Pharmaceutical BIOPROCESSING

Getting CHO host cell protein analysis up to speed

Aim: Host cell proteins represent a major process-related impurity in recombinant biotherapeutics. Present work reports about a fully automated platform based on a cobas instrument (Roche Diagnostic GmbH, Mannheim, Germany) allowing high-throughput determination of host cell proteins. **Materials & methods**: The instrument combines automated sample preparation with electrochemiluminescence-based detection technology, facilitating highly sensitive and accurate detection. **Results:** Our data shows that the assay performs readily comparable to conventional ELISAs, but outperforms in speed, sample throughput and by its superior linear range. Comparison of ELISA and electrochemiluminescence immunoassay validation results from more than ten independent validations of clinical product testing methods supports our comparability claim. **Conclusion:** The electrochemiluminescence immunoassay represents a milestone for bioprocess impurity testing and abolishes the sample throughput limitations posed by conventional ELISA.

Keywords: Chinese hamster ovary host cell proteins • cobas e 411 analyzer platform • electrochemiluminescence immunoassay • ELISA • high-throughput quantification • in-process control

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Production of recombinant therapeutic proteins is inextricably associated with coexpression of endogenous proteins by the host cell, so called host cell proteins (HCPs). Made up by a multiplicity of proteins that possess a broad variety of physicochemical and immunological features, HCPs represent a major process-related impurity. The majority of products evaluated and approved by health authorities contains HCPs at levels lower than 100 parts per million (ppm) [1,2], since HCP may potentially trigger adverse effects such as eliciting immune responses against the HCP itself or against the therapeutic drug product [3–5]. Assessment of HCP contaminants in protein drug solutions requires a highly sensitive and specific analytical test method. The predominant test method fulfilling this requirement is typically an ELISA, which represents the well-established standard for the quantification of HCP content in all bioprocess production steps [1]. The favorable characteristics of this immunoassay format include the sensitivity, precision and cost–effectiveness required to support valid determination of process-related impurity levels. However, this method suffers from drawbacks such as total procedure time and operator workload. Contemporary, particular Quality by Design (QbD)-driven process development approaches and process characterization and validation studies result in high sample numbers. The sheer number of samples impede the desired real-time HCP monitoring throughout the purification chain due to assay throughput limitations and capacity constraints. Recently, automated solutions to de-bottleneck this situation have become available, which allow for a speedy work-off of high sample numbers [6,7]. However, the implementation of these automated ELISA platforms can be quite challenging. Both, set up and maintenance of these systems requires highly skilled and experienced operators and

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Key terms

Host cell proteins: Undesired contamination in recombinantly expressed therapeutic products, made up by endogenous proteins of the host cell.

Electrochemiluminescence immunoassay (ECLIA): Combining the traditional sandwich immunoassay with an electrochemically initiated luminescence reaction to facilitate a highly sensitive detection of analytes over a wide linear concentration range.

significant investments to be made [6,8]. In this study, we present the development of a fully automated platform approach based on an electrochemiluminescence immunoassay (ECLIA) principle for HCP determination [9]. While being capable of highest sample throughput, the system (cobas e 411 analyzer, Roche Diagnostics GmbH, Mannheim, Germany) provides sensitivity, accuracy and precision readily comparable with the performance characteristics of ELISA-based assays. However, the ECLIA outperforms the ELISA in ease of use and speed of sample processing, and, most notable, by its large dynamic measuring range, a characteristic based on the applied electrochemiluminescence-based detection technology, which exceeds the dynamic range provided by the classical colorimetric detection approach of the ELISA by orders of magnitude. This feature of the ECLIA facilitates the simultaneous determination of impurity levels in various process intermediate and final bulk samples within a single run, without the need of numerous sample dilution steps to be performed. Used for routine testing of in-process intermediates, this platform enables us to handle numerous highly heterogeneous samples typically generated during contemporary bioprocess development. In addition, the system enables to perform high-throughput applications that could not be executed by conventional ELISA-based approaches.

Materials & methods

Generation of platform assay reagents for active pharmaceutical ingredient assessment

The polyclonal, affinity-purified anti-Chinese hamster ovary (CHO)-HCP antibodies used for both, the ECLIA- and the ELISA-based assay formats were generated in-house by immunization of sheep with CHO antigens. The respective antigens were derived from the harvested cell culture fluid (HCCF) of a CHO mock fermentation run, which was representative for our in-house upstream platform process.

