Research Article

Pharmaceutical BIOPROCESSING

A microfluidic approach to high throughput quantification of host cell protein impurities for bioprocess development

Background: Analysis of process-related impurities is critical for the control of biopharmaceutical processes and the quality of final biological products. Residual impurities in monoclonal antibody products such as host cell proteins (HCPs) increase the risk of immunogenicity and may directly affect drug potency. Commonly used HCP ELISA often involves complicated sample preparation, lengthy operation, and large volumes of reagent. To overcome these challenges, a fully automated CHO HCP assay was developed using a microfluidic platform (MFP) system and compared with existing plate-based ELISA for quantification of HCP in monoclonal antibody purification intermediates. **Results:** The automated MFP based assay approach enabled an improved throughput (5–10-times faster), broader dynamic range (100-times) and decreased sample consumption, hands on time and duration for assay development compared with Tecan plate-based ELISA. **Conclusion:** The newly developed microfluidic assay demonstrated its advantages over plate-based ELISA for the in-process HCP clearance monitoring and the quantification of final HCP in drug substance.

Jun Hyuk Heo*[,1], Xiaodun Mou[1], Fengqiang Wang[1], John M Troisi[1], Christopher W Sandifer[1], Suzanne Kirby[1], Dennis Driscoll[1], Suzanne Mercorelli[1] & David J Pollard[1]

[1]Bioprocess Development, Merck Research Laboratories, Merck & Co., Inc. 2000 Galloping Hill Rd. Kenilworth, NJ 07033, USA

*Author for correspondence:
Tel.: +1 908 740 4386
Fax: +1 908 740 4364
jun.heo@merck.com

Recombinant therapeutic proteins such as monoclonal antibodies are produced from cell culture or fermentation of genetically modified prokaryotic or eukaryotic host cells. The biologic products are purified by downstream manufacturing processing to remove impurities derived from either the process or the product itself. One of the major process-related impurities is the host cell protein (HCP) derived from the host cells producing therapeutic proteins. Although reports of adverse effects due to HCP are rare, the clinical safety concerns still persist [1–4]. Analysis of intermediate samples from bioprocesses is required in order to demonstrate that residual host cell impurities are reduced or eliminated during purification [5,6]. The testing requirements during late stage development to confirm the reduction of residual HCP to within allowable limits is a significant resource burden. During process development, the reduction of HCP can also serve as an indication of purification efficiency along with the levels of other impurities such as residual DNA and protein A ligand. Therefore, the analysis of process-related impurities is critical for both the development of the purification process and the control of biological drug product.

The challenges of HCP analysis include their large variety, wide concentration range, and complex biological properties of the analytes [3,7]. Substantial sample dilution is required to span the million-fold range from initial purification to final drug substance, in order to fit samples into the testing range. HCPs often exist as a complex mixture of various proteins with different concentrations and immuoreactivity [7]. HCP levels in the final product are usually low so it requires an assay with sufficient sensitivity (sub 10 ng/ml). The ELISA has been widely utilized to quantify HCP but the assay usually has narrow dynamic range, relatively high variability, lengthy operation time, and high reagent consumption. Despite these limitations, ELISA assays remain highly desired for the HCP clearance information necessary for the approval of a new product [7].

With the dominance of biological drugs in the current market, the industry's effort

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to accelerate bioprocess development has led to significant improvement in automation and throughput [8,9]. Consequently, the acceleration in bioprocess development has driven demands for fast and cost-effective analytical tools. As the ligand binding assay (LBA) is one of the major analytical tools for analyzing biologics, many new LBA technologies, such as Biacore, Octet, AlphaLISA, Meso Scale Discovery (MSD), Proximity Ligation Assay (PLA), and Gyrolab, are implemented to increase the throughput and control costs. Among these new technologies, the Gyrolab microfluidic immunoassay system shows great potential to replace traditional ELISA for higher throughput and better assay performance [10]. The innovation of the Gyros technology lies within the CD-shaped assay plate that contains nano-scale microfluidic columns packed with streptavidin coated beads (Figure 1) [10]. This allows for affinity binding of proteins labeled with biotin. In this study, we employed this microfluidic immunoassay technology to develop a fully automated HCP assay to overcome the obstacles of traditional plate-based ELISA assays. Process streams from monoclonal antibody production were used to develop a new method optimized for different types of microfluidic CDs, diluent buffers, spin speeds, and reagent concentrations. The selectivity, accuracy, and precision of the assay were evaluated by testing dilutional linearity, spike recovery, and inter/intra CD precision. The optimized assay was then compared with manual ELISA and Tecan-automated ELISA for the analysis of HCP with therapeutic antibody process streams.

Experimental

Chemicals & reagents

CHO HCP antigen concentrate and affinity-purified goat anti-CHO HCP were purchased from Cygnus (Southport, NC, USA). The manual and Tecan ELISAs used the CHO HCP ELISA kit purchased from Cygnus. Alexa Fluor 647 purchased from Invitrogen (Carlsbad, CA, USA) was used to label detection antibody; EZ-Link Sulfo-NHS-LC-Biotin, No-Weigh Format, purchased from Pierce (Rockford, IL, USA) was used to biotinylate the capture antibody. Protein Desalting Spin Columns with cut off 7 kDa purchased from Pierce (Rockford, IL, USA) was used to remove the free biotin. Nanosep 30K OMEGA Centrifugal Devices purchased from Pall Life Sciences (Port Washington, NY, USA) was used to exchange the buffer after the labeling reaction. The sample diluent (Rexxip A), the detection reagent diluent (Rexxip F), Bioaffy 200, and Bioaffy 1000 were purchased from Gyros (Uppsala, Sweden). PBS at pH 7.4 was purchased from Life Technologies (Philadelphia, PA, USA) and Tween 20 was purchased from National Diagnostics (Atlanta, GA, USA).

