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A DOE "Real Life" Example: Solid Phase Optimization of a Diagnostic Immunoassay

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ABSTRACT

Experimental design as used for immunoassay development in the *in vitro* diagnostics industry requires a thorough understanding of all aspects of a given product's composition and manufacturing. For immunoassays, a multiplicity of interactions between reagent components, sample composition, and process parameters make characterization of the process so complex and time consuming that the advantages of experimental design, or DOE, become immediately apparent. It is the strength of DOE to use statistics to define and/or optimize processes with fewer resources than standard experimental approaches.

This paper will first present a brief background on immunoassays to provide a context for the example that follows. The specific problem illustrates the use of DOE in a manufacturing problem that includes optimization of that process. The coating of a microparticle solid phase with antibodies for a specific analyte failed to yield the performance necessary as assessed by three different metrics (responses). Simultaneous optimization with only two DOE experiments corrected the problems, characterized and optimized the manufacturing process, and allowed the product to go to market.

ALTERNATE TITLE

Turning a Software Developer into an Immunoassay Developer in One Easy Lesson

INTRODUCTION

This paper assumes some minimal knowledge of DOE and so seeks to provide a background in the basics of *in vitro* diagnostic immunoassay development that will allow for more complete understanding and appreciation of the DOE example that follows. (Thus the alternate title suggested above.) That example, described in the second half of this paper, showcases the benefits and power of DOE as one particularly useful tool in addressing such multifaceted problems.

WHAT IS AN IMMUNOASSAY?[1]

DEFINITIONS

The first thing we need to do is establish our definitions:

Immunoassays are tests that use antibody and antigen complexes (also called immunocomplexes) to measure the presence of a specific analyte in a sample. "Immuno" refers to an immune response that causes the body to generate antibodies, and "assay" refers to a test. Thus, an immunoassay is a test that utilizes immunocomplexing when antibodies and antigens are brought together.

Immunoassays are different from other types of laboratory tests, such as colorimetric tests, because they use antibody:antigen complexes to generate a signal that can be measured. In contrast, most routine clinical chemistry tests utilize chemical reactions between the reagent (a solution of chemicals or other agents) and patient sample to generate a test result.

Antibodies (Abs) are proteins that are normally produced by the immune system in response to an "invading" (foreign) substance.[2] Antibodies are produced as part of the body's immune response to protect itself. For instance, some immunoassays test for the presence of antibodies to virus molecules. Thus, if the antibodies are present, it means invading virus is too. Their ability to recognize specific portions of their target molecules, called antigens (see next definition), can be exquisitely specific, aimed at single amino acid substitutions or, more rarely, at three dimensional polypeptide structures.

Antibodies are a type of protein called immunoglobulins. The most common one is immunoglobulin G (IgG). IgG is a protein comprised of two main structural and functional regions (Figure 1):

[1] Much of this section comes from *Learning Guide: Immunoassays,* 2008, Abbott Laboratories, 98-0897/R2-1.5 Jan 2008

[2] An exception is the case of autoimmune diseases, where the body produces antibodies to naturally occurring proteins rather than foreign substances.

- **Fab region:** Contains the antigen (Ag) binding site that varies between different antibodies.
- **Fc region:** Region of constant structure within an antibody class.

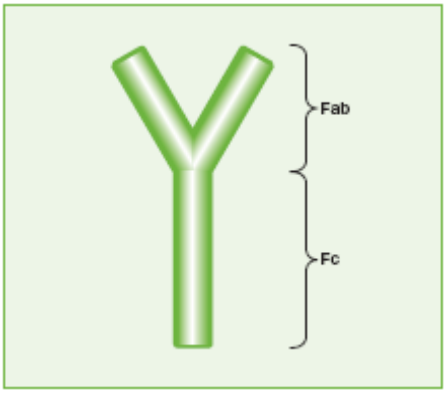


Figure 1: IgG molecule

Antibody preparations are either **polyclonal antisera**, which recognize multiple sites on antigens, or **monoclonal antibodies**, which recognize single sites on antigens.