Electrochemiluminescence immunoassay

The residual CHO-HCP content in IPC-samples (in process control samples) and drug substance solutions was quantified using an ECLIA-based assay format run on a cobas e 411 analyzer platform device (Roche Diagnostics GmbH). Assay procedure: in a first incubation step (40.5 min), CHO-HCP from a drug substance sample and biotinylated polyclonal CHO-HCP-specific antibodies (Roche Diagnostics GmbH, Germany) form a complex, which is captured on streptavidin-coated, ferrous microparticles (Roche Diagnostics GmbH) via interaction of biotin with streptavidin. In a second incubation step (18 min), a ternary sandwich complex is formed on the microparticles after addition of polyclonal CHO-HCP-specific antibody labeled with a ruthenium complex (Tris(2,2′-bipyridyl)ruthenium(II)-complex; Roche Diagnostics GmbH, Germany). The reaction mixture is aspirated into the measuring cell, where the microparticles are magnetically captured onto the surface of an electrode. Unbound substances are removed by a washing step and tris-propylamine is provided for the electrochemiluminescence (ECL) reaction as ProCell buffer (Roche Diagnostics GmbH, Germany) is applied. Application of voltage to the electrode then induces chemiluminescence, which is measured by a photomultiplier. CleanCell buffer (Roche Diagnostics GmbH, Germany) is applied by the system to clean the measuring cell. A typical assay duration accounts for approximately 60 min per sample.

The concentration of CHO-HCP in the test sample is finally calculated from a CHO-HCP standard curve of known concentration ranging from 5–10,000 ng/ml. Test samples are measured in several dilutions. Each test run includes controls, of which two individual dilutions are measured in duplicates, and a series of standards, each measured in duplicates. Results are corrected for dilution factors and the highest values are reported.

ELISA

The residual CHO-HCP content in drug substance solutions is quantified using an ELISA.

The streptavidin pre-coated microtiter plate (MTP; MicroCoat Biotechnologie GmbH, Bernried, Germany) is first coated with biotinylated polyclonal CHO-HCP specific antibodies (Roche Diagnostics GmbH, Germany) for 1 h. The MTP is washed (50 mM K_2HPO_4/KH_2PO_4, 0.1% polysorbate 20, 150 mM NaCl) using a Skan Washer 400 device (Molecular Devices, CA, USA), and subsequently incubated with the test sample at 4°C overnight. After a second washing step, a sandwich complex is allowed to be formed with digoxygen-labeled, polyclonal CHO-HCP-specific antibodies (Roche Diagnostics GmbH, Germany) by incubation for 2 h. For the formation of a detection sandwich, the MTP is first washed and incubated for 1 h with polyclonal, horseradish peroxidase-labeled anti-Digoxygenin Fab-Fragment (Roche

Diagnostics GmbH, Germany). After a final washing step, the substrate ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid; Roche Diagnostics GmbH, Germany) is added, and colorimetric read out is performed at 405 nm using a plate reader (VersaMax, Molecular Devices).

The concentration of CHO-HCP in the test sample is finally calculated from a CHO-HCP standard curve of known concentration ranging from 2.0–20 ng/ml using the software SoftMaxPro (Molecular Devices) for 4-parameter curve fit. Two individual dilutions of each test sample are measured in duplicates. In addition, the same sample dilutions are measured with defined spikes added to allow calculation of spike recovery. Each test run includes controls, measured in duplicate and standards, measured in triplicates, respectively. Results are corrected based on dilution factors and the mean values are reported. The limit of detection (LOD; ng/ml) and limit of quantification (LOQ; ng/ml) values are calculated for both methods according to the following formula: LOQ = 10 × standard deviation (SD)/slope. Values for slope and residual standard deviation were determined by linear regression analysis of signal versus CHO-HCP concentration in spike samples containing 1.4–2.0 ng/ml CHO-HCP. LOD was determined similarly, applying the following formula: LOD = 3.3 × SD/slope, this time with measurement results obtained from spike samples with CHO-HCP contents ranging from 0 to 2.0 ng/ml.

Spike recovery studies

Dilutions of sample solution and placebo, respectively, are spiked with CHO-HCP reference antigen at concentration covering the range of the respective assay (ELISA: 2.0, 10.0 and 20.0 ng/ml; ECL IA: 5.0, 5000 and 10000 ng/ml). A placebo sample is included in the spiking experiment to assess potential matrix interference that may influence the assay performance. To assess the recovery of spiked CHO-HCP, the CHO-HCP concentrations determined in spiked samples are corrected for the CHO-HCP concentrations measured for nonspiked samples obtained in the same measurement series.

Human IgG quantification

The human IgG content was quantified using a COBAS INTEGRA system (Roche Diagnostics GmbH, Germany). In this particular test, human IgG forms a precipitate with a specific antiserum, which is determined turbidimetrically at 340 nm. The immunoturbidimetric assay was performed using a COBAS INTEGRA Immunoglobulin G (Turbidimetric; IGGT) cassette (Roche Diagnostics GmbH, Germany) according to the manufacturer's instructions.

Cation exchange chromatography

Cation exchange chromatography (cIEX) was performed using Dionex ProPac WCX-10 Semi Prep column (9 × 250 mm; Thermo Scientific GmbH, Dreieich, Germany) on an ÄKTAexplorer 10 system (GE Healthcare, Munich, Germany). Prior to chromatography, samples were buffer exchanged in 10 mM sodium phosphate buffer, pH 6.5 using Amicon Ultra Centrifugal Filters Ultracel (MWCO 3kDa; Merck Millipore, Darmstadt, Germany). The applied protein load is displayed in the figures. A gradient of 0–60% in 7.5 column volumes at a flow rate of 3 ml/min at 25°C was applied using 10 mM sodium phosphate buffer, 500 mM NaCl, pH 6.5 as eluent B. Fractions (1.5 ml each) were collected.