For the capture reagent, anti-CHO HCP antibody was biotinylated according to standard N-hydroxysuccinimidyl ester (NHS) chemistry [11]. For the detection reagent, anti-CHO HCP antibody was labeled with Alex Flour 647 by primary amine reaction with NHS [11]. Final concentration and the labeling efficiency was determined by measuring A230 and A650 with Nanodrop 2000. The wash buffer was 0.01% TWEEN in PBS by volume.

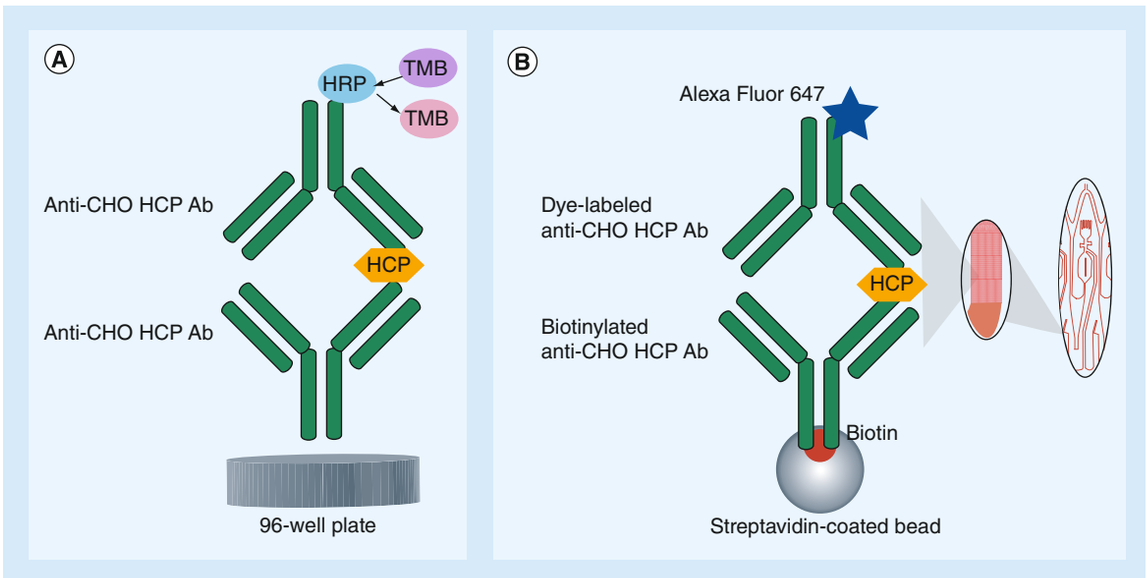

Figure 1. Binding format of sandwich ELISA for host cell protein detection. (A) Detection on 96-well plate and (B) on streptavidin-coated beads packed into a nanocolumn of the microfluidic flow path [10].

In-process therapeutic monoclonal antibodies

All in-process therapeutic monoclonal antibodies were prepared by the Bioprocess Development group at Merck Research Laboratories (Rahway, NJ, USA). Two mAbs were used along with the associated process intermediates from various stages of purification. The estimated HCP concentration varied from 100 to 300,000 ng/ml.

Instrumentation & general assay procedure

Similar to the plate-based ELISA in sandwich format, HCP was captured between biotinylated anti-CHO HCP antibody and Alexa Flour 647 labeled anti-CHO HCP antibody (Figure 1). However, each step of the assay was automatically performed by the instrument according to the user-defined protocol. The liquid handler transfers reagents and samples from the microplate into the inlet of microfluidic channels, and after reagents enter the microfluidic channels, the spinning speed of the CD dictated the liquid flow into the column.

First, all columns were conditioned with 10 nl of 0.01% PBS-TWEEN. Then 10 nl of biotinylated anti-CHO HCP antibody was added to each column. After three washes with 0.01% PBST, HCP antigen was added. All columns were then washed three-times with 0.01% PBST, and the background signal was measured. Lastly, anti-CHO HCP antibody labeled with Alexa Flour 647 was added and the fluorescence was measured by a detector for HeNe laser-induced fluorescence at 633 nm. All experiments were performed by the same instrument protocol unless noted otherwise.

Assay development

The column-based binding assay was based upon chromatographic principles, which showed different issues from plate-based assays. The most important parameters considered during the development were: sensitivity of microfluidic CDs; assay buffer effects; the concentrations of the capture and detection reagents; and the CD spin speed. The microfluidic CD chambers hold a different volume of samples for a different working range. For example, Bioaffy 200 CD holds 200 nl and Bioaffy 1000 holds 1000 nl [10]. The sample dilution buffer can affect the microfluidic property of samples and the detection buffer can affect the fluorescent signal detection. Multiple concentrations of capture antibody were tested to find the optimum amount that saturates the columns. The detection antibody concentration was tested to find the signal that gave the best signal/background ratio. The CD spin speed was adjusted to control sample flow to allow enough time for binding. Although the development methods of the Gyrolab may be different from traditional plate-based ELISA, ICH guidelines are applicable for defining the parameters and criteria for the Gyrolab assay [12–14].

