

Rapid Assay Optimization Using DOE and Laboratory Automation

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Automated Assay Development



Manual Assays

Bioprocess R&D



Automation
Development



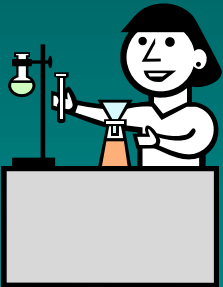
Automated Assays

- Early product development
- Non-validated
- Low throughput
- Semi-optimized

- Later product development
- Validation
- High throughput
- True walk-away automation

Automated Assay Development

We need more robustness!



Labs



Assay Automation

Development is too slow!



Management

Justify your assay conditions!



QA/QC

Automated Assay Development

DOE!



Assay Automation

DOE Design

- Wanted a platform design
 - Consultation with statisticians
- 5 “Plate” variables
 - Incubation times
 - Interactions not very likely
- 4 “Well” variables
 - Concentrations
 - Interactions possible
- Interactions between plate and well variables possible

Plate Factors – Incubation Times

2^{5-2} Fractional Factorial

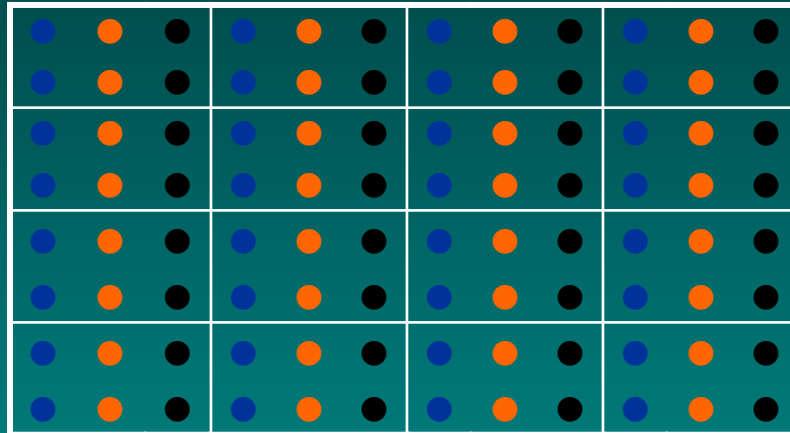
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2	+	-	-	-	-
3	-	+	-	-	+
4	+	+	-	+	-
5	-	-	+	+	-
6	+	-	+	-	+
7	-	+	+	-	-
8	+	+	+	+	+

Well Factors - Concentrations

2⁴ Full Factorial

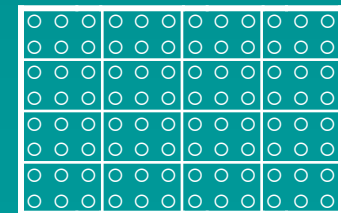
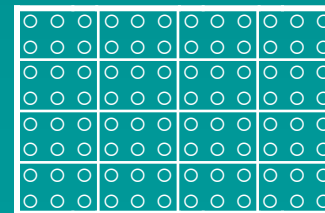
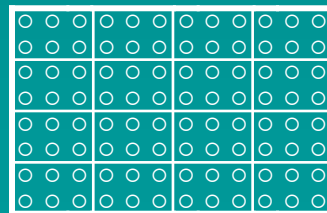
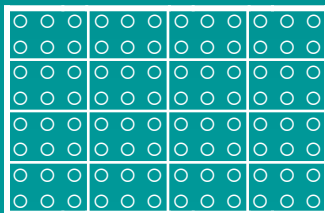
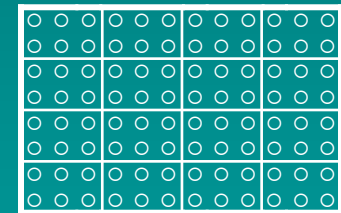
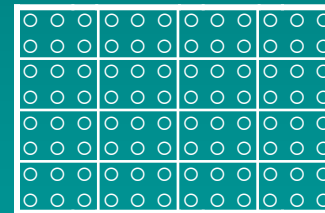
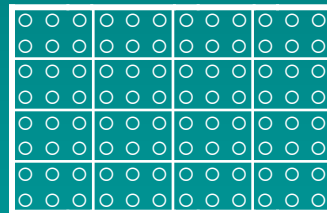
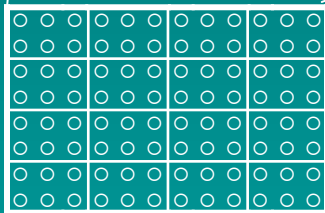
Standard Order	F	G	H	I
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2	+	+	+	-
3	+	+	-	+
4	+	+	-	-
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6	+	-	+	-
7	+	-	-	+
8	+	-	-	-
9	-	+	+	+
10	-	+	+	-
11	-	+	-	+
12	-	+	-	-
13	-	-	+	+
14	-	-	+	-
15	-	-	-	+
16	-	-	-	-