Immunofractionation

The CHO-HCP profile was assessed by a combination of the cIEX method described above and an ECLIA-based CHO-HCP and COBAS INTEGRA-based IgG quantification (Roche Diagnostics GmbH, Germany). Fractions obtained by cIEX were collected and subjected to ECLIA measurement. For data evaluation, the measured CHO-HCP content of each fraction was plotted against the elution volume of each LC fraction using the software OriginPro 9.0 (OriginLab Corporation, MA, USA).

Statistical evaluation of validation experiments

Validat Software package p1 (03/2014) Version 5.59.1623 (iCD. GmbH & Co. KG 2005–2010, Frechen, Germany) was used to evaluate the results of the validation experiments. Statistical parameters were calculated following a validated workflow designed in compliance with corporate guidelines on statistical interpretation of validation results.

Results & discussion

Part 1: comparison of HCP quantitation by ELISA & ECLIA: validation data

Due to the relatively low sample throughput, studies with high sample numbers cannot be reasonably supported by conventional, manually performed ELISA-based methods. To significantly increase the sample throughput, we have selected a fully automated ECLIA-based assay platform for HCP testing. In Table 1, major performance characteristics of a manual ELISA- and the ECLIA-procedure are summarized. In addition, exemplary information on the performance of an automated ELISA, as described by Rey and Wendeler [6], is provided.

In order to demonstrate comparability between this novel approach and the conventional ELISA assay, the results of a series of validation studies across different

antibody process development projects performed with either assay formats are shown in Table 2.

Linearity, accuracy and repeatability of the ELISA test method was investigated by analysis of different drug substance formulation buffer dilutions and different downstream processing buffers spiked with various concentrations of CHO-HCP standard material, ranging from 2.0 to 20 ng/ml. The tested matrices were chosen to consider the worst case scenarios of potential matrix interferences. Dilutions for sample preparation ranged from 1:20 for formulated drug substance solution to 1:200.000 for HCCF centrifuge effluent. Each sample was tested three-times in duplicate measurements (n = 6 measurements/sample). Accuracy evaluation was performed by spike recovery determinations of spike samples at the lower (2.0 ng/ml), intermediate (10.0 ng/ml) and upper level (20.0 ng/ml) of the linear method range. Data obtained from accuracy determination were also used to assess the repeatability of the ELISA method. Therefore, the relative standard deviation of all measured values per concentration level was determined. For assessment of the intermediate precision, the results of six measurements performed on six different days by two independent analysts were used to calculate the relative standard deviation per concentration level. The corresponding acceptance criterion was set to less than equal to 30%. The range of the test method was defined based on assessment of accuracy, linearity and repeatability. The acceptance criteria for each of these three validation parameters were met within the range of 2.0–20.0 ng/ml. The LOQ of the method was specified at 2.0 ng/ml.

ECLIA method validation was performed similarly, whereby spike samples of CHO-HCP standard ranging from 5.0 to 10,000 ng/ml were used, demonstrating the broad linear range of the ECL method. Typically, a sample dilution of 1:2 was applied, with the exception of HCCF centrifuge effluent, which was typically diluted 1:400. LOQ (5.0 ng/ml) and LOD (1.2 ng/ml) determination for the ECLIA method revealed a minimal lower sensitivity compared with the ELISA system (LOD at 1.2 and 0.6 ng/ml, respectively). A summary of the results obtained from validation studies for both ELISA and ECLIA is provided in Table 2. All results meet the acceptance criteria for all sample types tested. Overall, the validation parameters of both HCP quantification methods are generally comparable, with the exception of the markedly broader linear range of the ECLIA.

To further corroborate our comparability claim independent of our in-house platform reagents, we performed additional experiments using a commercially available reference antigen from the 3G CHO-HCP-ELISA kit from Cygnus Technologies (NC, USA) as a sample. Figure 1 demonstrates the comparability of measurement results assessed by ELISA and ECLIA. As reference antigen, the 3G CHO-HCP-ELISA kit from Cygnus Technologies was analyzed using the same proprietary anti-CHO-HCP antibodies for both assay formats.

As an additional experiment to demonstrate method comparability, HCCF from three different

Table 1. Performance characteristics of manually and automatically performed anti-Chinese hamster ovary-host cell protein-ELISA assays compared with electrochemiluminescence immunoassay.

Performance characterstics	ELISA manual	ELISA automated [6]	ECLIA
Dilution	Manual	Automated (maximum dilution 1:200)	Automated (maximum dilution 1:400)
Measuring device	ELISA plate reader	ELISA plate reader	Cobas e 411 analyzer including sample dilution and measuring module
Sample throughput	Eight samples per 96-well MTP with standards, controls and respective dilutions	12 samples per MTP with standards, controls and respective dilutions	Samples are loaded into sample cassettes, which are continuously fed into the instrument
Duration of sample preparation (hands-on-time)	One complete 96-well MTP in 2.5 h	288 samples (48 × 6) in 30 min	96 samples in 30 min
Maximum number of reported sample values per 24 h	64 (8 × 96-MTP with eight samples each MTP)	48	250
First results available in	24 h	~5 h	60 min

CHO: Chinese hamster ovary; ECLIA: Electrochemiluminescence immunoassay; HCP: Host cell protein; MTP: Microtiter plate.