After the development, the assay was evaluated for its dynamic range, selectivity, accuracy and precision. Since the concentration of HCP was estimated to range from 5 to 400,000 ng/ml for in-process samples, it is important to have broad dynamic range to reduce the number of dilutions of highly concentrated samples and provide sufficient LOQ for lower ranged samples. It is widely understood that the condition of sample matrix may affect antibody and antigen binding. Such interference may come from the presence of nonspecific proteins, pH, ionic strength, or hydrophobicity of the sample matrix [15–17]. To assess the selectivity of the assay, dilutional linearity and spike recovery testing were performed with analytes diluted by different magnitudes. To minimize the matrix effect, samples should be used at low concentration although this will decrease the sensitivity of the assay and increase the dilutional error. Thus the minimum required dilution (MRD) of the therapeutic antibodies was determined. Acceptance criteria set for the spike recovery testing is 100 ± 20% of the initial spike value according to the ICH standard for the acceptable range suggested in ICH Q2A [12,14]. Precision of the assay can be determined by assessing intra/inter assay variation in experimental design.

Assay evaluation

Dynamic range & LLOQ

To determine the dynamic range of the assay, HCP antigen at 20,000 ng/ml was serially diluted by two-fold steps from 10,000 to 1.22 ng/ml in Rexxip A buffer. The LLOQ was determined by running HCP antigen standards at 4, 8, and 10 ng/ml in triplicate. The acceptance criteria were CV <20% and bias <20% for all samples.

Dilutional linearity

Eleven in-process monoclonal antibody samples with a range of HCP (50-2000 ng/ml) present in the matrix were diluted two-times, four-times, eight-times and 16-times in Rexxip A buffer and tested in duplicate. Then linearity of the HCP concentration was determined by the R^2 value. As a subsequent experiment, two in-process mAbs with estimated HCP concentration of 10,000 ng/ml were diluted two-times, ten-times, 50-times and 100-times and tested in duplicate.

Spike recovery

In order to evaluate matrix effect, drug substance at 50 mg/ml was used for spike recovery testing. All samples were diluted by two-times, five-times, ten-times, and 50-times in Rexxip A buffer and tested in

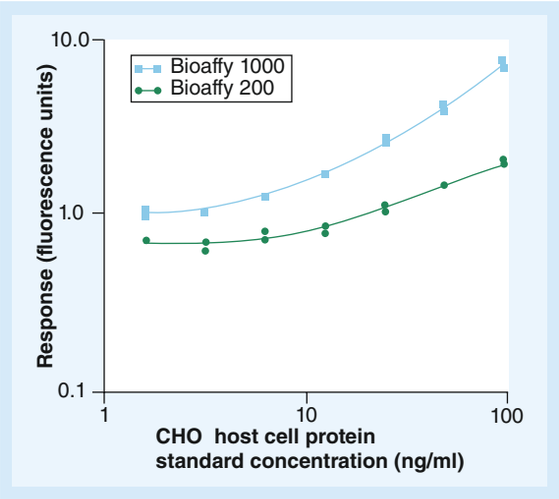

Figure 2. Selection of Bioaffy CD. Seven-point standard curve of CHO HCP from 100–1.56 ng/ml to compare sensitivity of two microfluidic columns. Bioaffy 1000 had the background response of 0.8 FU and the signal to background ratio from 1.2 to 8.8. Bioaffy 200 had the background response of 0.6 FU and the signal to background ratio from 1.1 to 3.3.

duplicates. HCP antigen was added into each sample to be 50 ug/ml and same volume of PBS was added into each sample as non-spiked controls. The percent spike recovery was calculated by subtracting the HCP concentration in unspiked control samples from the spiked samples. The difference was then divided by the spiked in value (50 ng/ml) and multiplied by 100%. The acceptance criterion was 100 ± 20% [12].

Inter- & intra-assay variation

In order to determine assay precision, inter and intra assay variability was evaluated. For intra assay variability, HCP antigen standard at 100 ng/ml was aliquoted in eight replicates and analyzed in the same Bioaffy CD. For inter assay variability, the same 8 replicates were ran in the other Bioaffy CD separately.

Assay comparison to plate based ELISA using in-process monoclonal antibodies

After the development and evaluation, the Gyrolab HCP assay was compared with two types of plate-based ELISAs. Manual ELISA was performed by an analyst using the Cygnus ELISA kit. Tecan ELISA was performed by Tecan automatic liquid handler using the same Cygnus ELISA kit. All three methods used the same type of Cygnus antibodies and antigens for parallel comparison. The eight-point standard curves (6–100 ng/ml) were used for all three methods. In addition, the Gyrolab assay was repeated using 13-point standard curve (4–10,000 ng/ml). The in-process mAb samples were from various purification steps, with estimated HCP concentrations ranging from 10 to 300,000 ng/ml. All samples were diluted to four different concentrations to find the optimum dilution that has a HCP value within the standard curve range. Among the values that fell within the range, the highest value was chosen.

Results & discussion

The HCP assay was developed on the Gyrolab system by testing the sensitivity of Bioaffy CDs, the concentration of capture and detection reagents, and flow speed. After the development of the assay, the selectivity, accuracy, and precision of the assay were evaluated by testing dilutional linearity, spike recovery, and inter/intra CD precision. The optimized assay was then compared with the manual ELISA and Tecan automated ELISA for the in-process analysis of therapeutic antibodies under development.

