GYROS PROTEIN Technologies

Combine DOE and Gyrolab system to quickly develop robust immunoassays

Case Study

Two assay development teams at Pfizer have combined the systematic approach of DOE and the Gyrolab platform to streamline the development of robust immunoassays.

Case Study 1: Design Of Experiments approach accelerates Gyrolab assay development for ADC studies

The PDM (Pharmacokinetics, Dynamics & Metabolism) group at Pfizer focuses on the development of innovative therapies such as Antibody-Drug Conjugates (ADC) through insights into targets, pathways and modeling for preclinical efficacy, and discrete toxicity. In one project, they needed to quickly develop high throughput toxicokinetic assays to measure serum and plasma concentrations of different components of their ADC – total antibody and conjugate (ADC). Aidong (Annette) Wu, a scientist working in the PDM group describes how they combined Gyrolab xP workstation and DOE to speed up assay development and improve precision and accuracy.

Method

The DOE process involved three main steps:

1. Antibody screening, defining ranges and minimum required dilution (MRD).
This was based on experience with plate-based assays.

2. Screening design and response surface design.
Many factors affect Ligand Binding Assays, including temperature and incubation time, but since Gyrolab system controls the majority of these factors, the screening step could be omitted.

In the response surface design (Response Surface Model, or RSM) step, the team set up a series of experiments to study two key factors – the concentrations of capture and detection reagents. Using JMP® (SAS) DOE software, two levels were set, high and low, plus one level in the middle in case the response was non-linear. This made a total of eight experiments that could be run on two Gyrolab™ Bioaffy CDs in two hours. Key responses were measured – Signal/Noise, that measured sensitivity, and Total Error for high QC and low QC, to measure accuracy and precision, and the data was used to create a model.

Table 1: *Experimental design*

	Capture	Detection	Signal/Noise	High TE*	Low TE
exp1	50	10	6.12	1.07	23.35
exp2	100	20	4.75	8.13	22.49
exp3	100	2	8.37	6.10	17.36
exp4	50	2	6.36	2.67	13.48
exp5	10	2	0.51	3.40	23.24
exp6	50	20	6.35	23.17	22.60
exp7	100	10	2.61	5.30	26.70
exp8	10	20	4.00	11.04	85.75

*Total Error (TE) is a measurement of accuracy and precision

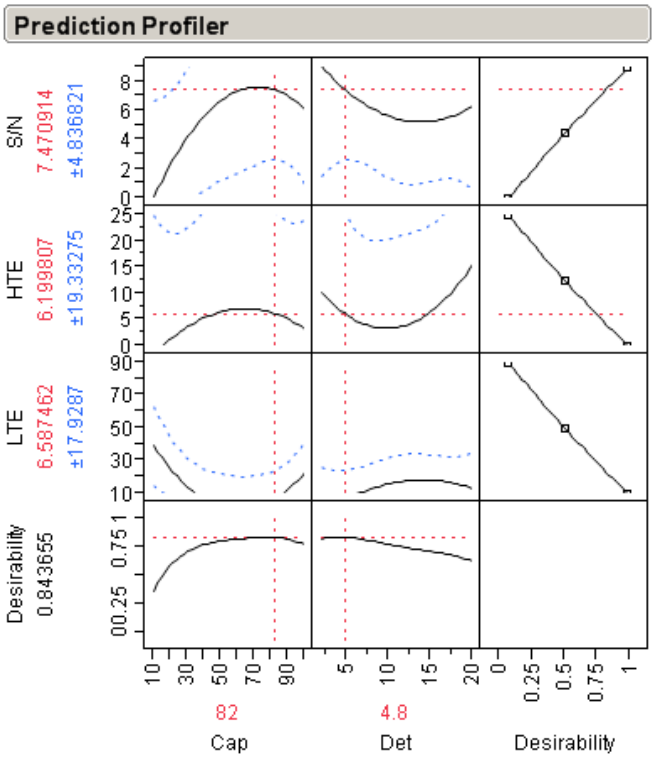

Figure 1: *JMP DOE software indicated the levels of capture and detection reagents needed to maximize signal/noise and minimize variability (total error).*

The goal was to maximize Signal/Noise (S/N), and minimize Total Error for Low QCs (LTE) and High QCs (HTE). The model indicated how these responses were affected by varying the concentrations of the capture and detection reagents, and the overall 'Desirability' of the result. The model indicated optimal concentrations of 82 µg/mL capture reagent and 4.8 µg/mL detection reagent, with predicted values of the responses as shown in Table 2.

