLBL2	18	No	High	Yes	Low
Cathepsin D	3	Yes	Low	Yes	Low

Scope for Use of IAC-MS as Central Risk Assessment Approach to HCP Control Strategy

The MS-based HCP risk assessment approach facilitates:

- Ranking of performance and usefulness of different generic HCP antisera for early clinical stages
- Root cause and investigational analyses of HCP irregularities in production processes or in product release and stability testing
- Demonstration of suitability of HCP antisera of platform ELISA assay for additional products, e.g., antibody-based new biological entities (NBEs)
- Performance evaluation of process-specific HCP ELISAs for late-stage clinical testing

Meet the Innovators

"The established IAC-MS approach provides a detailed analysis of HCPs detected by the respective antiserum used for ELISA. Compared to classical 2D-PAGE specificity coverage analysis, where typically you only obtain an overall percentage for coverage without identifying individual protein identities, this approach is a real game changer to judge the safety of your ELISA-based control strategy."

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