Gyrolab Demonstrated Shorter Assay Time and Broader Dynamic Range Compared to a Plate Based ELISA with a Generic Human IgG Assay in Rat Serum

Carolyn Mallozzi and Mania Kavosi

Pharmacokinetics, Dynamics and Metabolism: Pfizer Global Research and Development, Andover, MA

ABSTRACT

Due to throughput needs, the Gyrolab was investigated in an attempt to reduce assay run time and increase the dynamic range of a generic human IgG quantitative ELISA. A series of experiments were performed to transfer and qualify the ELISA assay to the Gyrolab platform. Parameters such as the dynamic range, sensitivity, accuracy, precision, sample volume and run time were examined. Overall, the ELISA assay was successfully transferred to the Gyrolab platform and demonstrated its ability to shorten assay run time, capability to reduce sample volume and broaden the dynamic range of the assay versus a plate based ELISA.

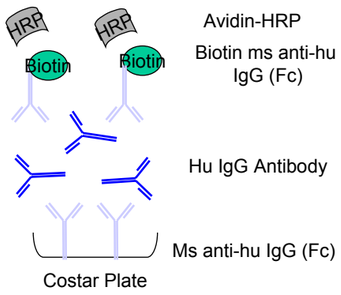
Figure 1: Gyrolab Instrument and Accessories

INTRODUCTION

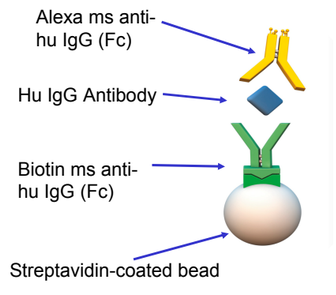
Gyrolab is a flexible instrument platform used for development and processing of miniaturized immunoassays for protein quantification. By leveraging two natural phenomena (centrifugal force and capillary action) and proprietary microfluidic technologies, immunoassays are run within microstructures of Gyrolab Bioaffy CDs under control of a Gyrolab workstation.

Previously, a generic quantitative assay to measure a human IgG antibody in rat serum was developed in an ELISA format. In order to examine the Gyrolab technology, the assay was transferred to the Gyrolab and qualified based on the lab's parameters.

Figure 2: Schematic of the Generic Assay for the Quantitation of Human IgG in Rodent Serum in: a) an ELISA platform, b) the Gyrolab platform



a. ELISA platform



b. Gyrolab platform

Table 1: Comparison of ELISA and Gyrolab Generic Assay Parameters

Parameter	Gyros	ELISA
Standard Point Range	1.68-16000 ng/mL	26.0-1000 ng/mL
Dynamic Range	10.5-6400 ng/mL	88.0-666 ng/mL
MRD	1:2	1:20

RESULTS

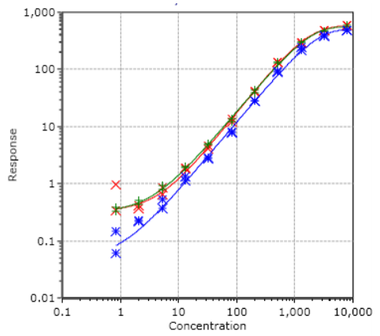


Figure 3: Reproducibility of Gyrolab Technology Illustrated with Overlay of Standard Curves.

Standard curves with pooled rat serum were interpolated with 5PL fitting with a response weighing factor. Three independent curves were run as part of qualification. Bias and CV were within acceptable limits in the dynamic range of each assay.

Table 2: Accuracy and Precision of Gyrolab Technology Demonstrated by QCs.

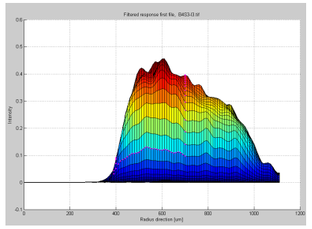
Pooled QCs were examined for all three runs of the qualification. The average values for each QC over the three days are shown below. One QC replicate (164 ng/mL) was masked due to an abnormal binding event on the column which caused a low reading of flourescence by the Gyrolab. The data for this QC is not included in the averages below.

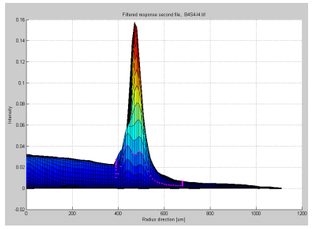
QC conc (ng/mL)	10.5	26.2	164	2560	6400
Mean Conc (ng/mL)	10.9	24.9	143	2567	5793
SD	2.10	3.13	11.0	340	1089
%CV	19.3	12.6	7.69	13.3	18.8
%Bias	3.56	-4.96	-13.0	0.260	-9.48

Six individual lots of Sprague Dawley rat serum were tested at the low QC concentration, high QC concentration and unspiked. All samples had acceptable CVs. The high and low QCs had acceptable biases for 5/6 individuals. 5/6 unspiked individual donors were below the limit of quantitation

Figure 4: Flourescence Graphs Illustrate Normal vs. Abnormal Binding to the Beads in the Column.

The flourescence graphs below represent the liquid binding on the column. The graph on the left has a broad peak with high intensity, which illustrates binding throughout the column. This is an example of the typical column binding for this assay. The second replicate of the same sample is shown on right, which has one narrow peak with a much smaller intensity (notice the differences in the scale). This is indicative to an abnormal binding pattern in this column, which was reason to exclude this data point.





CONCLUSION

Table 3: Comparison of Gyrolab vs. ELISA Platform With the Generic Assay.

Overall, the ELISA assay was successfully transferred to the Gyrolab platform and demonstrated its ability to shorten assay run time and broaden the dynamic range of the assay versus a plate based ELISA..

Parameter	Gyros	ELISA	Gyrolab Enhancement
Well Capacity	8 µL	100 µL	10X less volume
Assay Run Time (including o/n coating)	< 1 hour	10 hours	10X shorter run time
Dynamic Range	10.5-6400 ng/mL	88-666 ng/mL	8X broader dynamic range
MRD	1:2	1:20	10X lower MRD
Assay Development Time	3 days	1 week	2.3X faster development time
Built in Automation	Yes	No	Has built in automation
Frequent Interruptions from Deskwork	No	Yes	No interruptions from deskwork
Over night running	Yes	No	Capability to run overnight

Based on our evaluation, our lab purchased the Gyrolab instrument and the Gyrolab technology will be used when applicable.

ACKNOWLEDGEMENTS

Special thanks to Robert A. Durham from Gyrolab for his expertise. Thanks to Alison Joyce, Sheldon Leung, Terri Caiazzo, Kathleen Chapman and Boris Gorovits for their assistance.