GYROS PROTEIN Technologies

Accelerating bioprocess workflows with microfluidic immunoassay-based parallel impurity analysis

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BACKGROUND

Product titer and impurities levels are critical quality attributes (CQA) in biotherapeutic manufacturing. Minimizing the level of bioprocess impurities in the downstream purification process is a critical regulatory and safety requirement since they can induce adverse effects in patients. As the number of impurities requiring analysis continues to expand, executing accurate and precise bioassays of different types for large sample sets can cause workflow bottlenecks.

The Gyrolab® xPand system enables methods to be mixed and matched to meet specific needs, either by performing up to five CD runs with one assay and many samples, or by setting up a Gyroplex® panel, when up to 5 independent immunoassays are run sequentially in an automated walk-away format, reducing hands-on time and increasing productivity in bioprocess analytics. Since each assay is run independently with no cross-talk, re-runs of a single analyte is easy without having to sacrifice reagents and samples to repeat the assay for the other analytes.

RESULTS

In the present study, a MabSelect SuRe™ purification eluate from a Chinese Hamster Ovary (CHO) mAb culture was assessed for IgG titer and four different process related impurity levels in a Gyroplex panel generating results in 5 hours. (Data show dilutional linearity and spike recovery, which are important assay validation parameters in all stages of the biotherapeutic workflow (Fig. 2 and Table 1).) The Gyrolab software automatically calculates spike recovery from the dilution series of spiked and unspiked unknowns for each assay. This enables the accuracy and dilutional linearity of sample analysis to be verified. The system helps the user to set criteria for dilutions and spikes according to current guidelines (e.g. USP 38-NF 33 1132 Residual HCP; see Fig. 1).

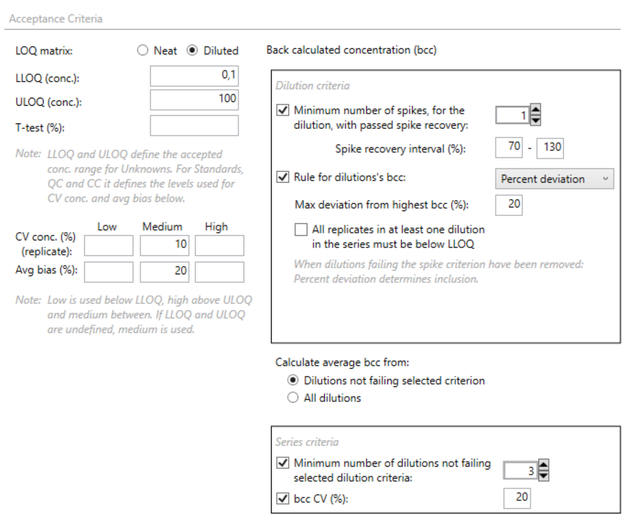
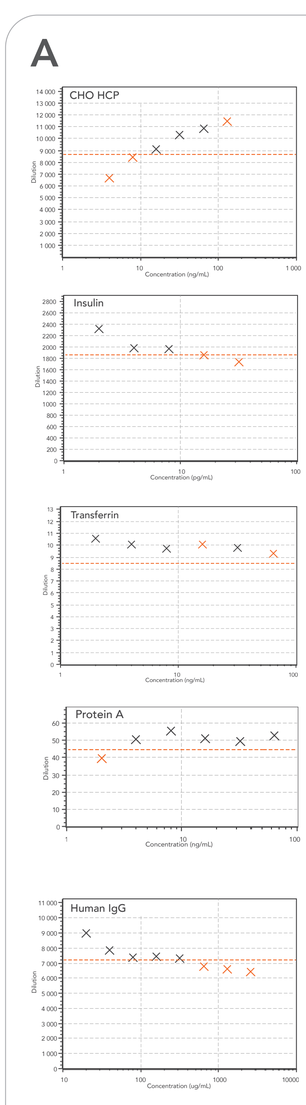


Figure 1. The acceptance criteria for dilution linearity for all assays was set in the Gyrolab Evaluator software to a maximum of 20% deviance from the highest valid dilution in the dilution series, represented by the dotted orange line in the plots (Fig. 2A). Greyed out marks are excluded data points due to either failed spike recovery or failed dilution linearity (below the 80% dotted line).



