Product Quantification (IgG Titer) Using an Automated Analytical Platform – Gyrolab Workstation

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Introduction

Antibody quantification (IgG titers) is critical to the cell line development and clone selection, as well as the optimization of bioreactor conditions in production. ProA-HPLC method has been widely used to determine antibody titers, which employs an Immunoaffinity HPLC column, followed by UV detection at 280nm. However, this method is time consuming, with low throughput.

In this study, a novel automated analytical platform-Gyrolab xP workstation is used to determine antibody titers. The immunoassays are automatically controlled in identical microstructures within a Gyrolab CD, in which an affinity capture column containing streptavidin-coated beads is employed to capture biotinylated antibodies. All the samples and reagents are transferred to each microstructure by spinning the CD at precisely controlled speed. Samples are processed simultaneously in parallel and the quantification is achieved by the integration of the laser-induced fluorescence. We have evaluated the capability of Gyrolab in linearity, precision, accuracy, and range, in comparison with the ProA-HPLC method. Gyrolab significantly improved turnaround time as well as reduced sample consumptions.

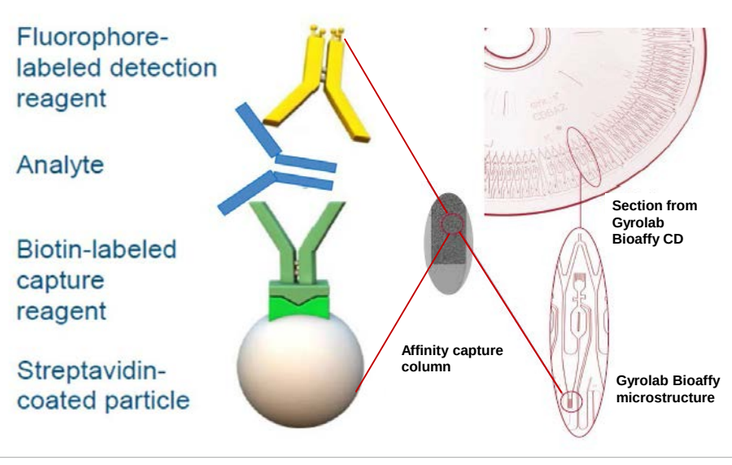
Experimental

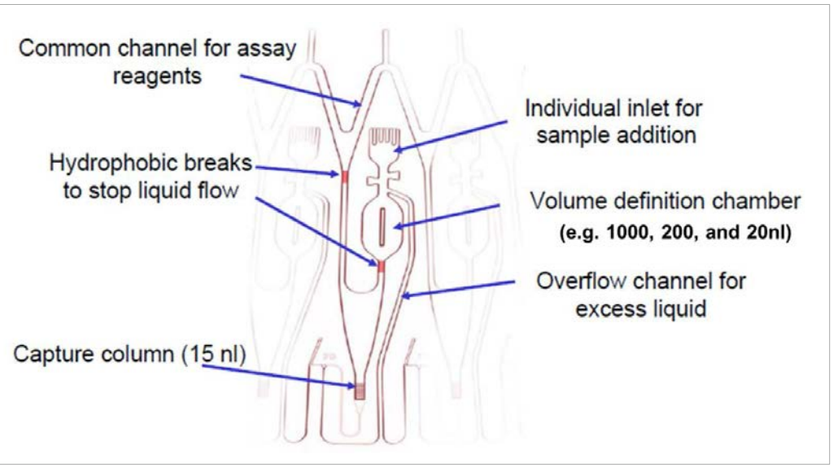
Materials and Methods:

Materials and Reagents: Buffers were purchased from Gyros US Inc. and antibodies were purchased from Jackson ImmunoResearch Laboratories Inc. Biotinylation reagents and reactive dyes were purchased from Pierce Biotechnology Inc. and Life Technologies, respectively.

Methods (sample and reagent preparation): Standards were prepared by pre-diluting the samples to 2.4 mg/mL in CCS buffer followed by 1:2 serial dilutions. Samples were prepared either by 1:2 serial or 1:5 single dilutions in CCS buffer. Biotinylation reagent (EZ-Link Sulfo-NHS-LC-Biotin) was used to label the capture antibody, and reactive dye (Alexa 647) was used to label the detection antibody (see references for details).

