Quantitative Ligand Binding Assay for Determination of Antibody Drug Conjugate Using Gyrolab Immunoassay Platform

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Abstract

PURPOSE: The antibody-drug conjugates (ADCs) is a new type of targeted therapy that combines the specificity of monoclonal antibodies (mAbs) with cytotoxic small molecule to target a tumor specific antigen. We developed a high throughput and streamlined process using design of experiment (DoE) and Gyrolab immunoassay platform to measure both total antibody and payloads of ADC to support exploratory toxicology (non-GLP) studies.

METHODS: Biotinylated anti-human IgG Fc mAb was used as a capture reagent. Alexa fluor 647 labeled anti-human kappa antibody was used as a detection antibody to measure total antibody. Alexa fluor 647 labeled anti-payload (custom made) antibodies were used to measure antibody drug conjugate concentrations. Multi-factorial DoE was used to optimize the conditions. A full standard curve and quality controls were tested against eight experimental conditions. The output parameters were included signal to noise ratio and total error (high and low QC). The goal was to maximize the S:N and minimize the total error. The most desirable conditions were applied to sample analysis and the performance characteristics of in-study QCs were compared to the JMP (SAS, Cary, NC) predictions.

RESULTS:

Table 1. DOE Predictions and Assay Performance Characteristics.

Assay	QC	DOE Predicted TE	In-study TE	In-study %CV	In-study Bias	DOE Predicted S:N	Mean In-study S:N	In-study Runs
1	Low	6.58 ± 17.9	12.9	9.8	3.1	7.47 ±4.83	7.45	83
	High	6.19 ± 19.3	9.6	8.1	1.5			
2	Low	7.68 ± 58.7	12.4	10.7	-1.7	8.98 ± 3.83	6.91	42
	High	2.58 ± 2.06	13.7	9.3	4.4			

CONCLUSIONS: The integration of DoE and Gyrolab platform has streamlined the process of developing immunoassays. The advantages of the Gyrolab in implementing DoE include: automated liquid handling, reduced reagent consumption, and easy to use interface.

GYROS IMMUNOASSAY PLATFORM

The Gyrolab is an automated and miniaturized immunoassay platform using compact disk technology, microfluidics, and a laser-based fluorescence-detection system to quantify biological drugs, proteins, and biomarkers from nanoliters of biological fluids.

The ADC and Assay format

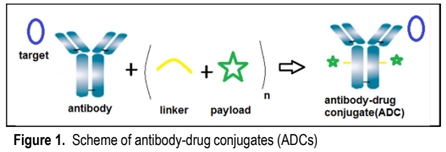


Figure 1. Scheme of antibody-drug conjugates (ADCs)

- Total antibody assay: capture with biotinylated goat anti-human IgG and detect with goat anti-human IgG kappa chain labeled with Alexa Fluor 647 (in house labeling).
- ADC assay: capture with biotinylated goat anti-human IgG (Same as TA assay) and detect with anti-payload reagents labeled with Alexa Fluor 647 (in-house labeling).
- For both assays,the range of quantification is from 81.9 ng/mL to 20,000 ng/mL using a 4-parameter logistic model. Quality controls (QCs) are at 4 concentration levels of 150, 250, 7500 and 15,000 ng/mL.

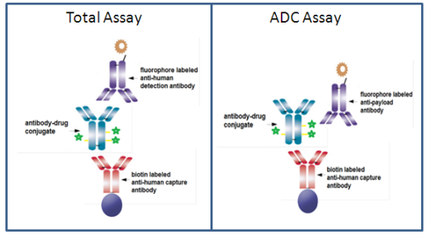
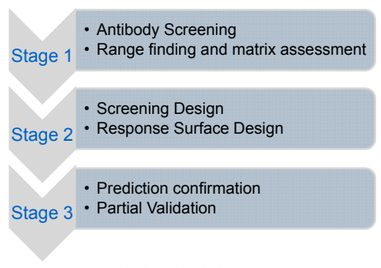


Figure 2. Scheme of Total assay and ADC assay on Gyrolab

DESIGN OF EXPERIMENT (DoE)

- The Design of Experiments (DoE) approach was utilized during method development to create an in-house generic human IgG assay and several payload detection assays on the Gyros platform.
- The strategy of experimentation



- There are many factors that contribute to the success of method development. The Stage 1 parameters are assessed based on previous plate base assay evaluations
- For this method development , we focused on 2 factors , to optimize appropriate capture and detection conditions.