B

CHO HCP

Unspiked Conc, Neat	Unspiked Conc, Diluted	Dilution Factor	Spiked Conc, Neat	Spiked Conc, Diluted	Spike Recovery [%]	Conc vs Max [%]	Dilution Used in Series Conc	Series Conc	CV Series Conc [%]
6.66E+003	1.66E+003	4.00	1.35E+004	3.38E+003	178	61.2	No	1.01E+004	8.77
8.42E+003	1.05E+003	8.00	1.28E+004	1.60E+003	109	77.4	No		
9.14E+003	571	16.0	1.39E+004	867	118	84.0	Yes		
1.03E+004	323	32.0	1.36E+004	424	80.7	95.0	Yes		
1.09E+004	170	64.0	1.54E+004	240	113	100	Yes		
1.15E+004	89.8	128	1.67E+004	131	131	106	No		

Insulin

Unspiked Conc, Neat [pg/mL]	Dilution Factor	Spiked Conc, Neat [pg/mL]	Spike Recovery [%]	Conc vs Max [%]	Dilution Used in Series Conc	Series Conc [pg/mL]	CV Series Conc [%]
2,33E+003	2,00	4,26E+003	96,5	100	Yes	2,10E+003	9,75
1,99E+003	4,00	4,12E+003	106	85,2	Yes		
1,97E+003	8,00	4,03E+003	103	84,5	Yes		
1,86E+003	16,0	4,22E+003	118	79,8	No		
1,74E+003	32,0	4,24E+003	125	74,4	No		
1,59E+003	64,0	4,26E+003	133	68,1	No		

Trasferrin

Unspiked Conc, Neat [ng/mL]	Dilution Factor	Spiked Conc, Neat [ng/mL]	Spike Recovery [%]	Conc vs Max [%]	Dilution Used in Series Conc	Series Conc [ng/mL]	CV Series Conc [%]
10,6	2,00	19,9	92,9	100	Yes	10,1	3,84
10,1	4,00	19,4	93,7	95,1	Yes		
9,74	8,00	19,5	97,7	92,0	Yes		
10,1	16,0	23,7	136	95,6	No		
9,81	32,0	21,6	118	92,6	Yes		
9,34	64,0			88,2	No		

Protein A

Unspiked Conc, Neat [ng/mL]	Unspiked Conc, Diluted...	Dilution Factor	Spiked Conc, Neat [ng/mL]	Spiked Conc, Dilute...	Spike Recovery [%]	Conc vs Max [%]	Dilution Used in Series Conc	Series Conc [ng/mL]	CV Series Conc [%]
39.6	19.8	2.00	83.1	41.5	72.4	71.2	No	52.0	4.38
50.9	12.7	4.00	96.6	24.2	76.3	91.5	Yes		
55.6	6.95	8.00	122	15.3	111	100	Yes		
51.1	3.19	16.0	116	7.25	108	91.9	Yes		
49.8	1.56	32.0	122	3.82	121	89.5	Yes		
52.8	0.825	64.0	120	1.87	112	95.0	Yes		

Human IgG

Unspiked Conc, Neat [ug/mL]	Unspiked Conc, Diluted...	Dilution Factor	Conc vs Max [%]	Dilution Used in Series Conc	Series Conc [ug/mL]	CV Series Conc [%]
> 1,00E+004	> ULOQ	10,0		No	7,82E+003	8,93
9,01E+003	451	20,0	100	Yes		
7,85E+003	196	40,0	87,1	Yes		
7,41E+003	92,6	80,0	82,2	Yes		
7,46E+003	46,6	160	82,8	Yes		
7,35E+003	23,0	320	81,5	Yes		
6,77E+003	10,6	640	75,1	No		
6,60E+003	5,16	1,28E+003	73,2	No		
6,46E+003	2,52	2,56E+003	71,6	No		
< 7,02E+003	< STD	5,12E+003		No		

C

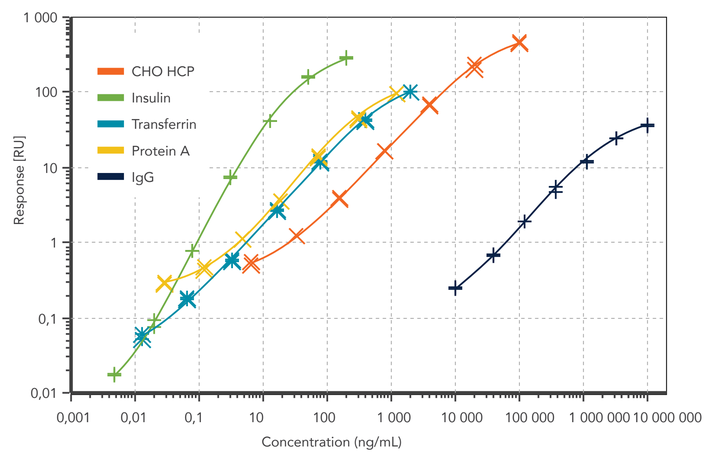


Figure 2. Dilution linearity plots (A), spike recovery and dilution linearity data (Conc vs Max (%) column) (B) and Standard curves (C), for CHO host cell proteins (HCP), Insulin, human Transferrin, Protein A and human IgG.