Table 2. Comparison of validation results between ELISA and electrochemiluminescence immunoassay.

Validation characteristics	Acceptance criteria†	Sample	ELISA‡	ECLIA‡
Linearity				
Coefficient of correlation	R ≥ 0.97	Bulk DS	0.9941	0.9991
		Protein A Eluate	0.9916	0.9994
Accuracy				
Recovery of spikes (minimum–maximum recovery)	70–130%	Bulk DS	80–102%	82–113%
		Protein A Eluate	81–125%	77–113%
		HCCF	86–118%	85–105%
Precision/repeatability				
Relative standard deviation (minimum–maximum RSD)	≤20%	Bulk DS	1–4%	3–13%
		Protein A Eluate	1– 4%	2–9%
		HCCF	3–9%	2–14%
Range				
Range	Linearity: R ≥ 0.97	Bulk DS	2.0–20 ng/ml	5.0–10,000 ng/ml
Defined on LOQ, linearity, accuracy and repeatability	Accuracy: 70–130%	Protein A Eluate		
	Repeatability RSD ≤20%	HCCF		
Intermediate precision				
RSD	≤30%	Bulk DS	18%	18%
		Protein A Eluate	18%	4%
		HCCF	NA	6%
LOD/LOQ				
LOD (ng/ml)	–	Bulk DS	0.6	1.2
		Protein A Eluate		
		HCCF		
LOQ (ng/ml)	Accuracy at LOQ: 70–130%	Bulk DS	2.0	5.0
	Repeatability at LOQ: RSD ≤20%	Protein A Eluate	Accuracy at LOQ: 80–125%	Accuracy at LOQ: 77–113%
		HCCF	Repeatability at LOQ: RSD = 1–11%	Repeatability at LOQ: RSD = 2–14%

†Acceptance criteria are based on internal guidelines for process-related impurity analytics.
‡ELISA and ECLIA both share the same set of anti-CHOP reagents, including an in-house CHO-HCP standard and proprietary anti-CHO-HCP antibodies. For the ECLIA, sample preparation steps (i.e., sample handling, dilutions, reagent incubations and washing steps), ECLIA procedure and data acquisition are performed automatically. The depicted data is representative for 14 individual ELISA and 12 ECLIA validation studies, respectively. Each validation parameter was assessed by six-times measurement of samples (n = 6 measurements per sample). Statistical interpretation of validation data was performed using VALIDAT software (iCD, Frechen, Germany).
Bulk DS: Bulk drug substance; CHO: Chinese hamster ovary; CHOP: Chinese hamster ovary cell protein; ECLIA: Electrochemiluminescence immunoassay, HCCF: Harvested cell culture fluid; HCP: Host cell protein; LOD: Limit of detection; LOQ: Limit of quantification; R: Correlation coefficient; RSD: Relative standard deviation.

products were measured applying both assay formats (Figure 2A). Comparison of the obtained results shows that both assays are in good agreement with regard to the determined HCP levels in samples from various products bioprocess pools. This outcome can be also visualized by a Bland-Altman plot (Figure 2B), comparing the ratio of means (mean ELICA/ mean ELISA) for samples of the Cygnus standard, as well as for HCCF pools of products A-C using the same datasets as depicted in Figures 1 & 2. The determined ratios range between 0.85 and 1.22 with a mean value of 1.02.

Our data indicate that the automated ECLIA assay platform represents a suitable alternative to ELISA-based assay formats, and shows that the ECLIA-based assay can be applied both for routine support of process development and clinical supply release testing. In the following sections we present some selected applications that take advantage of the excellent high-throughput capabilities of the ECL platform.

Part 2: applications in bioprocess development

HCP depletion through the purification process is one of the major subjects during the bioprocess development.

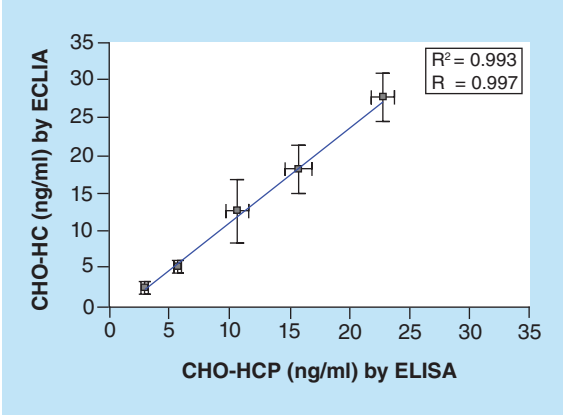

Figure 1. Determination of correlation between CHO-HCP-ELISA and electrochemiluminescence immunoassay, using a commercial CHO-HCP test kit as test sample. CHO-HCP levels and relative standard deviation of CHO-HCP levels determined by ELISA (x-axis) and ECLIA (y-axis) using the 3G CHO-HCP-ELISA standard of the 3G Cygnus Technologies (NC, USA) kit at various concentrations. The calibration was performed using the in-house CHO-HCP standard. The CHO-HCP concentration of the Cygnus CHO-HCP standard ranging from 2.5 to 20 ng/ml was determined. Each sample was measured in six independent measurements. The relative standard deviation of all values measured per concentration level and method was determined.
CHO: Chinese hamster ovary;
ECLIA: Electrochemiluminescence immunoassay;
HCP: Host cell protein; R: Correlation coefficient.