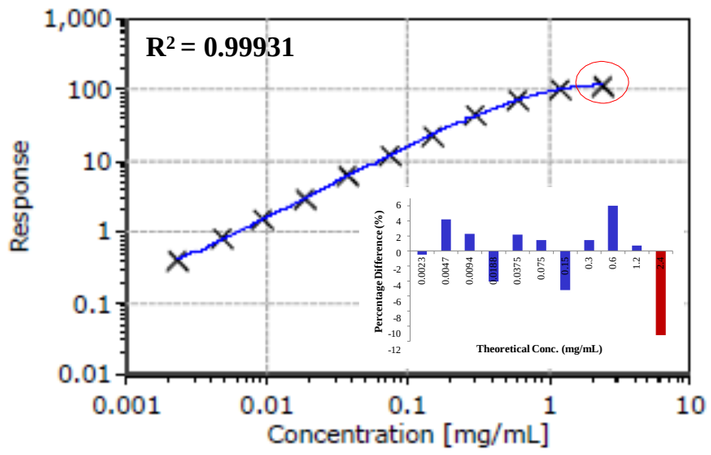
Instruments: Antibody titers were determined on Gyrolab xP workstation (Gyros US Inc), which were compared with the results measured on HPLC (Waters) with a ProA cartridge (Life Technologies). The concentrations of mAbs were measured at A280 by SoloVPE (C Technologies Inc).





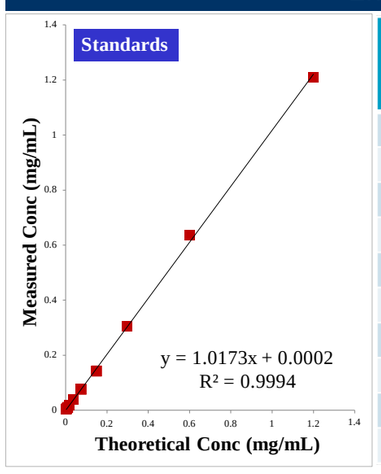
Results and Discussion

Standard Curve



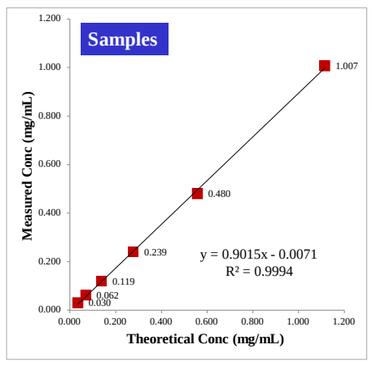
The standard curve (run in duplicate) was fitted with 5PL regression and concentrations ranged from 0.0023 mg/mL to 2.4 mg/mL. Most of the percentage differences were well below 10% except the highest one (2.4 mg/mL). Thus the range of this assay was determined to be 0.0023-1.2 mg/mL.

Linearity



Dilution Factor (Molecule A)	Theoretical Conc. (A280, mg/mL)	Measured Conc. (Gyrolab, mg/mL)	Percentage Difference (%)
2	1.2	1.21	0.9
4	0.6	0.636	6.0
8	0.3	0.304	1.4
16	0.15	0.142	-5.2
32	0.075	0.0761	1.4
64	0.0375	0.0383	2.2
128	0.0188	0.0180	-4.1
256	0.0094	0.00961	2.3
512	0.0047	0.00490	4.2
1024	0.0023	0.00229	-0.5

The linear regression analysis of standards was carried out by generating a plot of theoretical concentration vs measured concentration ranged from 0.0023 mg/mL to 1.2 mg/mL. The R^2 was 0.9994 showing a strong relationship between A280 and Gyrolab concentrations.



Dilution Factor (Molecule A)	Theoretical Conc. (mg/mL)	Measured Conc. (Gyrolab, mg/mL)	Percentage Difference (%)
2	1.007	1.007	NA
4	0.504	0.480	-4.8
8	0.252	0.239	-5.2
16	0.126	0.119	-5.6
32	0.063	0.062	-1.6
64	0.031	0.030	-3.2

Linear regression analysis of cell culture samples showed a good dilutional linearity (R^2 =0.9994) on Gyrolab, down to 0.030 mg/mL (64X dilution).