DOE Design – Strip Plot




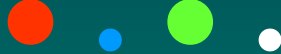














16 positions:
Intra-plate factors
(6 wells per position)

8 plates: Inter-plate
factors



Well Factor Design: Latin Square

Randomized Balanced Design

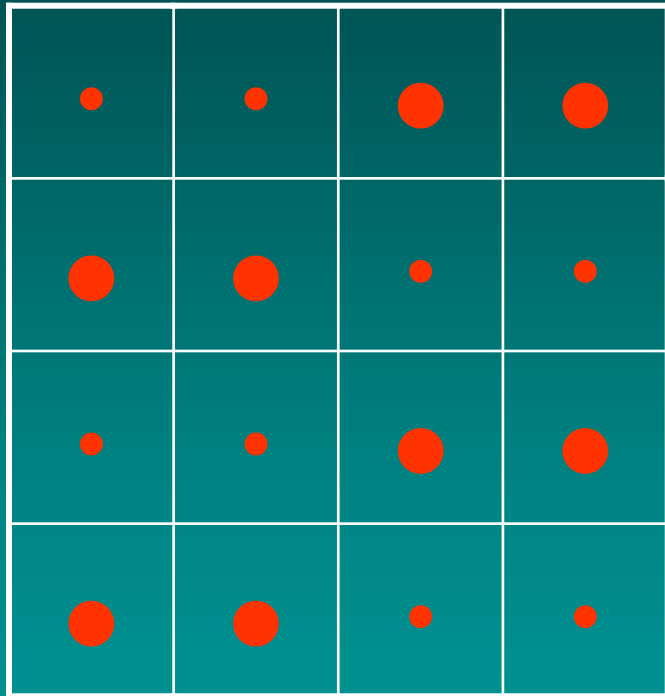


Plate 1

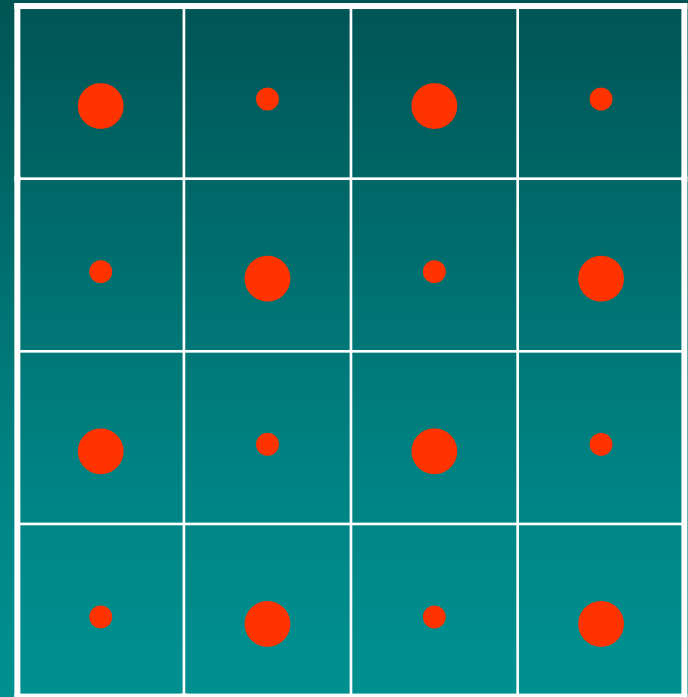
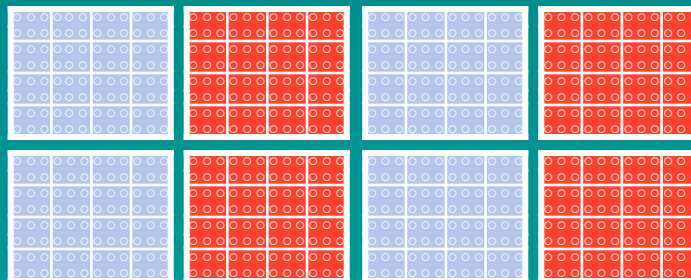
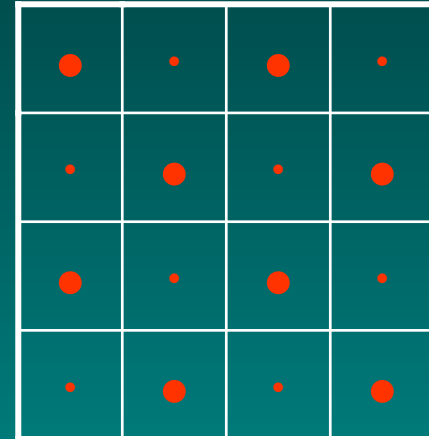


Plate 2 etc...

High Degree of Replication

- Intra-plate factors:
 - 48 replicates per concentration x 8 plates
 - 384 replicates total per level
- Inter-plate factors:
 - 96 replicates per time x 4 plates
 - 384 replicates total per level

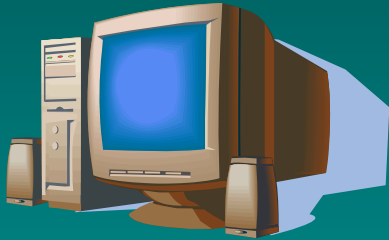


Implementation

- Impossible to do by hand
 - Too many variables
 - Randomization
 - Pipetting takes too long
- Strengths of Laboratory Automation
 - Randomization easy
 - “Interweaving” of plate timings possible
 - Two four-plate runs (1-2 days)

Implementation – Software Development

DOE controller software



- User enters timing levels
- Performs randomization
- Keeps track of plate timings

- Fully automated ELISA.
- Each DOE step performed by a short program.