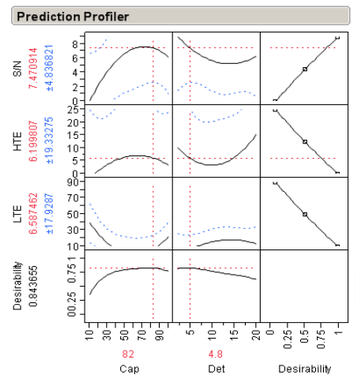
DoE Experiment and Results

- A minimum of 8 experiments are needed to optimized the response output (using TA assay as example).
- The results from signal-to-noise ratio (S/N) and total errors (TE) of high and low QCs are imported to JMP software to generate the optimization results.

Experiment	Factor (ug/mL)		Response		
	Capture	Detection	S/N	HQC-TE	LQC-TE
Exp 01	50	10	6.12	1.07	23.4
Exp 02	100	20	4.75	8.13	22.5
Exp 03	100	2.0	8.37	6.10	17.4
Exp 04	50	2.0	6.36	2.67	13.5
Exp 05	10	2.0	0.51	3.40	23.2
Exp 06	50	20	6.35	23.2	22.6
Exp 07	100	10	2.61	5.30	26.7
Exp 08	10	20	4.00	11.0	85.8

Table 1. The DoE design and results from generic human IgG assay on Gyrolab

DoE Experiment and Results Cont.



(µg/mL)	Capture	Detection
Total Assay	80	5

Figure 3. The prediction profiler from JMP software

ASSAY PERFORMANCE

Assay	QC	Predicted %TE (± error)	Upper %TE Range	In-Study %TE	Fold Difference from Predicted
1	Low	9.9 ± 15.4	25.3	12.1	1.2
	High	8.3 ± 1.9	10.2	5.3	0.6
2	Low	10.2 ± 21.3	31.5	9.7	1.0
	High	6.5 ± 9.5	16.0	10.4	1.6
3	Low	6.6 ± 17.9	24.5	12.9	2.0
	High	6.2 ± 19.3	25.5	9.6	1.6
4	Low	7.7 ± 58.7	66.4	12.4	1.6
	High	2.6 ± 2.1	4.7	13.7	5.3
5	Low	6.5 ± 33.5	40.0	12.7	2.0
	High	12.0 ± 19.4	31.4	11.2	0.9

Table 2. DoE Prediction vs. In-study Assay Performance

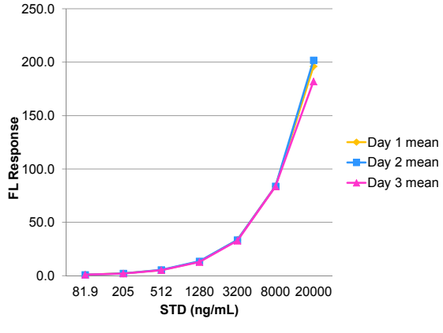


Figure 4. Standard Curve day-to-Day Comparison

RESULTS AND DISCUSSION

- Rat serum was collected from male Sprague-Dawley rats that were dosed intravenous for fourteen days with vehicle and antibody drug conjugate (ADC) analyte.
- The assays have clearly distinguish the PK for the fully intact ADC compared to the naked antibody.

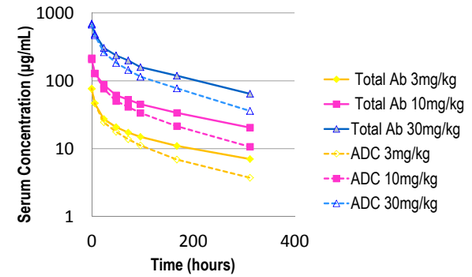


Figure 5. The serum concentration vs time of total Ab and ADC for rat toxicity study at three different doses

- The predicted concentrations shown below are based on previous study results using a traditional plate based ELISA assay. The observed concentrations are the results generated from the Gyros platform.

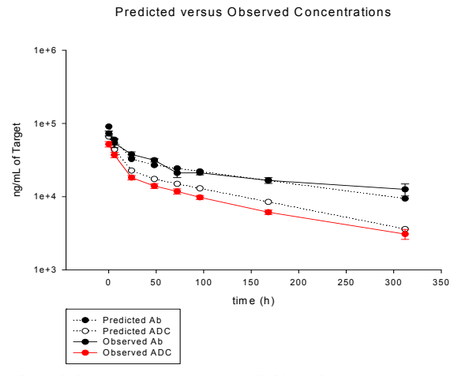


Figure 6. Comparison between plate base ELISA and Gyrolab results

CONCLUSIONS

- Immunoassays using the Gyrolab platform have been successfully applied to the measurement of ADCs.
- The integration of DoE and the Gyrolab platform has streamlined the process of developing immunoassays, adding assay robustness, in-study predictability, and improved reliability of measurement.
- The advantages of the Gyrolab include: automated liquid handling, reduced reagent consumption, and easy to use interface.
- It is also easy to convert plate base ELISA assay to Gyrolab platform.
- During a five month period, we had developed methods for 30+ ADC compounds and analyzed over seven thousand samples on one GyroLab instrument.