Selection of Bioaffy microfluidic CD

The first step in the development was to compare Bioaffy 200 and Bioaffy 1000 for its sensitivity at the range of 0 to 100 ng/ml. Figure 2, shows that Bioaffy 1000 gave better signal to background ratio and higher slope compared with Bioaffy 200. It was also suggested that using Bioaffy 1000 (1000 nl of analyte) gave better sensitivity at the range of interest (0–100 ng/ml) than Bioaffy 200. Since the sensitivity at low range (<100 ng/ml) is important but difficult to achieve, the Bioaffy 1000 was chosen for further evaluation work.

Binding reagent optimization

A range of capture and detection antibody concentration were tested to study binding capacity of the columns and find the optimal reagent concentration. Theoretically, an excessive amount of capture antibodies are needed to coat the entire microfluidic column. For the detection reagent, a higher concentration usually results in higher background, while the lower concentration will not give sufficient sensitivity. Four different concentrations of capture reagent in combination with three different concentrations of detection reagent were tested. Among all the four concentrations of capture antibody, 100 ug/ml gave the highest slope as shown in Figure 3A. Capture reagent at concentrations higher than 100 ug/ml gave a similar response curve, suggesting the column was saturated for capture reagent binding (Figure 3B) and the binding would not improve once the beads are fully coated. For the detection reagent, 2.5 ug/ml produced the steepest slope and the lowest background (Figure 3C). Based on such results, the best combination was determined to be 100 ug/ml of capture reagent to saturate the column and 2.5 ug/ml of detection reagent for the highest S/N ratio.

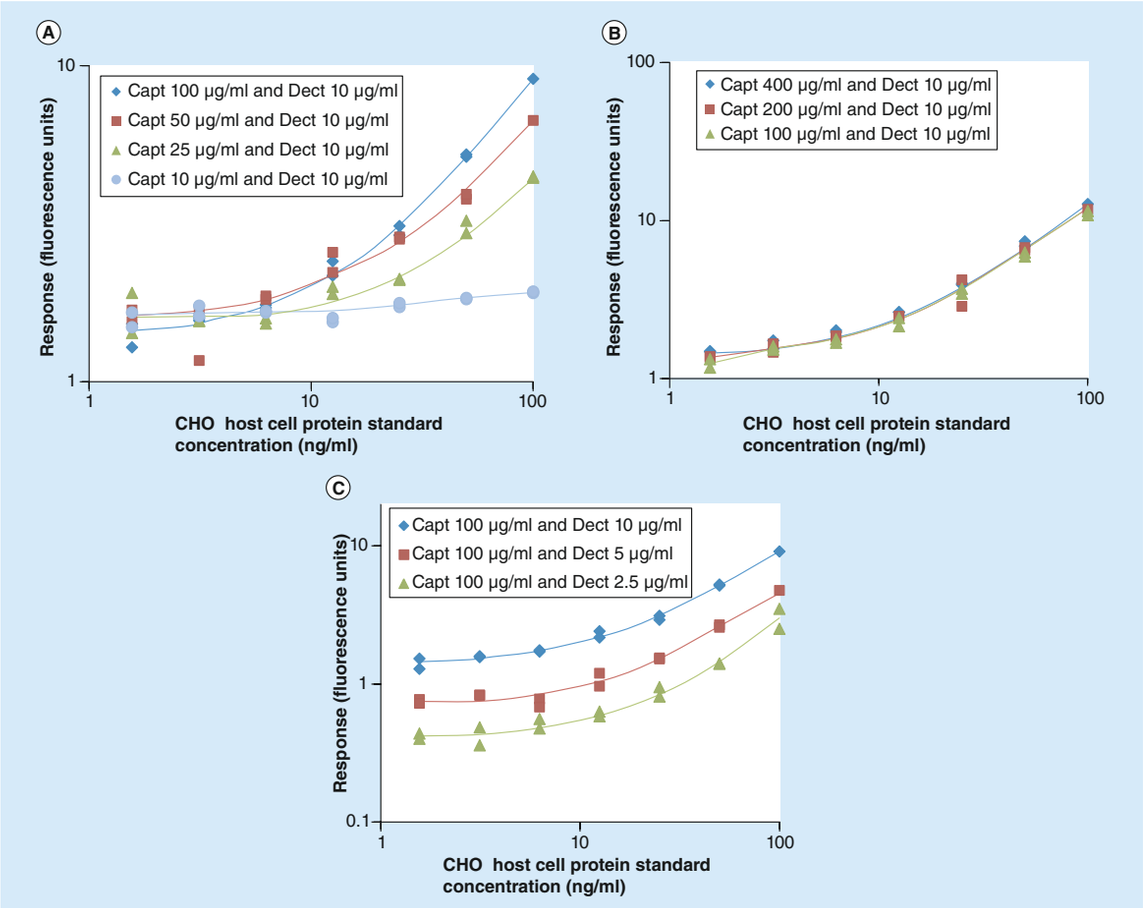

Figure 3. Capture and detection reagent optimization to identify the best sensitivity. (A) Titration of capture reagent concentration was from 12.5–100 ug/ml. (B) Testing higher concentration of the capture reagent (>100 ug/ml) to saturate the column. (C) Titration of detection reagent concentration from 2.5–10 ug/ml.