Antigens (Ags) are the molecules that antibodies bind to, which in the body could be an invading pathogen, or the foreign molecules injected into an animal to trigger the immune response. Some immunoassays test for antigens directly, rather than looking for the antibodies. In a test to measure the concentration of a therapeutic drug, for example, the drug is the antigen that binds to the antibody. When the antigen is a small molecule, such as one of the thyroid hormones, the entire molecule is the antigen. Protein antigens are often as large as, or larger than, the antibody binding to it. In this case, the specific region of the antigen recognized by the antibody is called an **epitope**.

An **analyte** is anything measured by a laboratory test. In immunoassay testing, the analyte may be either an antibody, or an antigen. Sometimes the analyte may be called a **marker**, or a **biomarker**, denoting the fact that the analyte is only present in a disease state or is otherwise a specific indication of something regarding the medical condition of the patient.

All immunoassays require the use of labeled material in order to measure the amount of antigen or antibody present. A **label** is a molecule that will react as part of the assay, so a change in signal can be measured in the blood:reagent solution. Examples of a label include a radioactive compound, an enzyme that causes a change of color in a solution, or a substance that produces light. The label can be applied during the manufacture of the reagent to either the antibody (Ab*) or antigen (Ag*), and the resulting molecule is called a **conjugate**.

Immunoassay technologies utilize different formats to distinguish the bound antigen-antibody complex from the free unbound label. Formats can be categorized as either competitive or noncompetitive. In **competitive immunoassays**, the amount of antigen is inversely proportional to the amount of signal. In **noncompetitive (sandwich) immunoassays**, the amount of antigen is directly proportional to the amount of signal. (Our example is noncompetitive.)

Noncompetitive assay formats generally provide the highest level of assay sensitivity and specificity and are applied to the measurement of critical analytes such as cardiac and hepatitis markers. This format is referred to as a “sandwich” assay because analyte is bound (sandwiched) between two highly specific antibody reagents (Figure 2).

Noncompetitive assay formats can also utilize either one step or two step methods, as with the competitive assay. The two step assay format employs wash steps in which the sandwich binding complex is isolated and washed to remove excess unbound labeled reagent and any other interfering substances.

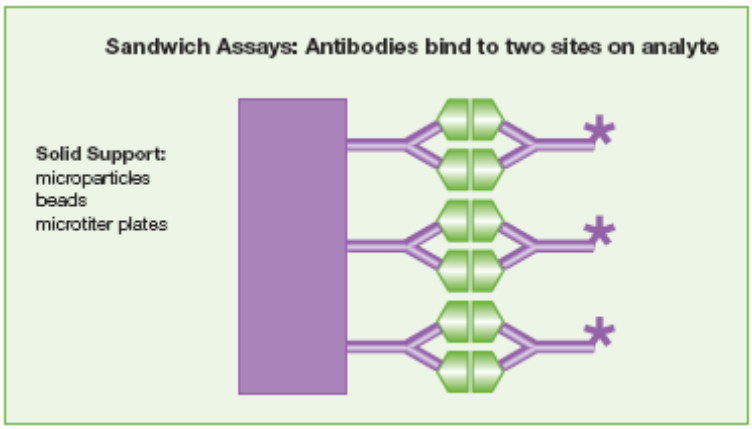


Figure 2: Noncompetitive sandwich method of immunoassay

Another categorization of immunoassays divides them into homogeneous and heterogeneous formats.

Homogeneous immunoassays do not require separation of unbound complexes from the bound complexes, and thus are faster and easier to perform than heterogeneous immunoassays. Homogeneous methods have been generally applied to the measurement of small analytes such as abused and therapeutic drugs.

Heterogeneous immunoassays require the separation of unbound complexes, often utilizing a solid phase reagent such as a magnetic particle or plastic bead (Figure 3). (Our example is heterogeneous, and combining the two categories, our example is a noncompetitive heterogeneous format.)