Application

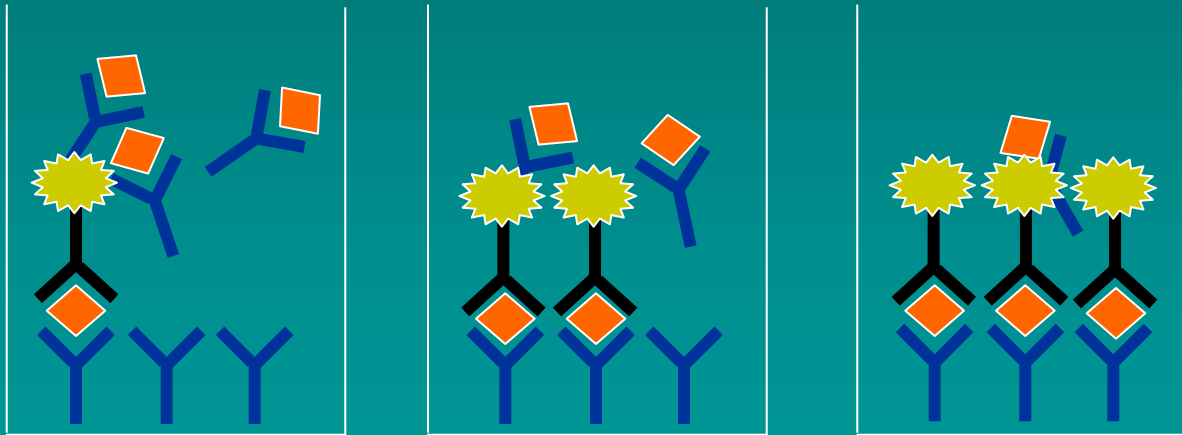
Competitive ELISA

Binding assay for a
monoclonal antibody project

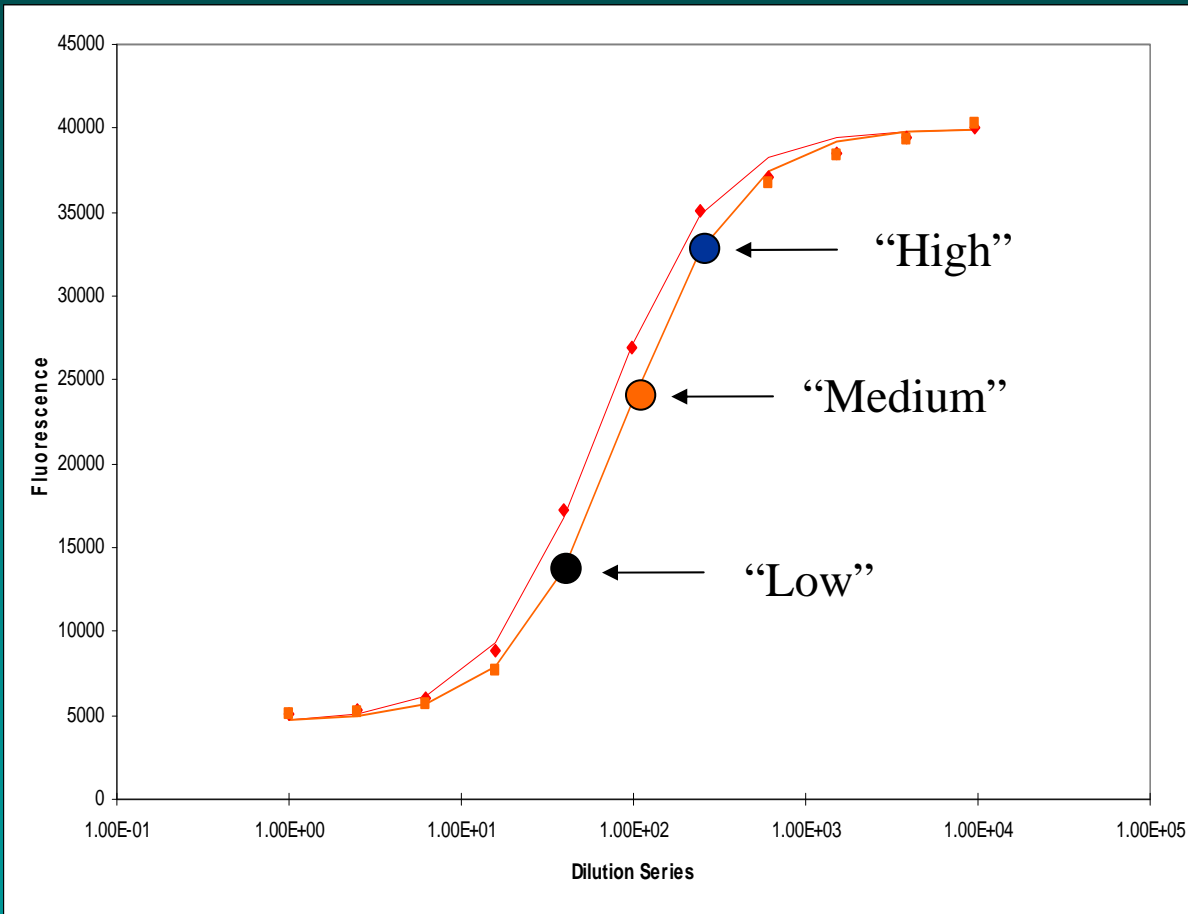
Competitive ELISA

Serial Dilution

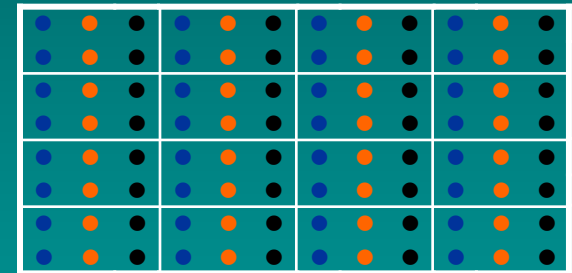
Signal level inverse of sample concentration



Dose Response Curve



DOE plate layout



Response

- Most important feature of these assays is inter-replicate variability.
 - Surrogate indicator of assay robustness.
