

Advanced Services for Host Cell Protein Analysis

Mass spectrometry services for analysis of HCPs



A critical task in biologics manufacturing is the control of process-related impurities particularly host cell proteins (HCPs), which co-purify with the drug substance and are known for adverse effects (e.g. immunogenicity) during patient treatment.

For decades, the method of choice to detect host cell proteins has been an immunoassay based on polyclonal antisera raised against a population of HCPs. However, there are some inevitable risk factors (e.g. antigens, animals, etc.) associated with this approach, and the final selectivity and sensitivity of the HCP assay strongly depends on the preparation work and materials used at the start.

PPS is offering following services for HCP analysis:

Protagen Protein Services GmbH (PPS) has extensive experience in providing structural analytical services to characterize antibody products and to provide data required by

- ▶ HCP-GAPexSM Services – Development of HCP assays with improved sensitivity and selectivity
- ▶ Mass spectrometric identification of HCPs by shotgun proteomics techniques as orthogonal method to immunoassays

Host Cell Protein Analysis

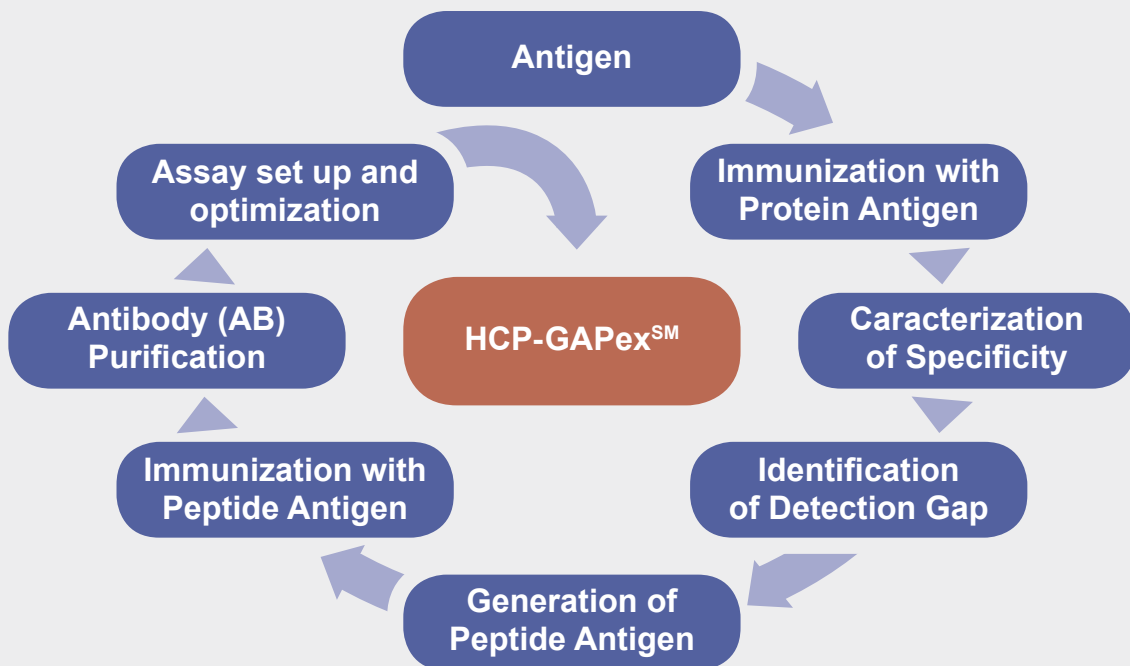
HCP-GAPexSM – Development of HCP assays with improved sensitivity and selectivity

HCPs not recognized by respective antisera used in immunoassay based release tests comprise significant risks for patients. HCP-GAPexSM addresses these detection gaps by following approaches:

- ▶ Proteins, which represent detection gaps can be unambiguously identified by a combination of high-