Matrix Interference

Theoretical Conc (Molecule A, mg/mL)	Dilution Factor	Measured Conc (mg/mL)	Accuracy (%)	CV Conc (%)
0.05mg/mL*	1	0.00476	10	26.3
		0.00623	12	
		0.00366	7	
	2	0.0571	114	6.4
		0.0557	111	
		0.0505	101	
	4	0.0491	98	5.8
		0.0542	108	
		0.0491	98	

* Drug substance of molecule A was diluted into feed media.

The sample was measured neat, and diluted 2 and 4 fold in triplicate. The very low spike recovery was observed for neat samples suggesting matrix interference.

Accuracy

Sample Name	Day	Unspiked Conc (mg/mL)	Expected Spiked Conc (mg/mL)	Calculated Spiked Conc (mg/mL) (mean, n=3)	Spike Recovery (%)	CV (%) (n=3)
A	1	1.81	3.01	3.15	105	1.7
	2	2.20	3.33	3.63	109	1.8
B	1	0.98	1.61	1.69	105	1.8
	2	1.17	1.79	2.00	112	1.6
C	1	0.18	0.268	0.266	99	2.1
	2	0.17	0.252	0.256	102	5.2

* Each concentration was measured in triplicate (5 fold dilution) and the result was an average of 3 data points (n=3).

Three different molecules were used to evaluate the assay accuracy on two different days. Spiking level was 50% for all the samples. The results showed good spike recoveries ranged from 99% to 112% demonstrating assay accuracy for samples between 0.2 and 2 mg/mL.

Repeatability and Intermediate Precision

Sample Name	Day	Mean Measured Conc (mg/mL, n=3 or 6)	% CV (Repeatability)	% CV (Intermediate Precision)
A	1	1.81	3.3	10.8 (n=12)
	2	2.20	3.7	
B	1	0.98	2.2	9.6 (n=12)
	2	1.17	1.5	
C	1	0.183	3.8	6.0 (n=6)
	2	0.166	1.8	

Cell culture samples from three molecules were tested to evaluate the repeatability. Each concentration (5x dilution) was measured in 3 (C) or 6 (A and B) replicates on two different days. The results showed good assay performance within each single day for repeatability and intermediate precision.

Gyrolab vs. ProA HPLC

Sample Name	Theoretical Conc (HPLC, mg/mL)	Mean Measured Conc (Gyrolab, mg/mL, n=6 or 12)	Percentage Difference (%)
A	2.25	2.01	-10.7
B	1.23	1.07	-13.0
C	0.171	0.175	2.3

The mean measured concentration was an average from two different days. The percentage difference of Gyrolab compared with ProA HPLC ranged from 2% to 13%.

	ProA HPLC		Gyrolab	
Sample volume(ul)	10 – 100 ul		4 ul (including dead volumes)	
Measurement range	0.02 – 5 mg/mL (usually 1–50 ug load); sample dilution can be avoided by adjustment of sample injection volumes		0.04 – 6 mg/mL; single 5X dilution for all samples	
Precision (%CV)	Intra (neat) ≤ 3%	Inter (neat) ≤ 3%	Intra (5x dilution) < 4%	Inter (5x dilution) < 11%
Assay time including sample preparation (e.g. 100 samples)	13 –16 hours		< 2 – 3 hours	
Accuracy	88 – 119%		99 – 112%	
	Percentage difference to ProA HPLC is 2 – 13%.			

Conclusions

- Sample and reagent consumptions have been significantly reduced by Gyrolab (by a factor of 25).
- The repeatability of Gyrolab (2 —4%) is comparable to ProA HPLC (≤ 3%) while the intermediate precision (6 —11%) is not as good as ProA HPLC (≤ 3%).
- The sample preparation of Gyrolab was substantially simplified by making a single 5X dilution for all the samples across the plate, which allowed for concentrations to fall into the linear range of the standard curve and overcame the effects of matrix interference.
- The dynamic range with current experimental set-up for Gyrolab is comparable to ProA HPLC.
- Gyrolab has been used to test three different molecules (mAbs) showing comparable results to ProA HPLC with a percentage difference of 2-13%.
- Gyrolab titer assay significantly improves turnaround time compared with ProA HPLC method.

Acknowledgement

- Kathi Williams (Gyros)
- Kenji Furuya, Ph.D. (Boehringer Ingelheim)

References:

1. Instruction of Alexa Fluor 647 mAb labeling kit from Molecular Probes.
2. Instruction of Sulfo-NHS-LC–Biotin labeling from Thermo Scientific.