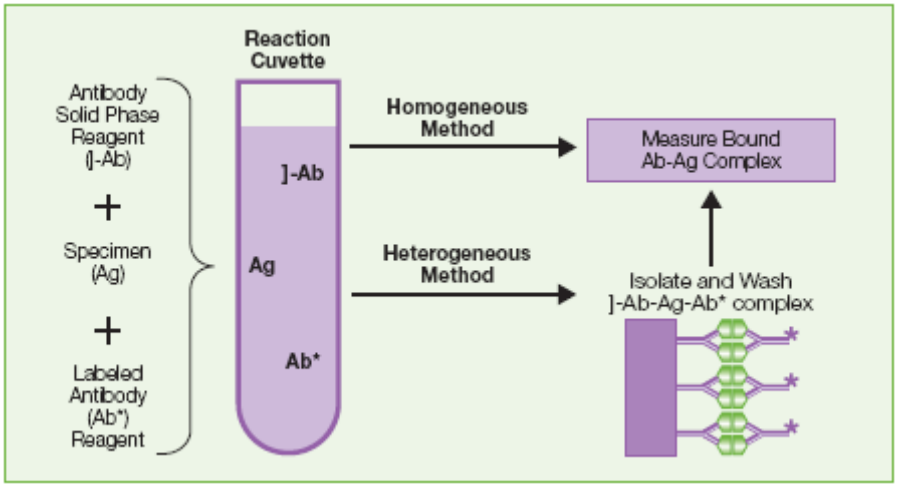


Figure 3: Homogeneous and heterogeneous immunoassays

IMMUNOASSAY DEVELOPMENT

DON'T BE FRIGHTENED…ALL I VANT IZ A LEETLE OF YOUR BLOOD

The body fluid most informative of a patient's health is generally his blood, and this is the "sample" used most often to test for the presence of antibodies or analyte. The reason blood is the most informative also makes it quite problematic: it is an incredibly complex matrix. About 55% of whole blood is blood **plasma**, a fluid that is the blood's liquid medium, which by itself is straw-yellow in color (Figure 4, left tube). The blood plasma volume totals of 2.7–3.0 liters (2.8–3.2 quarts) in an average human. It is essentially an aqueous solution containing 92% water, 8% blood plasma proteins, and trace amounts of other materials. Plasma circulates dissolved nutrients, such as glucose, amino acids, and fatty acids (dissolved in the blood or bound to plasma proteins), and removes waste products, such as carbon dioxide, urea, and lactic acid. Other important components include:

- Serum albumin
- Blood-clotting factors (to facilitate coagulation)

- Immunoglobulins (antibodies)
- Lipoprotein particles
- Various other proteins
- Various electrolytes (mainly sodium and chloride, but also trace minerals such as potassium, manganese, and calcium)
- Various hormones which vary in structure from small organic molecules (e.g., the thyroid hormones) to proteins of various molecular weights (e.g., hormones associated with pregnancy)

The term **serum** refers to plasma from which the clotting proteins have been removed by allowing the clotting mechanism to go to completion. Most of the proteins remaining are albumin and immunoglobulins. Most immunoassays can use either plasma or serum as samples for testing.

Two tubes of EDTA-anticoagulated blood.
Left tube: after standing, the RBCs have settled at the bottom of the tube.
Right tube: contains freshly drawn blood.

Figure 4: left tube = serum on top of red blood cell pellet; right tube = fresh whole blood

SO WHAT'S THE PROBLEM?

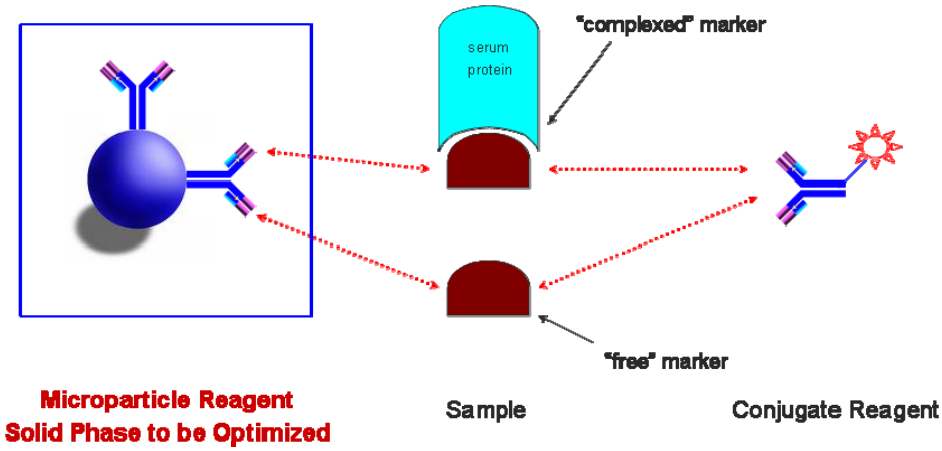
Because of the complexity of the sample and the nature of the reagents, there exist a large number of potential interferences in the reaction matrix to the specific detection of the desired analyte. As stated in the abstract, a multiplicity of interactions between reagent components, sample composition, and process parameters complicate the development of a functional diagnostic kit. One of the more common problems is called "nonspecific binding," where some protein or plasma component other than the analyte binds to the solid phase of the immunoassay kit, interfering with and sometimes even blocking entirely the desired interaction of specific antibody and the analyte to which the antibody is directed (i.e., the antigen). The following example provides an instructive story of how DOE enabled the successful resolution of a manufacturing problem for the solid phase of an immunoassay.