To facilitate bioprocess development toward enhanced reliability and consistency, it is of importance to gain information on: the extent of impurity depletion by the applied purification steps; potential batch specific impurity profiles to learn about batch to batch variance; and HCPs copurifying with the active pharmaceutical ingredients (API).

Figure 3A & B shows exemplary cIEX chromatograms of two different antibody purification steps. CHO-HCP content was determined from selected fractions by the described ECLIA system. Results are displayed in an overlay with the UV values measured from the respective fractions (absorbance at 280 nm). This type of process development application is generally used to assess optimal pooling limits for an optimal balance of maximized CHO-HCP clearance and product yield. The required analytics to determine CHO-HCP levels in these fractions using conventional ELISA-based assay formats represents the main time-limiting factor during bioprocess development. Thus, the application of high-throughput ECLIA technology essentially facilitates timely monitoring of the CHO-HCP level and accelerates downstream process development, particularly during small-scale purification studies making full use of robotics, when high sample numbers need to be leveraged.

CHO-HCP level assessment is also an integral part of routine in-process control analytics performed during production of biotherapeutic material for clinical use. The extent of HCP depletion is exemplarily demonstrated for three different antibody products at various downstream processing steps in Figure 4A. Starting from a level of approximately 10^4 ng CHO-HCP/mg API in HCCF, the decrease of CHO-HCP content in consecutive purification steps is routinely monitored. CHO-HCP levels decrease by up to two orders of magnitude in each single purification step, and usually reach contents below 50 ppm at final drug substance level. Evaluating the individual yields of HCP depletion helps to evaluate the capability of single-process steps and may indicate where purification process improvement measures need to be considered. For more detailed investigation of process capabilities, we implemented an approach combining a LC-based fractionation of in-process and API solutions, followed by ECLIA-based CHO-HCP and IgG content quantification of each LC-fraction, which is hereafter referred to as **immunofractionation**. Figure 4B illustrates an example for semipreparative fractionation based on cIEX of different in-process pools. The majority of remaining HCPs either coelute with the API main peak or with its more basic charge variants. Interestingly, it becomes apparent at later stages of the purification process that the majority of the CHO-HCP population seems to

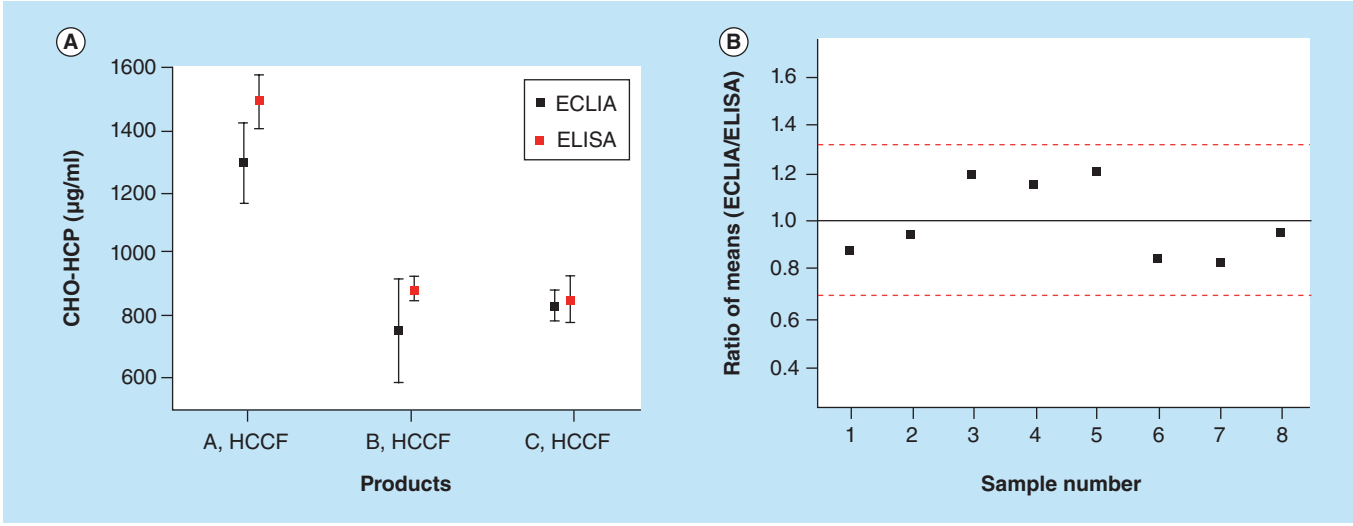

Figure 2. Comparison of CHO-HCP analysis results determined with electrochemiluminescence immunoassay and ELISA. (A) CHO-HCP levels and twofold standard deviation of CHO-HCP levels of three different products at HCCF level determined by ELISA and ECLIA. Each sample was tested three-times in duplicate measurements (n = 6 measurements/sample). The twofold standard deviation per concentration level and method is depicted for each product. **(B)** Bland-Altmann plot, sample number 1–5 cygnus standards, sample number 6–8 HCCF samples containing active pharmaceutical ingredient. The black line represents mean of ratio ECLIA/ELISA and the red dotted line represents twofold standard deviation of the mean ratio.
CHO: Chinese hamster ovary; ECLIA: Electrochemiluminescence immunoassay; HCCF: Harvested cell culture fluid; HCP: Host cell protein.