Table 1. Summary table of impurity levels in eluate after MabSelect SuRe purification

Impurity	Level (ng/mL)	CV (%)	ng impurity/ mg IgG product
CHO HCP	10000	8.8	1279
Insulin	2.1	9.8	0.268
Transferrin	0.01	3.8	0.0013
Protein A	52	4.4	6.65

THE GYROLAB SYSTEM

Gyrolab systems perform automated immunoassays within nanoliter-scale microfluidic structures in a Compact Disk (CD) format. Each structure on the CD comprises a 15-nanoliter affinity column pre-packed with streptavidin-coated particles, supporting a variety of assay types including sandwich and indirect antibody assays. While Gyrolab xPlore™ runs single CDs, Gyrolab xPand can run up to five CDs unattended. Consumption of sample and reagents is dramatically reduced compared with plate-based ELISA. Microfluidic control ensures that all samples on a CD are processed in parallel, giving consistent results. Each microstructure equates to one data point, eliminating cross talk. The control and analysis software enables 21 CFR part 11 compliance, ensuring that assays can be developed and transferred through to GMP and GLP environments.

MATERIALS AND METHODS

Automated three-step sandwich assays were used to measure titer as well as Protein A, CHO HCP, Insulin and transferrin impurities in a MabSelect SuRe™ purified therapeutic antibody sample* from a CHO transfected cell line. The ready-to-use Gyrolab CHO-HCP Kit, Gyrolab Protein A Kit and Gyrolab huIgG – High titer Kit were used. Reagents, CDs and buffers needed for all assays are summarized in Table 2.

Three different types of Gyrolab Bioaffy™ CDs were used, including a Gyrolab Mixing CD 96 in the Protein A kit that includes an automated mixing step to perform acid dissociation prior to measurement of Protein A.

Gyrolab Bioaffy CD

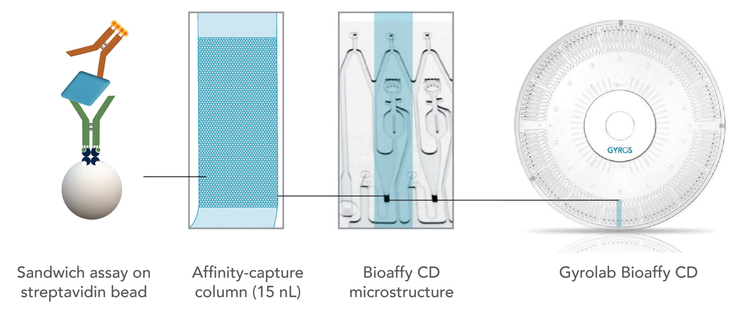


Table 2. Assay designs and assay performances. The huIgG, CHO HCP and Protein A are available as ready-to-use Gyrolab kits and the Insulin and Transferrin assays are available as Gyrolab assay protocols **. The assay performances of each assay are from respective IFU or protocol instruction.

Assay	Capture reagent	Detection reagent	Gyrolab Bioaffy CD	Analytical range (ng/mL)		Precision (CV %)	
				LLOQ	ULOQ	Intra-run	Inter-run
CHO HCP	Affinity purified goat antibody	Affinity purified goat antibody	1000 HC	~2	8 000	< 10	< 20
Insulin	mAb mouse anti insulin/proinsulin #1	mAb mouse anti insulin/proinsulin #2	1000	0.025	15	< 10	< 20
Transferrin (human)	anti-human transferrin mAb	anti-human transferrin pAb	1000	0.1	150	< 10	< 10
Protein A (MabSelect SuRe)	Affinity purified a-Pro A chicken pAb	Affinity purified a-Pro A chicken pAb	Mixing CD 96	~0.1	100	< 10	< 10
IgG Titer	Protein A derivate from Staphylococcus aureus	$F(ab')_2$ fragment of anti-human IgG	20 HC	300	1 000 000	< 10	< 10

*Gyros Protein Technologies wishes to thank POLYMUN Scientific Immunbiologische Forschung GmbH for generously supplying the samples for analysis.

** Gyrolab assay protocols for Insulin and human Transferrin can be downloaded from https://www.gyrosproteintechnologies.com/gyrolab-assays

SUMMARY

Human IgG titer and four independent bioprocess impurities were analyzed in a MabSelect SuRe eluate and evaluated in one single run using the Gyrolab xPand system. Sample concentration, dilutional linearity and spike recovery parameters were determined for culture-related impurities of CHO HCPs, transferrin, insulin, and Protein A leakage from chromatographic columns. Results were obtained in 5 hours, with dynamic assay ranges at least 1 log greater than manual ELISA and precision all under 10% CV, and with excellent dilutional linearity. The automated Gyrolab immunoassay system allowed minimum hands-on and in-lab time, streamlining impurity analysis.

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