- DOE analysis uses the average variability among replicates at all three dose response concentrations.
- Gives us the factors that have an overall effect on replicate variability.

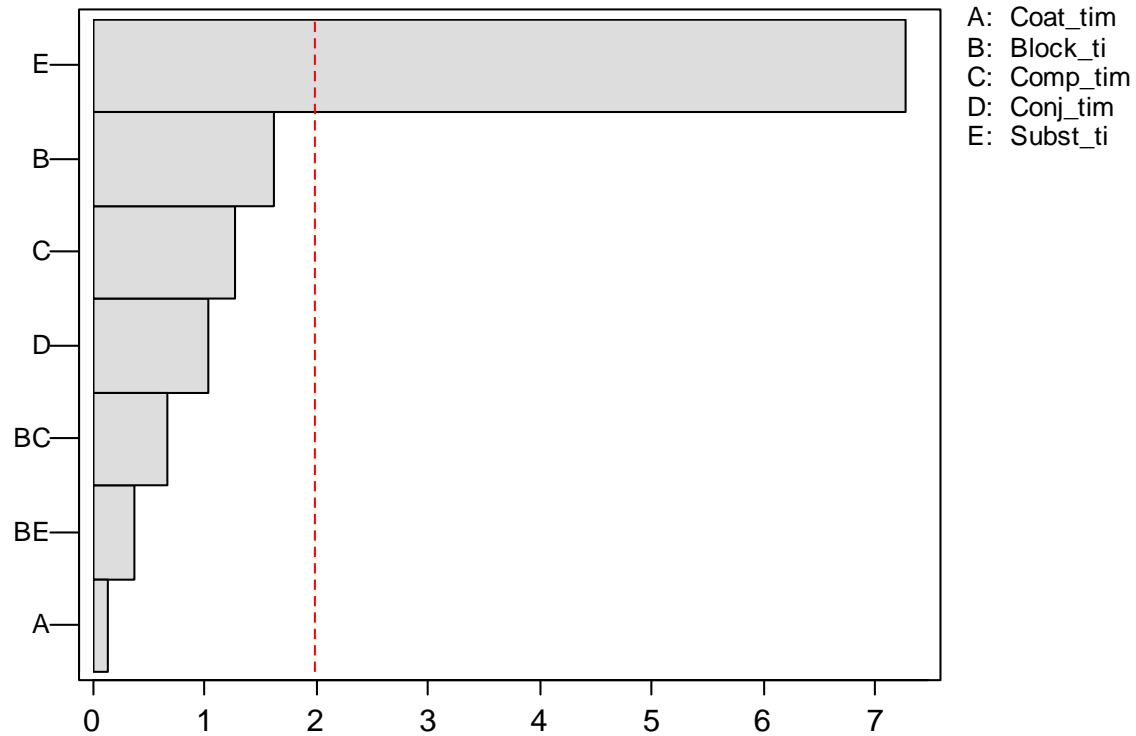
Factors

- 5 incubation times (60 min – 120 min)
 - Coating
 - Blocking
 - Competition
 - Detection antibody
 - Substrate development
- 3 concentration variables
 - Coating antibody
 - Antigen
 - Conjugate antibody