Optimization of flow speed

Two different analyte flow rates were evaluated to determine binding efficiency of the columns. The default instrument setting has a flow speed of 1 nl/s for the analyte and it was compared with a slower speed of 0.5 nl/s. As shown in Figure 4, the analyte applied at 0.5 nl/s showed improved overall response rate with a similar background. The signal to noise ratio was increased by 30–70% through applying the slower flow rate. The slower application of analyte gave more binding time which allowed for better assay performance. However, the slow spin method increased the run time for each CD by 20 min from the 60 min standard spin.

Determination of assay dynamic range & LLOQ

Under optimized conditions (capture reagent at 100 ug/ml, detection reagent at 2.5 ug/ml and spin speed at 0.5 nl/s), the dynamic range of the Gyrolab HCP assay was determined from 4 ng/ml to 10,000 ng/ml (Figure 5), which is 100-times broader than that of the manual ELISA (6–100 ng/ml). CHO HCP standards ran in triplicate at 4, 8, 10 ng/ml and all passed the 100 ± 20% acceptance criteria with ≈10% CV and bias. The acceptance criteria were CV < 20% and bias

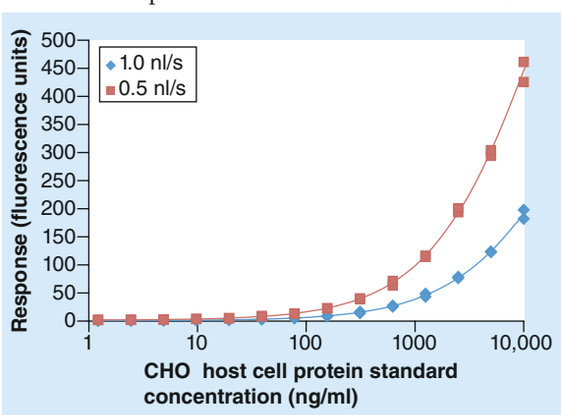

Figure 4. Comparison of analyte flow speed. 13-point CHO host cell protein standard curve from 10,000–2.44 ng/ml was run twice separately at 1 nl/s and 0.5 ng/s.

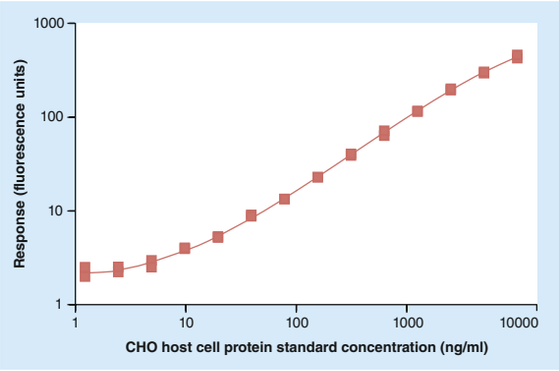

Figure 5. Linearity range of Gyrolab ELISA. The dynamic range of the Gyrolab assay under optimized condition was 4–10,000 ng/ml.

< 20% for all samples. The two lower points (2.4 and 1.2 ng/ml) did not meet the acceptance criteria.

Dilutional linearity & matrix evaluation

In order to test linearity of different dilutions, five in-process mAb samples with a range of HCP concentration (50-2000 ng/ml) were diluted by two-times, four-times, eight-times and 16-times in Rexxip A buffer.

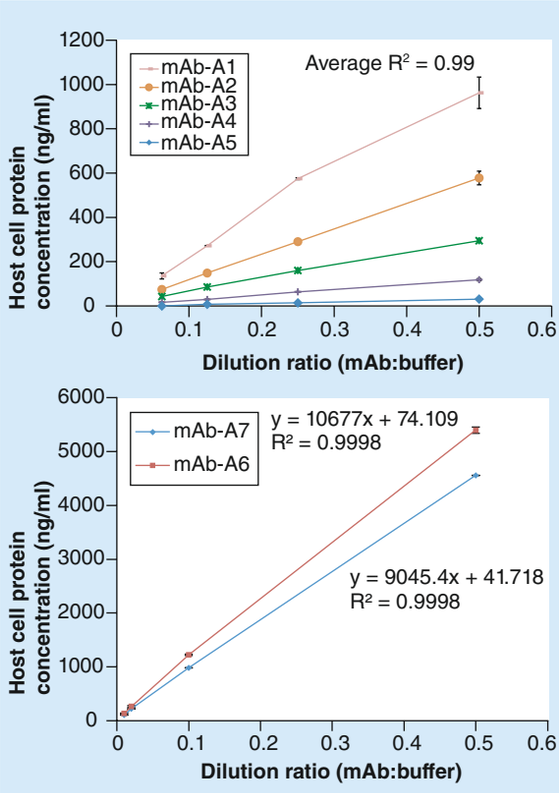

Figure 6. Dilutional linearity of in-process monoclonal antibodies. (A) In-process samples are in different purification buffers and contain 50–2000 ng of host cell protein/ml. (B) Two in-process samples at higher host cell protein concentration (≈10,000 ng/ml) were diluted by four different folds.