3. Prediction confirmation and partial validation

The recommended reagent concentrations were then used to determine in-study performance and confirm that the model was in line with real assay performance.

Table 2: Comparison of predicted and measured variation

Response	Predicted	In-study performance
High QC TE	6.2 %	7.2 %
Low QC TE	6.6 %	15.5 %
S/N	7.5 %	

The performance of the assay was evaluated by running three calibration curves per day for three days, and indicated that the assay was very robust. The overall variation for QC's was 4.6%, which was well within the accepted limit of 15%.

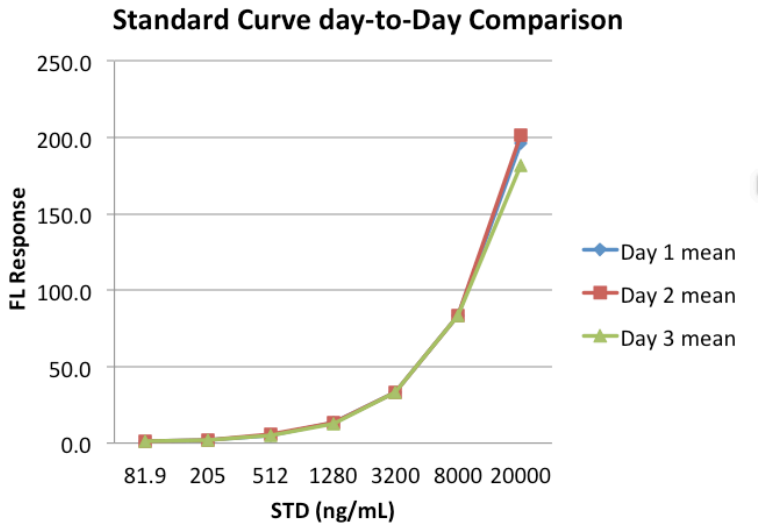

Figure 2: The Gyrolab assay was very robust – each curve is an average of three independent standard curves run on one day.

Summing up

This approach helped the PDM group at Pfizer to develop three assays to GxP level in six days. The automation of Gyrolab xP workstation then enabled them to assay 240 study samples using six CDs per day, and to complete the analysis of 1200 samples in six days. Gyrolab technology also reduced reagent consumption five-fold compared to plate-based ELISA, which was a key advantage considering the limited amount of reagents available. The team went on to develop assays for over 30 ADC compounds in five months and analyze over 7000 samples on one Gyrolab system.

Annette concluded that the integration of DOE and the Gyrolab platform has streamlined immunoassay development, adding assay robustness, in-study predictability, and improved measurement reliability.

Find out more

View seminar

Annette Wu in the PDM group at Pfizer, La Jolla, CA, USA, presented 'Case study: Antibody-drug conjugates TK study on Gyros immunoassay platform' at the Gyrolab Seminar, 2011, USA.
View Seminar >>

Download poster

Quantitative ligand binding assay for determination of antibody drug conjugate using Gyrolab immunoassay platform, available from the Downloads section >>

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Case study 2: Using DOE leads to a more robust assay for alpha fetoprotein

Allison Given and her colleagues at Pfizer Worldwide Research & Development, Pharmacodynamics Metabolics and Dynamics, in USA, needed to develop a Gyrolab assay to measure alpha fetoprotein as a diagnostic and prognostic biomarker for hepatocellular carcinoma. They started by screening seven antibodies to select the optimal capture and detection antibody pair based on high response and signal/noise ratio. The next step was to improve robustness by applying DOE to minimize assay error and maximize signal/background.