Results – Incubation times

Pareto Chart of the Standardized Effects

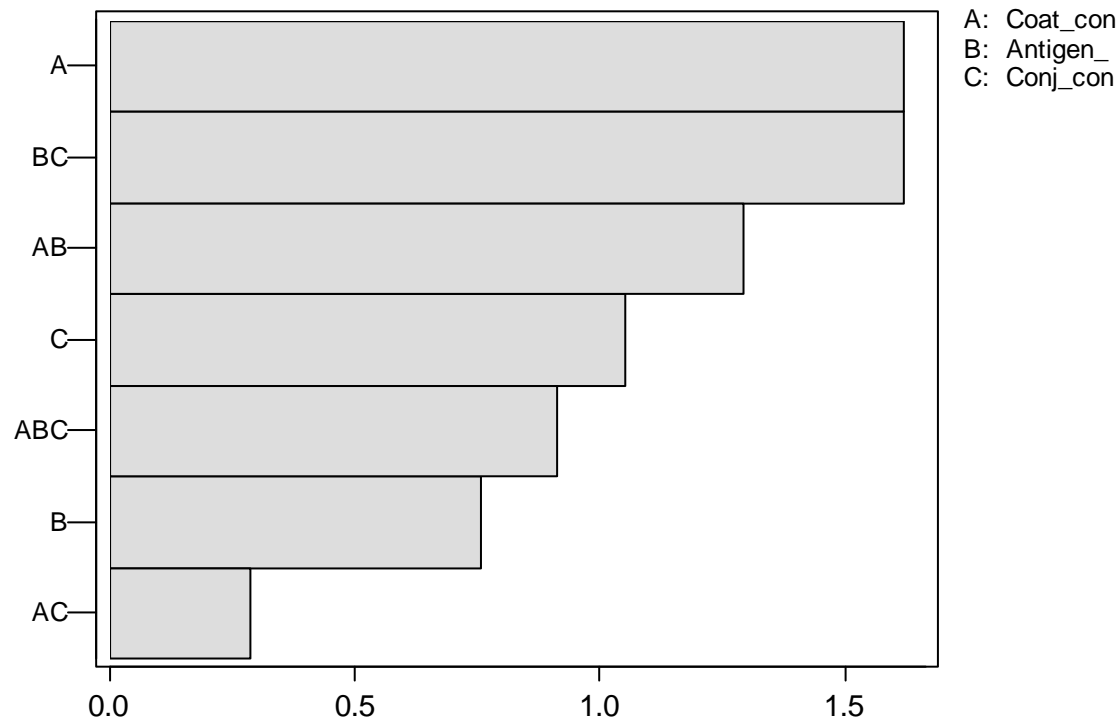
(response is response, Alpha = .05)