A DOE "REAL LIFE" EXAMPLE – SOLID PHASE OPTIMIZATION

THE CAST OF CHARACTERS

Figure 5 below gives us a simplified introduction and orientation to the problem at hand.

Figure 5: The Process



In the first step, the solid phase, a polystyrene microparticle coated with antibody (Ab) directed against the analyte, or marker, is incubated with the sample containing said marker. After some time for interaction, the solid phase is physically removed from contact with the sample and washed to remove other sample components, and then incubated with a conjugate, that is, another antibody tagged with an enzyme or some other chemical moiety used to generate a signal (e.g., light) that can be detected and quantified. In this case, an additional member of the cast is a serum protein that can bind our marker of interest, masking part of the surface of the marker that would otherwise be available to interact with a solid phase. For this particular marker, there is some clinical significance to quantitating both the amount of "free" marker, and the amount of "complexed" plus "free" marker (i.e., the "total" quantity of the analyte). The key to making this distinction is in the selection of monoclonal antibodies (MAb) for the solid phase and the conjugate. To measure all of the marker present, the two antibodies must be directed against different sites on the surface of the marker, neither of which are masked by the serum protein when it binds. To measure the quantity of the "free" marker

only, the capture antibody on the solid phase must recognize a site on the marker molecule to which the serum protein binds, thus preventing recognition of the complexed form of the marker.

THE PLOT

Preliminary protocols for making the reagents necessary for the immunoassay have been completed, and the initial performance evaluated. Three performance parameters were of particular importance:

1. Equimolarity = the ability to see complexed and free analyte equivalently, operationally defined as a signal ratio of 1.0 ± 0.1 for complex/free
2. Microparticle stability, defined as 100 ± 10% of 2-8ºC signal after 3 days storage at 45ºC
3. Panel[3] values = within 5% of target values across the dynamic range of the assay

The performance in the initial evaluate showed that panel values were within the specified goal, but the microparticles were not stable (signal loss greater than 10%) and the equimolarity performance was marginal. Thus, the experimental objective is to determine a manufacturing formula to meet all three of the above goals.

Additional investigations had shown that the loss in stability was related to free antibody coming off of the solid phase during storage as a function of time, suggesting a lack of covalent coupling of the antibody to the microparticles. Dropping the antibody concentration for coating improved the stability, but then the panel values read lower than acceptable, and equimolarity, already marginal, departed further from the target.

THE FACTORS

Four input factors to the microparticle manufacturing process were evaluated. The first three are the obvious "active ingredients" for the coupling process: the concentration of the coating antibody (in mg/mL), the concentration of the microparticles (in % solids), and the concentration of the coupling reagent, affectionately known as EDAC (a carbodiimide, for those chemically curious, in mg/mL). The fourth factor is the concentration of sodium chloride in mM. Ionic strength is known to impact the interaction of proteins with surfaces, and the concentration of NaCl is one easy way of manipulating this characteristic of the coupling reaction.

We already know the three critical output factors in which we have interest. Equimolarity is a simple ratio with a target of one. Panel values were evaluated with a high and a low panel, so this output consists of two ratios (panel value - target value)/target value with a target of zero (zero difference from standards). Stability is evaluated by averaging across calibrators (Cal B-F) and calculating the ratio of (heat stressed – cold storage)/cold storage with the target again zero difference from cold stored calibrators.