All samples maintained excellent linearity with average R^2 value of 0.99 for all four dilutions (Figure 6A). In addition, in-process samples at a different purification step with higher HCP concentration were also tested (Figure 6B). Two in-process mAbs with estimated HCP concentration of 10,000 ng/ml were diluted two-times, ten-times, 50-times and 100-times in Rexxip A buffer, respectively in duplicate. Both samples showed excellent linearity for all dilutions with average R^2 value of 0.99. Table 1 shows that dilution corrected HCP concentrations for all samples were consistent with less than 10% CV. Therefore, MRD was determined to be 2. High dilutions tend to minimize potential matrix interference and reflect the better estimation of HCP concentration but can also lead to over-estimation of the concentration. If the diluted sample concentration is at the lower end of the linear range, the response signal of the analyte can be unproportionally high. The affinity differences of the antibodies in the pool may lead to over-estimation or underestimation because the number of dilutions may change binding kinetic of each antibody differently [18]. Therefore, it is important to test the samples at different dilutions and determine the most consistent concentration.

In addition to in-process purification intermediates, the mAb drug substance at 40 mg/ml passed the spike recovery test for all three dilutions as shown in the Table 2. The percent spike recovery was all within the immunoassay variability. The result of dilutional linearity and spike recovery suggest that the column-based ELISA is not strongly affected by the matrix contents. Moreover, all other unbound contents will flow through the column, minimizing possibility of other binding interference.

Precision

The intra CD and inter CD variability was tested to assess the assay's precision. Eight QC HCP standards at 100 ng/ml were analyzed in two CDs. The QC samples had an intra-CD CV of 3.9% (n = 8) and inter-CD CV value of 4.1% (Table 3). Figure 7 represents the standard curves for five experiments completed over a 3-month period and shows excellent operational consistency.

Comparison of Gyrolab HCP ELISA & plate-based HCP assays for analysis of in-process mAb purification intermediates

A set of in-process mAb samples from typical purification processes were used to compare the performance across the manual ELISA, Tecan ELISA and Gyrolab. Overall, all methods resulted in similar values for all purification intermediates (Figure 8). The consistent quantification of all methods showed the trend of decreasing HCP levels through the purification pro-

Table 1. Dilution corrected host cell protein concentrations of in-process monoclonal antibodies.

Buffer ratio (mAb:buffer)	Dilution factor	Host cell protein concentration (ng/ml)	Dilution corrected host cell protein concentration (ng/ml)	CV
mAb-A1				
0.5	2	962	1925	7.3
0.25	4	575	2299	
0.125	8	272	2176	
0.0625	16	136	2171	
mAb-A2				
0.5	2	578	1156	1.7
0.25	4	290	1162	
0.125	8	149	1190	
0.0625	16	75	1196	
mAb-A3				
0.5	2	295	590	7.0
0.25	4	160	641	
0.125	8	86	687	
0.0625	16	43	685	
mAb-A4				
0.5	2	119	237	6.5
0.25	4	64	256	
0.125	8	29	236	
0.0625	16	17	270	
mAb-A5				
0.5	2	31	62	5.0
0.25	4	14	56	
0.125	8	7	60	
0.0625	16	<4 ng/ml (below LOQ)	<64 ng/ml	
mAb-A6				
0.5	2	5398	10,796	9.9
0.1	10	1224	12,240	
0.02	50	266	13,300	
0.01	100	135	13,500	
mAb-A7				
0.5	2	4558	9116	8.3
0.1	10	982	9815	
0.02	50	221	11,050	
0.01	100	105	10,500	

cess leading to acceptable log reduction HCP clearance. Further evaluation of the method focused on the impact of sample dilution.

The Table 4 shows the standard curve range of each method, the magnitude of sample dilution, and an example measured HCP concentration of P1 sample. For the plate-based ELISA, only one out of four dilutions fell within the detectable range so the dilutional linearity could not be determined. However the Gyrolab had the advantage of a broad dynamic range and was able to detect 6 out of 8 dilutions with an acceptable consistency of 5% CV. At HCP concentrations

Table 2. Spike recovery test of 50 ng/ml host cell protein in a drug substance at two-, five- and ten-fold dilutions.

DF	Unspiked control host cell protein concentration (ng/ml)	Final host cell protein concentration (ng/ml)	Host cell protein content (ng/ml)	Spike recovery (%)	Pass or fail
mAb @ 40 mg/ml					
2	126.6	186.6	60.0	120	Pass
5	183.0	234.0	51.0	102	Pass
10	216.0	256.0	40.0	80	Pass

Table 3. Variability of eight QC samples within CD and between 2 CDs.

Variability	CHO HCP Concentration (ng/ml)	CV (%)
Intra CD	100	3.9% (n = 8)
Inter CD	100	4.1% (n = 16)

< 2000 ng/ml (samples P3 - P6), Gyrolab generally detected more HCP amount than the plate-based ELISA method and is therefore more sensitive.

