HCP Coverage by Immunocapture and LC-MS/MS

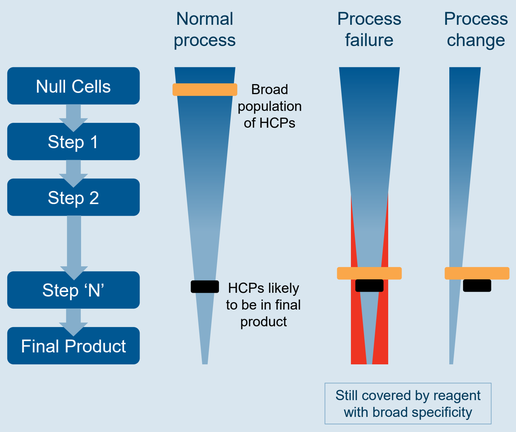
Combining ELISA and Mass Spectrometry

Katrine Pilely[1], Rikke Raaen Lund[1], Solveig Beck Nielsen[1], Anette Draborg[1,2], Maiken Lumby Henriksen[2], Søren Werner Karlskov Hansen[2], Lars Skriver[3] and Ejvind Mørtz[1]
[1]Alphalyse A/S, [2]Department of Molecular Medicine, University of Southern Denmark, [3]Savara ApS

HCP ELISAs

HCP ELISAs are important process monitoring tools. It is essential that the chosen assay has a broad coverage of HCPs in the process (yellow), as well as a specific coverage of the HCPs in the purified drug substance (black). Selecting a suitable HCP assay is important to avoid delays in time-to-market.

A broad coverage secures that the ELISA will detect alterations in HCPs if the process is changed, or in case of process failure.



Adapted from Alexey Khrenov, FDA and Denise Krawitz, CMC Paradigms

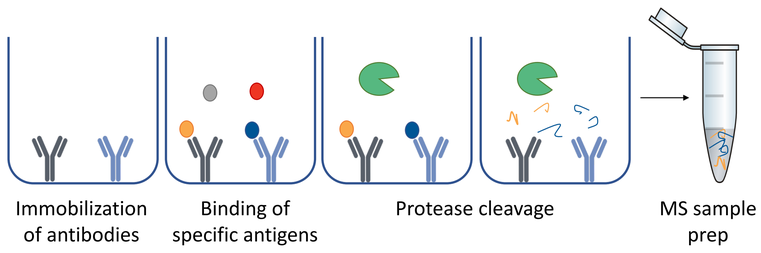
Coverage Analysis in a Plate

The method presented here is an improved coverage analysis based on immunocapture using ELISA antibodies immobilized in a plate combined with LC-MS/MS protein identification and quantification.

The analysis provides a list of individual HCPs recognized by the ELISA antibodies, as well as a coverage percent. The list of proteins covered can be compared to the actual HCPs identified in the purified drug substance to obtain a specific coverage.

Experimental Setup

Anti-HCP antibodies and isotype/negative control antibodies, were immobilized to the plates and incubated with antigen from product specific mock or early process sample. The bound antigens were digested in the plate before MS preparation. An aliquot of the antigen was digested in parallel and served as a reference for total protein content. All samples were analyzed by mass spectrometry using a TripleTOF 6600 System (Sciex®). Protein lists were generated from IDA LC-MS/MS runs searched against the relevant proteome.



This Analysis Provides

- List of individual proteins recognized by HCP antibodies in ELISA
- List of individual proteins in mock or early process sample
- HCP coverage percent
- Specific coverage of HCPs in drug substance

This Analysis is Relevant for

- Fit-for-purpose validation of a given ELISA for a specific bioprocess
- Selection of ELISA antibodies with the broadest coverage of mock or early process sample
- Documentation of HCP coverage in the purified drug substance

Evaluation of 3 Commercial HCP ELISAs

The coverage of three commercial HCP ELISAs was evaluated using an early process sample. 1265 proteins were identified in the early process sample, of these, 952 proteins were covered by ELISA A, 917 proteins by ELISA B and 662 proteins by ELISA C, corresponding to a coverage of 75%, 72% and 52% (Figure 1).

The protein lists were compared to HCPs previously identified in the corresponding drug substance by SWATH® LC-MS/MS. Table 1 shows the coverage of top 10 HCPs identified in the Drug substance. ELISA A showed the best coverage of both the process sample (75%) as well as the drug substance (9 of the 10 most abundant HCPs).

Figure 1: HCP Coverage of 3 Commercial ELISAs

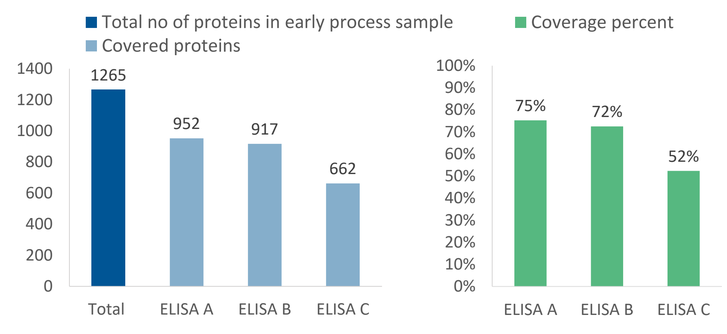


Table 1: Coverage of HCPs in Drug Substance

	ELISA A	ELISA B	ELISA C
Oxidoreductase A	+	+	+
Protein B	+	+	-
C Reductase	+	+	+
Cyclohydrolase D	+	-	+
Regulation protein E	+	-	-
Heat shock protein F	+	+	-
Protein G	-	+	-
Dehydrogenase H	+	+	+
Protein I	+	+	+
Chaperone J	+	+	+
Specific coverage	**9/10**	**8/10**	**6/10**