Results – Concentrations

Pareto Chart of the Standardized Effects

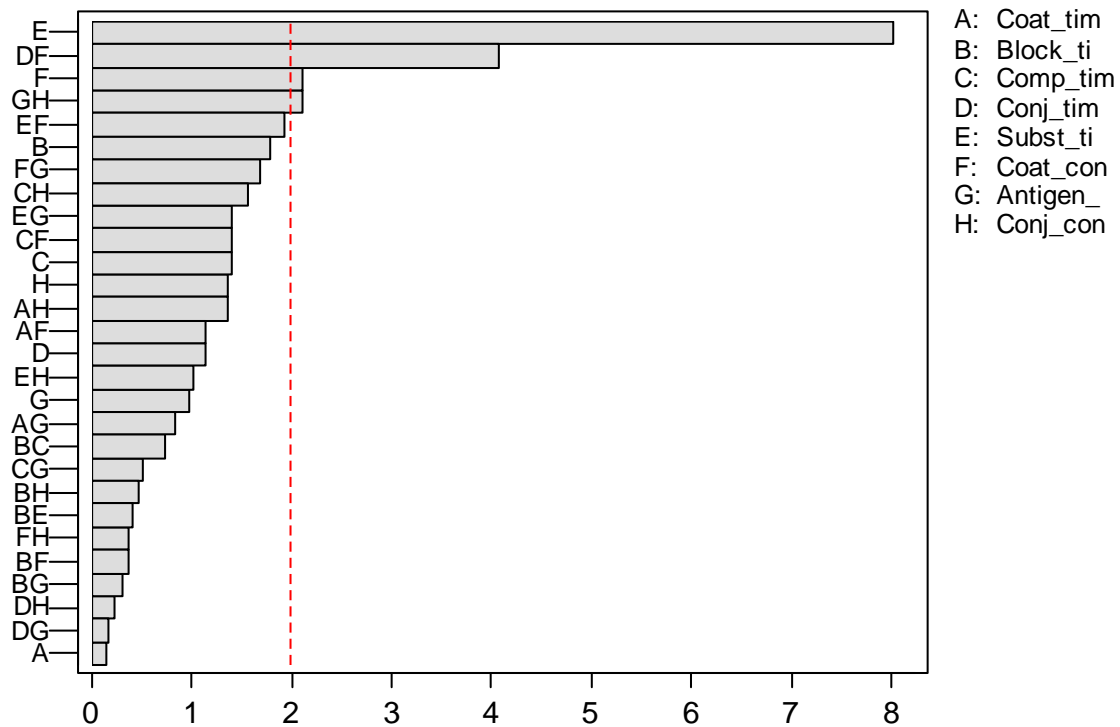
(response is response, Alpha = .05)



Results – Interactions

Pareto Chart of the Standardized Effects

(response is response, Alpha = .05)



Main Effect – Substrate Development

Design-Expert® Software
Transformed Scale
Ln(Resp)

