A quick and efficient approach to immunogenicity assay development and optimization using Design of Experiments

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Background

Immunogenicity assessment of therapeutic proteins is a critical part of drug development. Anti-drug antibodies (ADA) generated by a host immune system may result in altered pharmacokinetic profiles, neutralization of biological activity or even anaphylaxis. Sensitive and robust immunoassays must be efficiently developed to measure ADA during preclinical and clinical drug development.

Traditional method development characterizes variables in sequential univariate or bivariate experiments. This approach can be resource intensive and does not necessarily yield the most robust method since it does not study multivariate interactions. Design of Experiments (DOE) offer ease in multivariate experimental design, data analyses, and the prediction of optimum assay conditions. Here we report the development and optimization of an ECL based homogeneous bridging assay to measure anti-XMab® antibody antibodies in monkey and human serum samples.

Using Design of Experiments (DOE), we optimized critical assay conditions including concentrations of capture and detection reagents and assay incubation time. The assay conditions predicted by response surface model analysis of the data were experimentally verified and validated for sensitivity, drug tolerance, matrix interference and statistical cut point.

Materials & Methods

Software

JMP software (JMP 11) was used to design and optimize assay conditions.

Reagents and assay format

XMab® antibody was labeled with each biotin (Bt) and ruthenium (Ru) using N-hydroxysuccinimide esters chemistry. Samples containing ADA were first diluted in acid (1:10 MRD in 300mM acetic acid pH 3.0) then neutralized with 1M Tris HCl pH 9.5 containing equimolar concentrations of Bt-XMab® antibody and Ru-XMab® antibody. Samples were then loaded on 96-well MSD GOLD streptavidin coated plates and the ECL signal measured on MSD SECTOR Imager 6000.

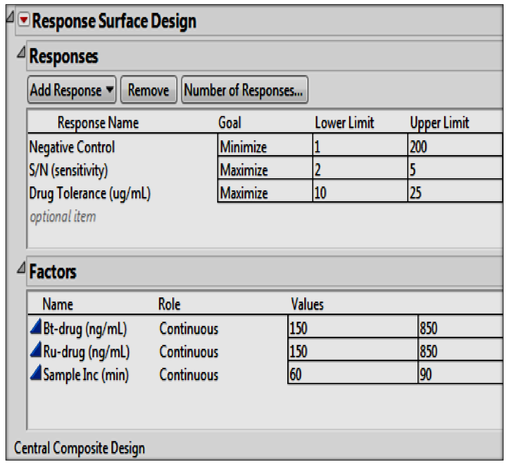
Assay conditions and design

Assay conditions selected for optimization;
1) Concentration of capture reagent (Bt-XMab® antibody)
2) Concentration of detection reagent (Ru-XMab® antibody)
3) Sample incubation time for optimization.

Assay responses;
a) Background signal, b) sensitivity and c) drug tolerance.

Each of three assay conditions were tested at three levels (low, mid and high) in a total of sixteen combinations on three streptavidin gold 96-well assay plates using a central composite design (CCD) from JMP software (Figure 1).

Design of Experiment (DOE)



		Factors		
Condition	Pattern	Bt-drug (ng/mL)	Ru-drug (ng/mL)	Sample Inc (min)
Condition 1	−−−	150	150	60
Condition 2	+−−	850	150	60
Condition 3	00a	500	500	60
Condition 4	−+−	150	850	60
Condition 5	++−	850	850	60
Condition 6	0a0	500	150	75
Condition 7	a00	150	500	75
Condition 8	0	500	500	75
Condition 9	0	500	500	75
Condition 10	A00	850	500	75
Condition 11	0A0	500	850	75
Condition 12	−−+	150	150	90
Condition 13	+−+	850	150	90
Condition 14	00A	500	500	90
Condition 15	−++	150	850	90
Condition 16	+++	850	850	90

Figure 1. Layout of Response Surface Design. Three assay variables (Bt-drug concentration, Ru-drug concentration, and incubation time) were selected for optimization with negative control, sensitivity and drug tolerance as assay responses (left). Each variable was tested at three levels with a total of sixteen conditions (right).