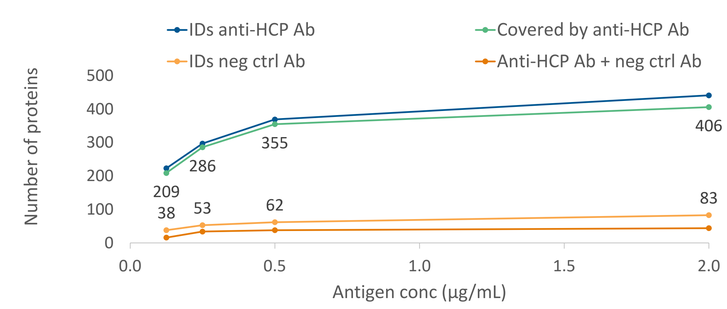
Tight Control of Unspecific Binding

As the amount of antigen increased, higher unspecific binding was observed, using a commercial antibody and a mock lysate (Figure 2). The unspecific binding is monitored by:

A) Optimization of the antibody and antigen concentrations using sandwich ELISA.

B) Comparison of the protein identifications (IDs) in the negative control to the proteins recognized by the anti-HCP antibodies.

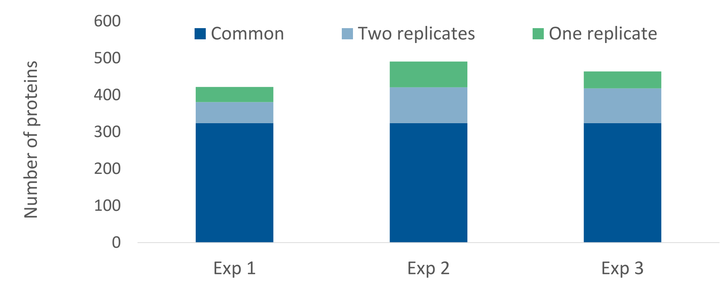
Figure 2: Low Unspecific Binding in Negative Control



Repeatability

Three independent experiments showed high repeatability in the number of protein identifications: CVs of <8% (Figure 4). Further, 89% of the identified proteins were identified in at least two replicates and 71% were identified in all three replicates (Figure 3).

Figure 3: High Repeatability among Replicates

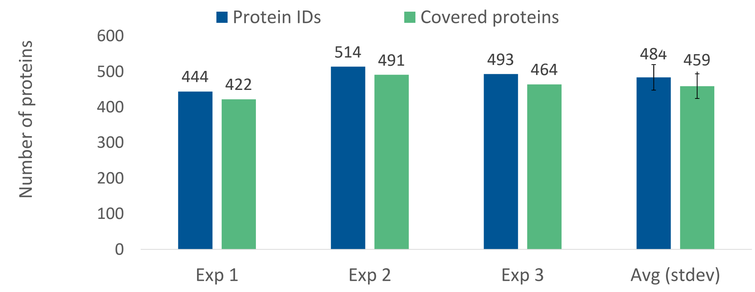


Combining assays would further increase the coverage of the process sample (Table 2).

Table 2: Combined ELISA for Higher Coverage

	Proteins covered	Coverage
ELISA A	952	75%
ELISA A and B	1015	80%
ELISA A, B and C	1036	82%

Figure 4: High Repeatability in Number of Protein IDs



Comparison of Coverage Methods

The coverage of HCP ELISA antibodies is typically determined by comparing protein spots, detected in 2-D PAGE of a mock cell lysate, to the spots detected using the ELISA antibodies in a corresponding Western blot or a 2-D PAGE after immunoaffinity binding. But according to the U.S. Pharmacopeia (Table 3) those techniques have several limitations compared to coverage analysis by ELISA immunocapture / LC-MS/MS (Table 4).

Table 3 *USP 39 Published General Chapter. Table 3, <1132>, Residual Host Cell Protein Measurement in Biopharmaceuticals. May 1, 2016*

	Pros	Cons
2-D SDS-PAGE/ Western blot	Good separation of individual HCPs allows for individual spot counting.	HCP antigens are denatured, may not represent what is seen in the immunological assays (e.g., ELISA).
		High variability – numerical "percent coverage" values vary widely with the same material tested within a single lab and between different laboratories.
		Transfer efficiency of a broad range of HCPs difficult to optimize, leading to underestimates due to over-transfer through the membrane or failure to transfer from gel, dependent on molecular weight.
		Matching spots between blots and gels are difficult and not standardized.
Immunoaffinity binding/1- or 2-D SDS-PAGE	HCPs bind to antibody resin in solution under native conditions similar to the sandwich immunoassay.	May underrepresent some HCPs if they are bound too tightly and do not elute, resulting in an underestimation of coverage.
	Analysis of spots in gels does not require immunoblotting and is simpler because the problems of transfer are avoided.	Preparation of anti-HCP resin must be done carefully and may be difficult to reproduce. Results can be dependent on resin loading and elution conditions.
		Proteins at low concentration that only shows up in Westerns will not be detected.

Table 4

Pros of ELISA immunocapture / LC-MS/MS

HCPs bind to antibodies under native conditions as in the sandwich ELISA.

Low variability, high repeatability.

No problems with protein transfer efficiency.

No spot counting.

The unspecific binding is tightly controlled and can be distinguished from specific binding.

Provides a theoretical coverage of HCPs in substance.

Provides a list of individual proteins recognized by the antibodies and a list of HCPs in mock cell lysate or early process sample.