exhibit a similar pI as the API. These results let assume that the impurities present in the final product either share major physicochemical properties (e.g., distribution of charge heterogeneities, hydrophobicity and size, among others) with the API or, alternatively, may take part in strong interactions with the API, and thus remain unaffected by the most commonly applied purification modes in biotherapeutic purification processes [10].

Consistent HCP removal along the consecutive purification steps is certainly a hallmark of a robust bioprocess. In order to evaluate these characteristics, it may be advantageous to compare CHO-HCP levels from different batches of the same product at different purification steps. It is conceivable that each specific bioprocess may yield comparable HCP profiles in final drug substance batches. The corresponding HCP profiles of each batch can be assessed by various means, for example, by using 2D SDS-PAGE-based methodologies [11,12] or combinations of 1/2D SDS-PAGE and LC-MS-based technologies [10,13–15], respectively. Immunofractionation can serve as capacity efficient means to supplement HCP profile characterization performed by the aforementioned approaches. Minor HCP profile deviations observed between different batches of one product can be evaluated by comparison of individual immunofractionation results obtained from different purification process steps. Our data indicate that the immunofractionation approach is a sound method to assess potential alterations of the CHO-HCP distribution pattern of different batches, taking relevant biophysical properties such as, for example, charge, hydrophobicity or size into account.

Three characteristic cIEX immunofractionation-profiles of purified batches (batches 1–3) from product B are visualized exemplary in Figure 5. While being largely comparable between different batches, there are essentially two major HCP populations that can be observed in each of the batches (at ~30–50 ml retention volume), suggesting the presence of two characteristic CHO-HCP species not being cleared by the underlying bioprocess. The ability of the immunofractionation approach to detect slight differences between individual CHO-HCP profiles, for example, as observed at 90–95 ml retention volume, indicates the capability of the method to detect small batch-specific differences with sufficient resolution.

Conclusion

The ECL platform provides a combination of fully automated sample preparation, rapid measurement and data analysis which enables to perform a variety of extensive experiments that would not be practicable to

Key term

Immunofractionation: Combining liquid chromatographic separation of process-related impurities with subsequent quantification of the impurity and active pharmaceutical ingredient content in the obtained fractions by immunoassay-based detection methods.

be performed employing a conventional ELISA assay format. The ECLIA instrument used in the present study has been widely utilized for more than a decade in clinical laboratories for routine diagnostics. Due to its user-friendliness, the systems has an edge on automated ELISA platforms [6], which are complex, custom-made solutions that require highly trained personnel, extensive maintenance and are tedious to qualify for use in a highly regulated GMP environment. The system is highly expandable to any immunoassay-based impurity testing, in other words, assays for residual Protein A and growth hormones, among others, can be readily implemented. Here we describe total HCP testing as an exemplary application established on our ECL platform, which has been successfully implemented in our facilities for routine testing of process intermediate pools and clinical supply release testing. Though a high-throughput method, our data demonstrates that the automated platform test meets the performance characteristics of the routinely used ELISA system, but exceeds the linear range of the ELISA-based assay format by nearly 3 orders of magnitude. The impact of this feature becomes apparent, when different samples containing a broad variety of different HCP levels (e.g., intermediate process pool samples from different bioprocess steps) need to be analyzed within a single run. Given the comparably narrow dynamic range (range over which there is a linear relationship between the analyte concentration and the absorbance reading) of the ELISA, several subsequential dilution steps may be necessary to obtain valid results, which in turn increases the amount of samples that need to be analyzed when applying the ELISA. While the ECLIA outperforms the ELISA-based assay format in linear range, it has to be noted that the latter features a slightly higher sensitivity, which manifests in an approximately two-times lower LOQ as compared with the ECLIA. This may be related to the applied sample amount, which can be increased to enhance sensitivity. Both methods described here – ELISA and ECLIA – are performed utilizing the same set of reagents, which consists of a pool of CHO-antigens used as standard and anti-CHO polyclonal antibodies to facilitate detection. The reagents have been characterized by means of orthogonal methods (i.e., 2D-SDS PAGE, immunoblotting and extensive LC-MS characterization), demonstrating a high degree of antigen coverage (data not shown). Thus, a sensitive and accurate detection of HCPs – within the inherent limitations of methods based on immunological detection – can be postulated. Taking into consideration, that HCP levels are generally reported in parts per million (ppm; ng HCP/mg API), the difference in LOQ between ECLIA and ELISA of 5.0 ng/ml and 2.0 ng/ml, respectively, results in a ppm value less than 1 ppm for both methods, assuming an API concentration more than 5.0 mg/ml, which is the case for the majority of tested biotherapeuticals. Taken together, the slight disadvantage of the ECLIA-based assay format in terms of sensitivity can be disregarded and it is outweighed by the platforms many times faster sample turnover (up to 86 samples/h). In addition to that, the ECLIA platform offers the possibility to perform serial sample dilution in an automated fashion, resulting in an increased precision and accuracy in sample handling and preparation when compared with a manually performed ELISA.