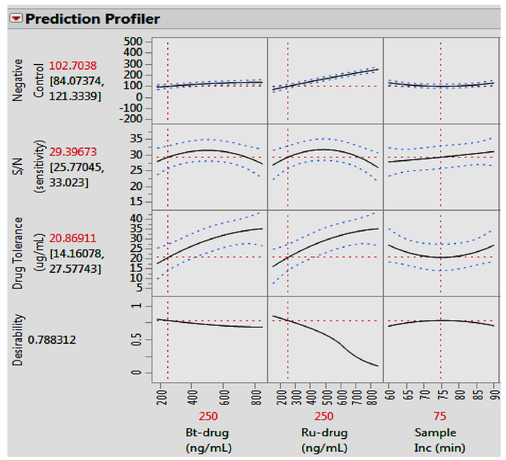


Fig. 2. Data Analysis and Prediction.
Response surface analysis of data predicted the optimal assay conditions to be:
- 250 ng/mL of capture (Bt-drug)
- 250 ng/mL of detection (Ru-drug)
- 75 min incubation

Verification and Validation

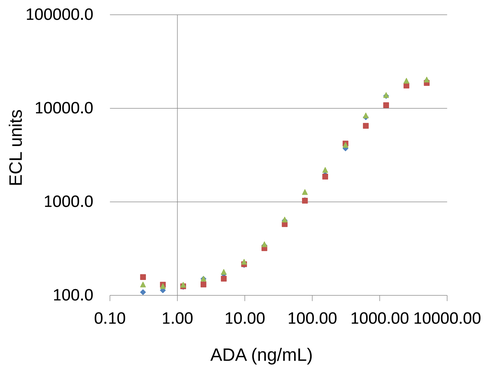


Fig. 3. Sensitivity. Optimization of conditions yielded a highly sensitive assay (~1.4 ng/mL).

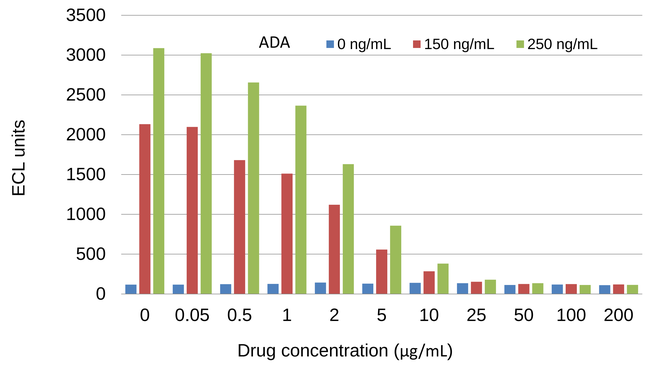


Fig. 4. Drug tolerance. Drug tolerance of more than 30 µg/mL was achieved at both 150ng/mL and 250ng/mL of ADA.

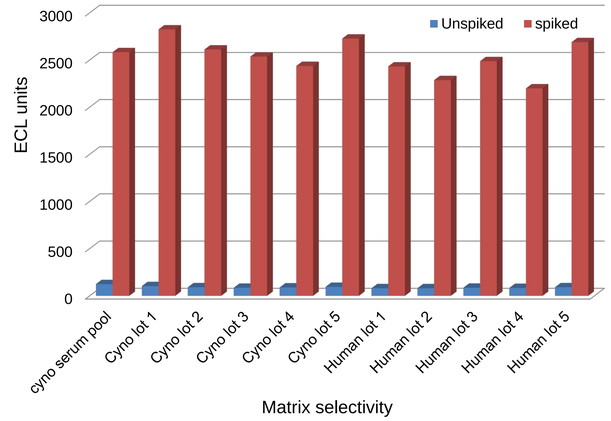


Fig. 5. Matrix selectivity. Individual lots of cyno and human serum samples were spiked at 100 ng/mL ADA. Assay showed consistent performance in both cyno monkey and human matrix

Summary:

1. JMP Software was used to design an experiment of three assay variables, each tested at three levels for a total of sixteen conditions.
2. The resulting data were analyzed in the Response Surface Model, which indicated that:
   a. the concentration of the detector (Ru-XMab® antibody) had a significant effect on the assay performance
   b. the optimal assay conditions would include 250 ng/mL of each Bt-XMab® antibody and Ru-XMab® antibody incubated for 75 minutes
3. The predicted optimal conditions were verified and validated

Bt-drug:	250ng/mL
Ru-drug:	250ng/mL
Acid dissociation:	25-30 min
Neutralization:	50-60 min
Sample incubation	75-85 min

Conclusion:

By applying DOE in assay development, we have optimized highly sensitive and robust assays for immunogenicity testing of the XMab® antibody. DOE design significantly eased assay optimization and data analysis compared to conventional checkerboard optimization, making assay development and optimization more efficient. Both cynomolgus monkey and human serum showed consistent ADA recovery thereby enabling us to simultaneously develop two robust assays to support immunogenicity testing of the XMab® antibody in Preclinical and Clinical studies.