THE DESIGN

A screening design was chosen first to minimize the number of runs and determine the most important factors. Table 1 shows the inputs, and Table 2 shows the design that was run (a screening design: 4 factors, 2 levels with midpoint: 11 unique preps including 5 duplicate preps for a total of 16):

TABLE 1: Design Inputs

Factor	*Low Level*	*High Level*	*Midpoint*	*Current*
[Ab], mg/mL	0.02	1.0	0.51	2.0
[EDAC], mg/mL	0.1	5.0	2.55	1.0
[NaCl], mM	0.0	500	250	0.0
% solids	0.5	2.0	1.25	1.0

[3] Panels are samples at known defined values for an immunoassay used to monitor performance.

TABLE 2: Design

Pattern	Trial #	Ab mg/mL	EDAC mg/mL	NaCl mM	% Solids
+−++	1	1	0.1	500	2
−+−−	2	0.02	5	0	0.5
−+++	3	0.02	5	500	2
+−−−	4	1	0.1	0	0.5
+++−	5	1	5	500	0.5
−−−+	6	0.02	0.1	0	2
++−+	7	1	5	0	2
−−+−	8	0.02	0.1	500	0.5
++++	9	1	5	500	2
−−−−	10	0.02	0.1	0	0.5
0000	11	0.51	2.55	250	1.25
+−++	1	1	0.1	500	2
−+−−	2	0.02	5	0	0.5
−+++	3	0.02	5	500	2
+−−−	4	1	0.1	0	0.5
+++−	5	1	5	500	0.5

Note: For the “Pattern” in the above table, low, midpoint, and high settings are shown as minus, zero, and plus signs.

THE RESULTS WITH CHECKPOINTS

One of the first diagnostics of fit is the plot of predicted versus actual values. The R squared values give an objective measure of that fit, while the plots give a good visual indication of outliers and the overall fit. R square values do not have to be close to one for the model to have predictive power, which is why looking at the plot is helpful in making the decision whether or not to use the model predictions for next steps. Figures 6 and 7 show these results for the two panels. Figure 8 shows the measure of stability used, and figure 9, the equimolarity results.