Conclusion

The Gyrolab was used to develop an automated high throughput HCP assay suitable for testing in-process samples of monoclonal antibodies supporting downstream process development of therapeutic monoclonal antibodies. This study identified important assay parameters unique to the Gyros microfluidic platform. The optimized condition was achieved through the largest sample chamber volume, saturation of the beads with capture antibody, and slow application of the analyte. The developed assay demonstrated excellent selectivity, accuracy, and precision with in-process sample analysis. The dilutional linearity and spike recovery results suggested that Gyrolab's chromatographic mechanism minimizes the matrix effect and well-controlled microfluidics gives excellent consistency. All three methods, manual ELISA, Tecan ELISA and Gyrolab measured similar HCP concentration for purification intermediates, proving efficient clearance of HCP throughout the purification processes. The differences of absolute values among the assays were further investigated and Gyrolab was found to maintain an excellent consistency for all dilutions. The findings can be explained that the measured HCP concentration may vary depending upon the

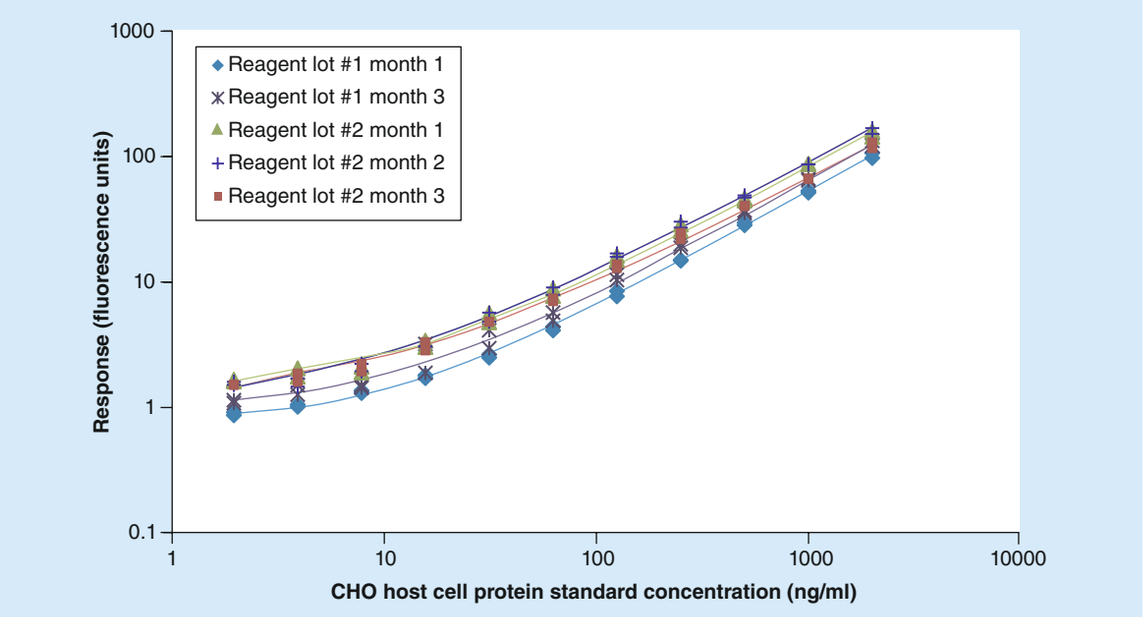

Figure 7. Comparison of 11-point standard curves for five experiments done over 3-month period. Two reagent lots prepared separately were used to draw CHO host cell protein standard curve from 2000 to 1.95 ng/ml.

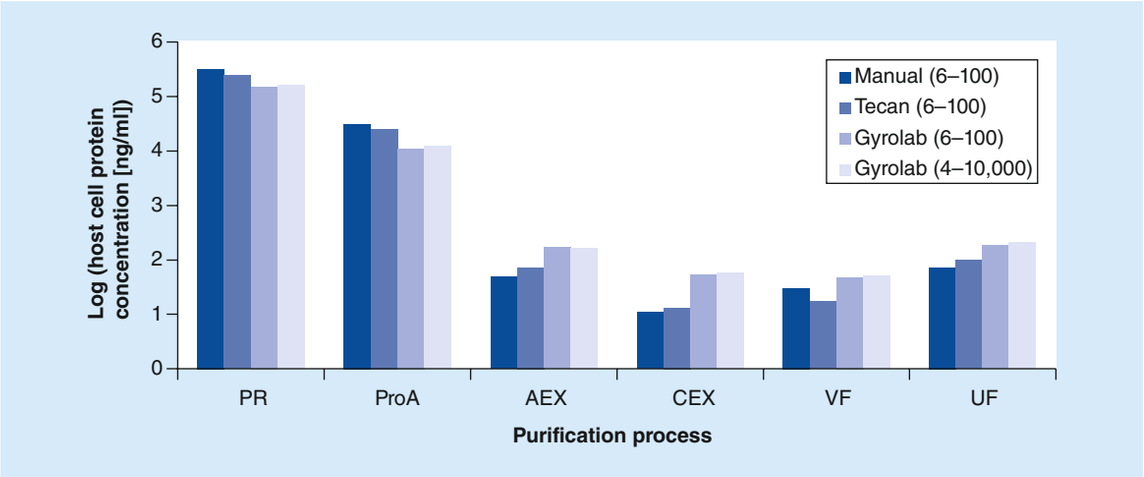

Figure 8. Comparison of Gyrolab host cell protein assay and plate-based host cell protein assay. In-process purification samples measured by manual plate-based ELISA, Tecan plate-based ELISA, and Gyrolab ELISA.

matrix content and the type of detection method [7,15,18]. A recent presentation in 2011 FDA/IABS [18] addressed the strategy to determine the validity of HCP concentration and emphasized the importance of dilutional linearity and spike recovery as key factors that may give inaccurate quantification. With the broad dynamic range, Gyrolab was found to be suitable to screen purification intermediates with HCP concentrations spanning 6 logs. In addition to the excellent dilutional linearity, the Gyrolab detected higher HCP levels in samples with low HCP concentrations suggesting the higher sensitivity.