The ECLIA platform facilitates a speedy routine analysis of CHO-HCP contents of in-process pools and final product samples. It allows leveraging highest sample loads and provides a suitable means to study product-specific HCP profiles when combined with LC-based separation techniques.

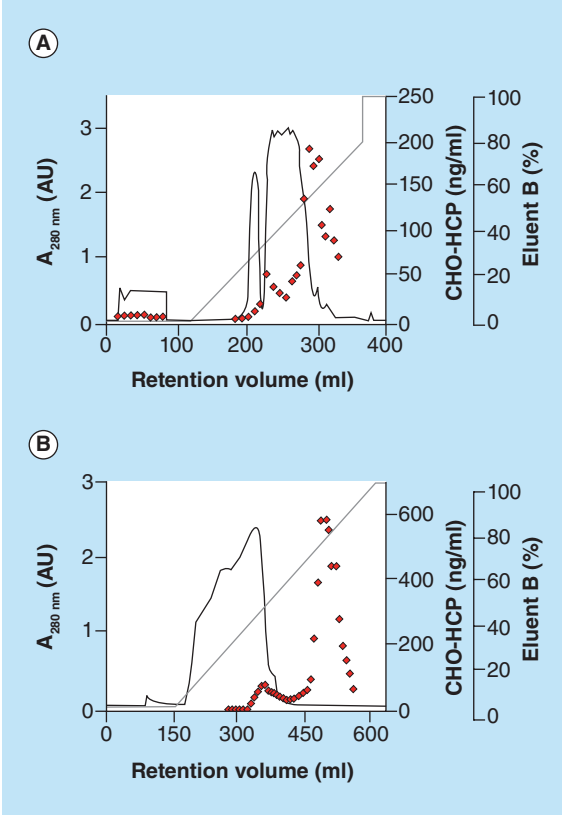

Figure 3. Exemplary cation exchange chromatography chromatograms of different downstream process development experiments including CHO-HCP analysis by electrochemiluminescence immunoassay in fractions of interest. **(A)** Example one, **(B)** example two. Exemplary cation exchange chromatograms of two different downstream process development experiments **(A & B)** including CHO-HCP analysis by electrochemiluminescence immunoassay for fractions of interest.
CHO: Chinese hamster ovary; HCP: Host cell protein.