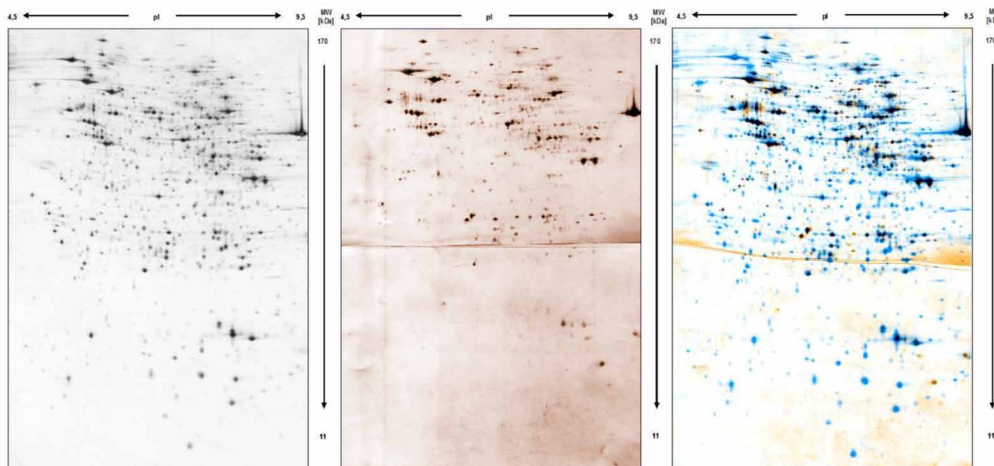
resolution 2D Western Blotting with subsequent mass spectrometric analysis

- ▶ Identified proteins are screened for immunogenic peptides, which are basis for design and production of protein specific antigens
- ▶ Protein specific antibodies recognizing previous HCP gaps are combined with antiserum in order to obtain maximum detection coverage and patient safety



HCP-GAPexSM is a combined service of PPS in our strategic alliance with Charles River Labs - Biopharmaceutical Services

Case Study: Assessment of specificity by high-resolution 2D-Western Blotting



CHO cell lysate analyzed by high resolution 2D PAGE and Western Blotting (overlay: blue-silver stain, yellow-Western Blotting)

Mass spectrometry as orthogonal method to immunoassays



Significant improvements in mass spectrometry facilitate its use as a complementary method to immunoassays in order to gain more detailed information on individual HCP patterns and species.

For identification of HCP during DSP and in the final drug product solution digestion and separation techniques (e.g. RP-HPLC or ion exchange chromatography) are combined with online analysis on highly sensitive mass spectrometers such as Orbitrap and QTOF systems.

Methods for HCP analysis	Sensitivity	Main advantage(s)	Main disadvantage(s)	Eligibility for release purposes
Immunoassay	+++	<ul style="list-style-type: none"> • Clear endpoint • High sensitivity • Validation feasible 	<ul style="list-style-type: none"> • Black-box detection • HCP detection gaps 	Yes
2D gel-electrophoresis combined with WB and MS	+	<ul style="list-style-type: none"> • Overview on HCP pattern • Identification of HCPs by MS • Improvement tool for immunoassays 	<ul style="list-style-type: none"> • Lack of sensitivity 	Theoretically Yes but more useful as supporting tools for immunoassay development and improvement
Shotgun proteomics approaches (1D or 2D separation, Orbitrap or QTOF detection)	++	<ul style="list-style-type: none"> • Multi-dimensional detection of HCP patterns • Identification of individual HCPs • Excellent sensitivity for particular HCPs 	<ul style="list-style-type: none"> • Lack of sensitivity for particular HCPs • Lack of robustness due to complexity of method • Validation not feasible yet 	Not yet due to complexity and limited robustness
Targeted proteomics approach (LC-MRM analysis)	+++	<ul style="list-style-type: none"> • Robust and sensitive quantification of particular HCPs • Sensitivity for particular HCPs comparable with immunoassays 	<ul style="list-style-type: none"> • Lack of robustness due to complexity of method • Validation not feasible yet 	Not yet due to complexity and limited robustness

Summary

- ▶ Immunoassays are, due to high sensitivity and clear detection endpoints, still the method of choice for HCP release testing in protein drugs
- ▶ Some HCPs might be undetectable due to immunological gaps in the detection antiserum
- ▶ These HCPs can be unambiguously identified by a combination of high-resolution 2D Western Blotting with subsequent mass spectrometric analyses
- ▶ Protein identity of HCP gaps facilitates generation of protein specific antibodies
- ▶ Combination of these antibodies with antiserum closes detection gaps (HCP-GAPexSM)
- ▶ Mass spectrometry as orthogonal method to immunoassays is useful for HCP pattern comparison after process changes (ICH Q5E) or during biosimilar development as well as for DSP optimization
- ▶ Combining immunological tools with powerful mass spectrometric methods provides broadest coverage for detection of HCP and therefore maximum safety for patients during clinical stages and after drug approval



At Protagen Protein Services

Quality is one of the major pillars in the company's mission. It is an integral part of the company, also in non-GMP projects. It is based on a long standing scientific expertise.



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