Screening design

The team screened four factors:

- Concentration of capture antibody
- Concentration of detection antibody
- Minimum Required Dilution
- Assay Buffer (4 variants)

They used JMP DOE software to create the initial screening design, which involved 16 of the 32 possible experiments:

Capture Conc. (µmL)	Detection Conc. (µmL)	MRD	Assay Buffer
100	20	5	Superblock
100	2	20	Superblock
100	2	5	Superblock
10	20	5	Superblock + NaCl
100	2	20	Superblock + NaCl
10	20	20	Superblock
10	2	20	Superblock + NaCl
10	2	5	Superblock + NaCl
10	20	20	Superblock + NaCl
10	20	5	Superblock
10	2	5	Superblock
100	20	20	Superblock + NaCl
10	2	20	Superblock
100	20	20	Superblock
100	20	5	Superblock + NaCl
100	2	5	Superblock + NaCl

The results of the experiments run on Gyrolab system were evaluated using Prediction Profiler in JMP DOE software to determine the combination of factors that would minimize error and maximize Signal/Background (Signal:Noise or S:N) when using a 'desirability' function (Figure 3).

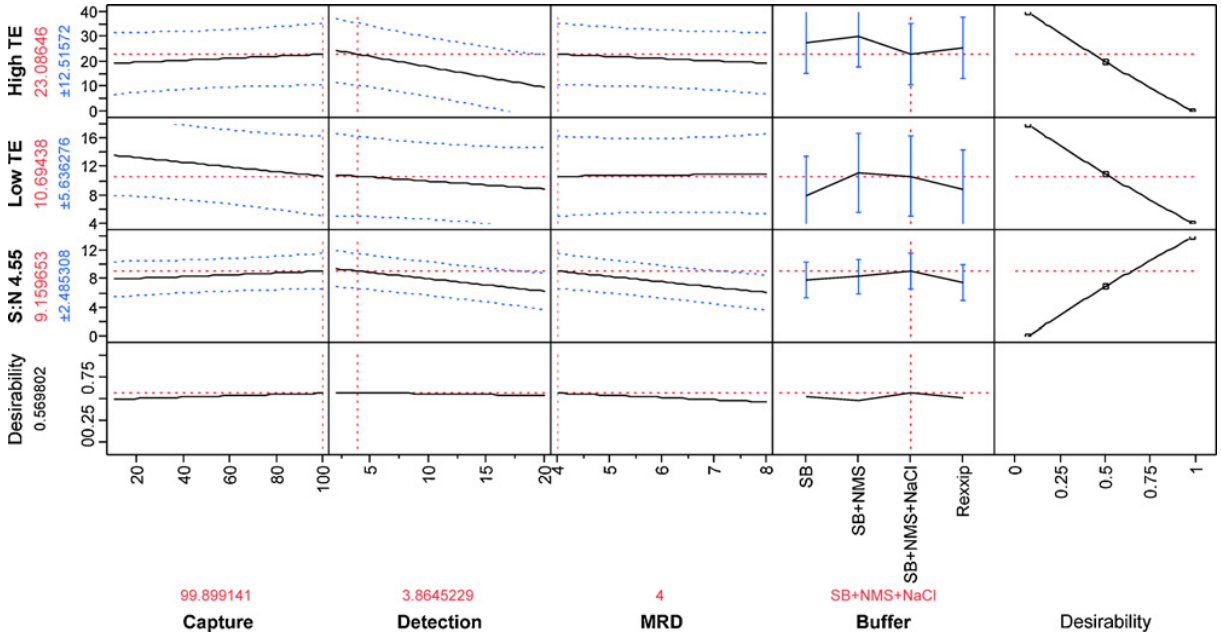

Figure 3: Initial screening experiment using JMP DOE software. Data from Given et al, 2012

This analysis indicated that the optimal combination was 100 µg/mL capture antibody, 3.83 µg/mL detection antibody, MRD of 4, and with the buffer Superblock with 5% NMS and 500 nM NaCl.