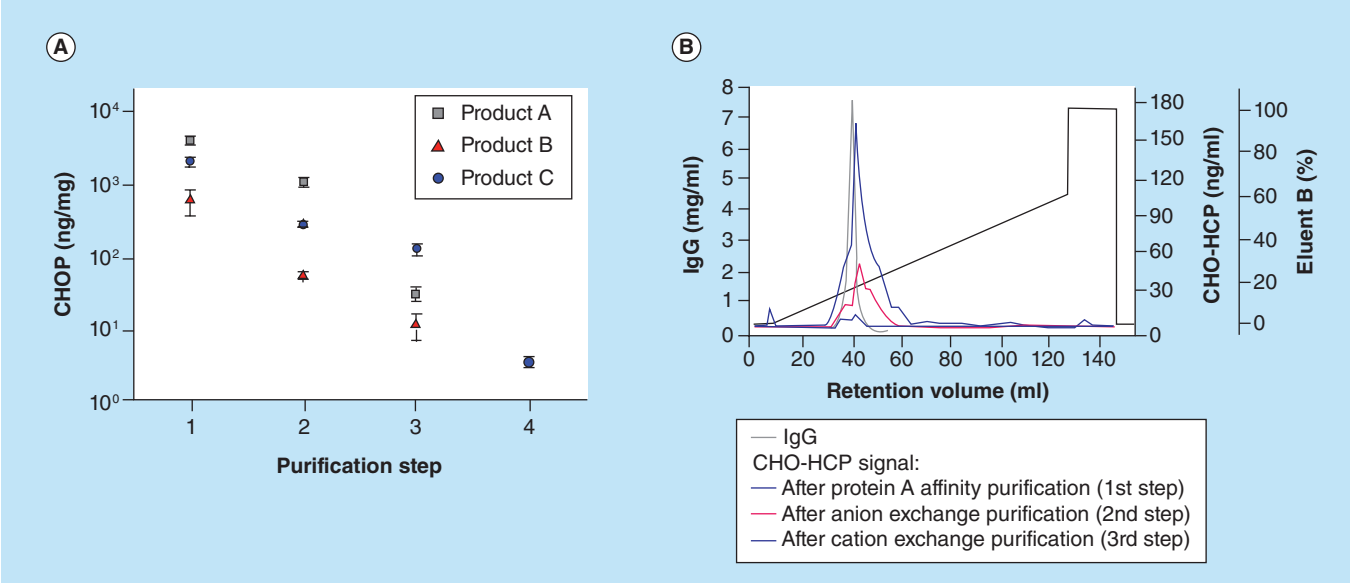

Figure 4. CHO-HCP depletion during downstream processing. **(A)** CHO-HCP depletion during downstream processing for three different therapeutic antibodies (product A–C). Host cell protein content was measured in process samples (harvested cell culture fluid and eluates of the respective purification chromatographies) by electrochemiluminescence immunoassay. Depicted are the average batch values with the respective standard deviation (product A: 4 batches; product B: 3 batches; product C: 6 batches). Of note, product C has a four-step purification process. Relative deviation ranges between 7.2 and 40.2%. **(B)** Immunofractionation: CHO-HCP profiles of cation exchange chromatography-fractionated product B at three different purification levels determined with electrochemiluminescence immunoassay. A protein load of 25 mg was applied. Of note, fractionation of harvested cell culture fluid is not displayed for better visualization.
CHO: Chinese hamster ovary; CHOP: Chinese hamster ovary cell protein; HCP: Host cell protein.

Taken together, this approach supports process development, process economics and broadens product knowledge.

Future perspective

Sandwich immunoassays are considered the gold standard in the industry for assessment of process-related impurities derived from pharmaceutical biotherapeutics production. They provide the precision, accuracy and sensitivity needed to support bioprocess development and Good Manufacturing Practice lot release. Automated approaches, like the one introduced in the present study, help to leverage the steadily growing numbers of samples that need to be evaluated during development of a biological therapeutic. However, immunoassays have some decisive drawbacks, particularly with regard to detection of less immunogenic impurities, which requires an additional set of orthogonal tools (i.e., 2D SDS-PAGE, immunoblotting) to verify the reported levels of impurities. Recent advances in the field of LC-MS/MS demonstrate that proteomics methods are rapidly closing the sensitivity gap to immunoassays, which used to hamper their applicability for process-related impurity analytics in the past. In comparison to immunoassay-based formats, LC-MS/MS offers the advantage of being capable to detect individual, not immunogenic HCPs. They have therefore become an excellent orthogonal tool to complement the classical toolbox of quality control departments, helping to minimize the chance that an individual HCP escapes

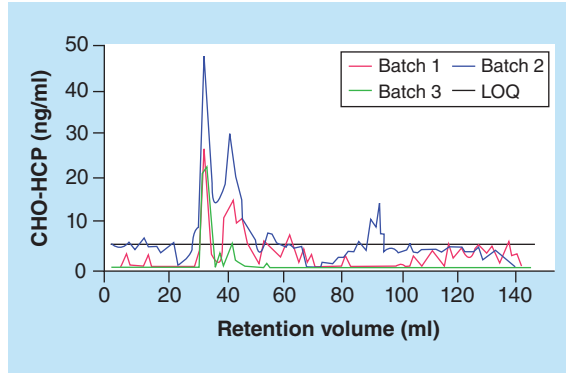

Figure 5. Batch to batch comparison. Three batches were assessed. CHO-HCP content off ion exchange chromatography-elution profile was determined by electrochemiluminescence immunoassay. 90 mg unconditioned bulk (=concentrated drug substance bulk solution prior to the final bulk formulation step) for each batch was loaded. Absorbance channel, HPLC gradient and IgG content for each fraction are not depicted here. This application supports comparability assessments, process characterization and process validation studies.
CHO: Chinese hamster ovary; HCP: Host cell protein; LOQ: Limit of quantification.

detection. Considering the enormous technological leaps this technology has made in the past, it is conceivable that the importance of LC-MS/MS for HCP analysis will continue to increase.

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Executive summary

Aims

- An automated, high-throughput system applying electrochemiluminescence-based detection technology (i.e., electrochemiluminescence immunoassay [ECLIA]) was introduced to measure host cell proteins in biotherapeutics, providing an alternative to well-established, conventional ELISA-based detection techniques.

Materials & methods

- To evaluate the suitability of the ECLIA method as a substitute for ELISA-based assay formats, a series of validation studies with different Chinese hamster ovary cell line derived biotherapeutic products and their corresponding in-process intermediates was conducted.
- Immunofractionation was introduced as an application in support of biotherapeutics downstream process development, which was anticipated to take benefit of the high-throughput capacity of the ECLIA system.

Results & conclusion

- ECLIA exhibited a comparable performance to manually performed ELISA testing, as indicated by accuracy and precision data available from more than ten independent validation studies for each assay format.
- Compared with ELISA-based assay formats, the applied antihost cell proteins ECLIA provides a 3 orders of magnitude higher dynamic range, which facilitates simultaneous measurement of samples with highly varying protein concentrations, such as in process intermediates and final product samples, in a single run.
- The study demonstrates that the presented ECLIA-based assay is a suitable alternative to conventional ELISA-based methods for supporting pharmaceutical bioprocess development and Good Manufacturing Practice lot release.

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