As shown in Table 5, Gyrolab automated HCP analysis with 5x higher sample throughput than Tecan ELISA. Gyrolab is capable of 96 data points per hour and 960 data points per day. In contrast, Tecan assay generated 192 data points per 5 h run. In addition to improvements in automation and throughput, 2 logs broader dynamic range was another major advantage of Gyrolab HCP assay for in-process sample analysis, minimizing the need for large dilutions and avoiding re-runs. Unlike plate-based ELISA, in which reagents and samples are incubated in the wells and unbound proteins are rinsed, the reagents and samples of the Gyrolab assay flow through the column for more efficient binding and minimum matrix effect.. The nano-column based assay required five-times less sample and reagents saving valuable in-process samples and costly HCP reagents. Such reagent savings compensated for the cost of the Bioaffy

Table 4. Example of the host cell protein measurement for purification process 1 (P1) sample.

Method (standard curve range, ng/ml)	Dilutions	Host cell protein concentration (ng/ml)	Dilution corrected host cell protein concentration (ng/ml)	CV (%)
Tecan ELISA (6-100)	100	>LOQ	>LOQ	N/A
	500	>LOQ	>LOQ	
	2500	>LOQ	>LOQ	
	12,500	19.33	241,623	
Gyrolab (4-100)	100	>LOQ	>LOQ	20
	500	>LOQ	>LOQ	
	2500	44.0	109,957	
	12,500	11.7	146,185	
Gyrolab (4-10,000)	20	7139	142,786	4.3
	40	3629	145,161	
	80	1945	155,624	
	160	1001	160,114	

Table 5. Comparison summary of plate-based host cell protein assays and Gyrolab host cell protein assay.

Host cell protein assay	Manual ELISA	Tecan ELISA	Gyrolab ELISA
Assay time (run)	5 h (192 data points)	5 h (192 data points)	1 h (96 data points)
Throughput (day)	192 data points	384 data points	960 data points
Overnight operation	No	Yes (up to 192 data points)	Yes (up to 480 data points)
Hands-on time	3 h with frequent intermission	30 min with one time operation	30 min with one time operation
Dynamic range (ng/ml)	7–100	7–100	4–10,000
Minimum required sample	50	50	8
Relative assay cost	×	2×	×

CD and the Gyrolab HCP assay cost became comparable with manually performed commercial HCP ELISA kit. Tecan liquid handler required the higher working volume therefore more reagent was used. In addition, the automation and throughput of Gyrolab system also save the labor and time. Since the system is in a highly controlled environment with minimal human interference, the data consistency was improved from the plate-based assays. All in all, this well-controlled automated assay provides greatly enhanced throughput, consistency and reliability, delivering fast and cost effective results to support downstream process development. The throughput improvement and cost reduction align well with the industry's goal to rapidly deliver drugs to market with minimum resources.

Future perspective

The Gyrolab system has been proven to be a reliable and effective immunoassay platform for in-process HCP clearance monitoring and the quantification

Executive Summary

Background

- The analysis of host cell-derived proteins is critical for the control of biopharmaceutical process and the quality of final biological products. Commonly used ELISA is lengthy, labor intensive and often shows inadequate assay performance. The high-throughput residual host cell protein (HCP) assay with minimum hands-on time and better performance was developed using Gyros technology to facilitate in-process development of biologics.

Assay development

- Bioaffy CDs with different sample chamber size showed significant sensitivity difference. Desired LLOQ (<10 ng/ml) was achieved using Bioaffy 1000
- 100 ug/ml of capture antibody saturates column and gives the best binding profile. Higher concentration does not improve the assay performance
- Higher sensitivity can be achieved by decreasing flow rate of the analyte.

Assay evaluation

- Gyrolab has a broad dynamic range of 4–10,000 ng/ml with possibility of extending ULOQ.
- Affinity chromatography in Gyrolab's columns minimizes the matrix effect, showing great dilutional linearity of in-process mAbs and spike recovery of mAb drug substances
- Well-controlled assay conditions in Gyrolab's system gives superior consistency (≈5% intra and inter assay CV)

Assay platform comparison

- Three assay platforms, Gyrolab ELISA, manual plate-based ELISA, and Tecan automated plate-based ELISA, were compared using in-process therapeutic antibodies from a series of purification processes.
- All three methods showed a good correlation and provided quantitative analysis to prove effective clearance of HCP.

Improvements

- Gyrolab improved throughput (five- to ten-times), cost (up to 1/2), sample consumption (1/5) compared with plate-based ELISA.
- Gyrolab HCP assay provided high quality data and minimized repeats (100-times broader dynamic range, high consistency, better sensitivity)
- One-hour immunoassay allowed for rapid assay development, screening optimal conditions as fast as 3 h.

of final HCP in drug substance. It has shown great potential to be used as a release assay for QC work. The Gyrolab has a capability to impact other areas of bioprocess development where high throughput immunoassays are in critical need. Although regular Bioaffy CDs can process only simple ligand binding assays, the more advanced microfluidic channel design will allow it to perform more complicated assays such as residual protein A ligand assay. In addition, the potential to execute complex digestion assays will allow broader applications of the Gyros technology.

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Financial & competing interests disclosure

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval. No animal or human subject was used in the experiment.

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