Response Surface Model (RSM)

The team refined the assay by fixing the buffer and MRD and using a response surface design with capture and detection reagents set at three levels (high, medium, low). This gave a total of eight experiments that were run on the Gyrolab system and analyzed using JMP DOE software. The results indicated that capture antibody should be fixed at 100 µg/mL and the detection antibody fixed at 11.1 µg/mL (Figure 4).

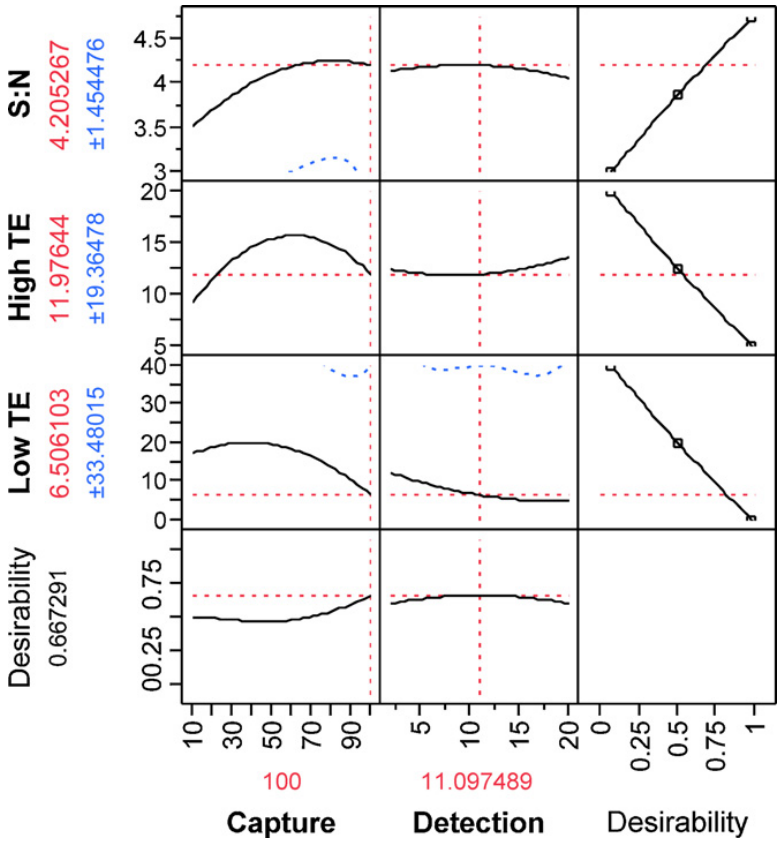

Figure 4: A response surface design was used to determine the combination of concentrations of capture and detection reagent that maximized S:N and minimized error.

Confirming predicted performance

The accuracy of the DOE prediction was then tested by running standards and QCs using the recommended assay, and the results showed good agreement between the actual and predicted values.

Response (AFP concentration)	Predicted	Observed
TE High level (100 ng/mL)	11.98 ± 19.36	15.1
TE Low level (3.5 ng/mL)	6.51 ± 33.48	5.1
S/N (2.52 ng/mL)	4.21 ± 1.45	4.5

Summing up

The reliability of the assay design resulting from the DOE approach was confirmed after optimization, including prediction confirmation, pre-study validation, and in-study validation. Added to that, the team found the assay to be very robust, with a 100% pass rate when applying the industry standard 4–6–X approach to acceptance/rejection.

Integrating the DOE approach and Gyros technology enabled the Allison Given and her colleagues to develop the assay quickly – six days from initial antibody screening to confirming the prediction of the DOE software. The Gyrolab software wizard also proved to be very valuable in quickly translating the experimental design into practice, and the total run time of one hour meant that several experiments could be run in a single day. The team concluded that, *"The combination of software and hardware has reduced many of the barriers of implementing DOE into immunoassay development."*

Further reading

Development and validation of an alpha fetoprotein immunoassay using Gyros technology. Given AM *et al.* J Pharm Biomed Anal. 2012 May; 64-65:8-15.
Pubmed: https://www.ncbi.nlm.nih.gov/pubmed/22386211