X1 = E: Substrate time

Actual Factors

A: Coat time = 0.00

B: Block time = 0.00

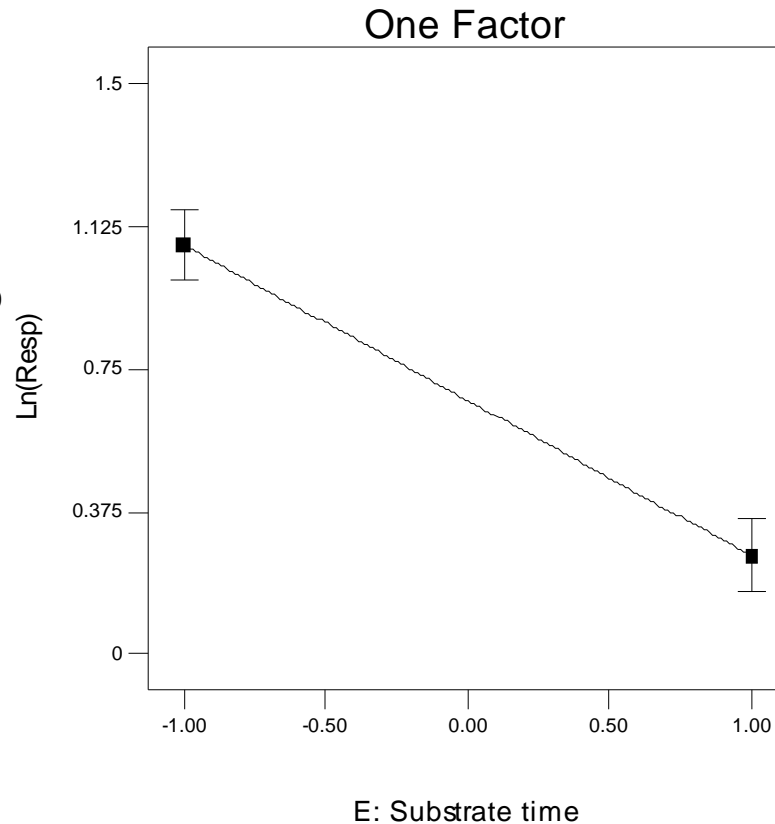
C: Competition time = 0.00

D: Conjugate time = 0.00

F: Coat conc = 0.00

G: Antigen conc = 0.00

H: Conj conc = 0.00



Note – Aliased with an interaction between coating time and competition time

Interaction: Coating concentration with Conjugate time

Design-Expert® Software
Transformed Scale
Ln(Resp)

■ F- -1.000
▲ F+ 1.000

X1 = D: Conjugate time
X2 = F: Coat conc

Actual Factors

A: Coat time = 0.00

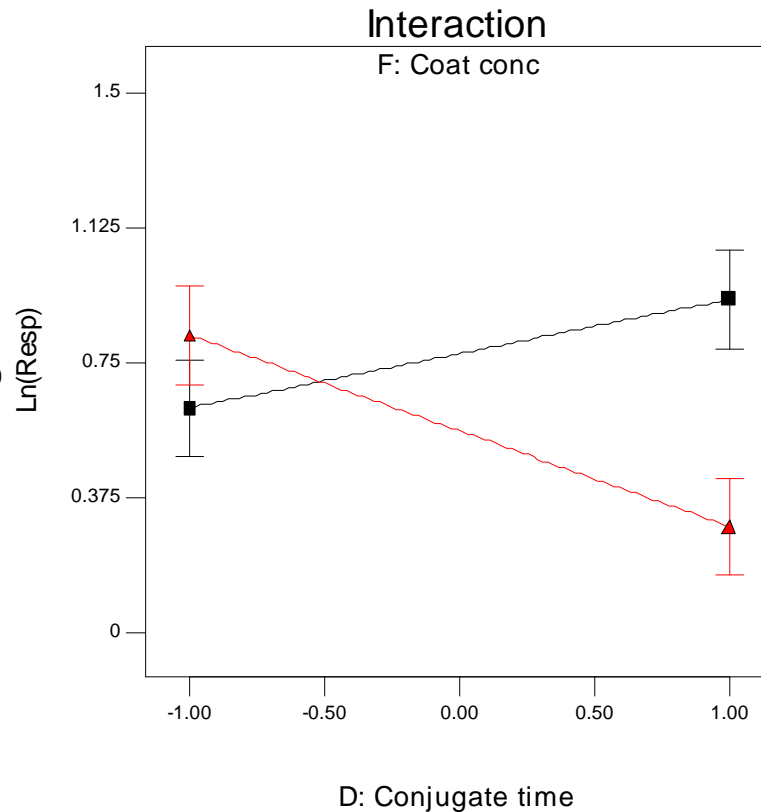
B: Block time = 0.00

C: Competition time = 0.00

E: Substrate time = 0.00

G: Antigen conc = 0.00

H: Conj conc = 0.00



Next Steps

- Confirm DOE results in the lab
 - Reality check
 - Reduction of estimated “root-variability” of the assay by $\sim 1/3$ (from 3.6% RSD to 2.4% RSD).
- Further optimization of significant factors
 - Possible further improvement of assay performance

Conclusions

- Developed a platform approach for a rapid measurement of robustness and optimization of automated immunoassays.
- Each of our goals were met:
 - Labs
 - Found robust conditions
 - Non-critical factors are robust
 - QA/QC
 - Assay settings easily justified
 - Management
 - Quicker assay development
 - Only 2 days necessary to perform DOE