Figure 6: Panel C Predicted vs. Actual

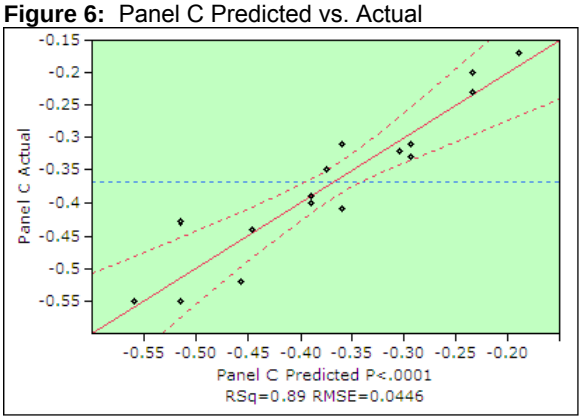


Figure 7: Panel I Predicted vs. Actual

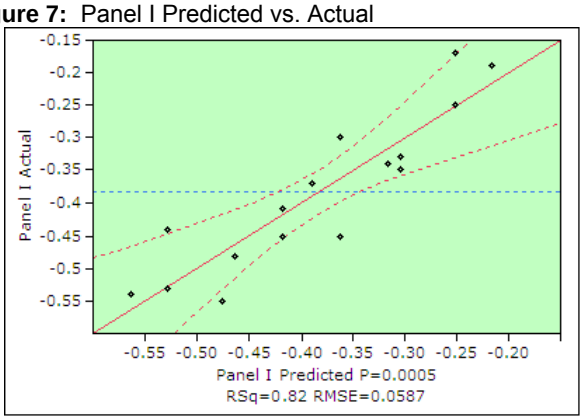


Figure 8: Cal B-F Stability Predicted vs. Actual

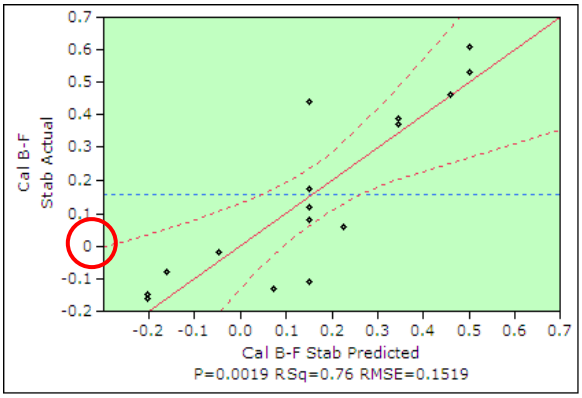
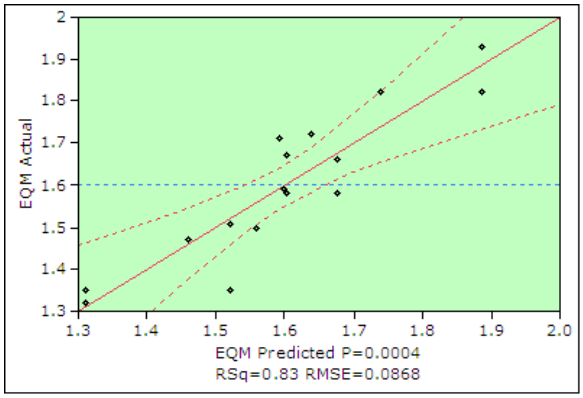


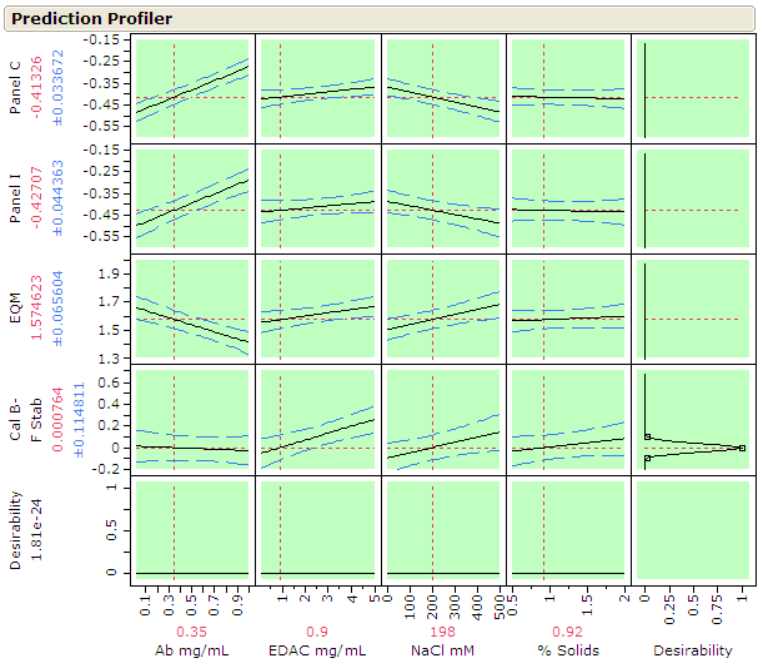
Figure 9: Equimolarity Predicted vs. Actual



These plots show a reasonable ability to predict these responses of interest, with most R Square values above 0.80. Note that the only target actually met in the data collected for this design is for stability (zero difference from control; red circle in Figure 8). Thus, it was decided to attempt optimization using these results first for stability. To do this, we turn to the Prediction Profiler tool (Figure 10). With this tool, the inputs are plotted separately along the x-axes with the range of values used in the design. On the far right is the Desirability function used to optimize the responses (see the JMP tutorial materials for instructions on how to use this plot). The y-axes are the outputs. Plotted this way, you can immediately see visually the impact of each input on each output and verify with your eyes what the p-values and other statistics have told you about your process. For example, we see immediately that the % solids have little influence in any of the responses here.

Of particular relevance here is the flat line for the Desirability function for the panels and the equimolarity responses. This is because the target is not on the y-axes for these responses, indicating that the input factors cannot be adjusted to any setting within these ranges that will yield the desired response.

Figure 10: Prediction Profiler optimized for stability



Optimizing the responses for the best stability yielded the following inputs:

- [Ab] = 0.35 mg/mL
- [EDAC] = 0.9 mg/mL
- [NaCl] = 198 mM
- % solids = 0.92%

Since these were not conditions used to create the model, a checkpoint preparation was made and tested. It was found that, consistent with predictions (i.e., the model was indeed good enough to act as a response surface even though the design was a simpler screening design), the stability was 100%. Unfortunately, panel values were now 40% below target (-0.4) and equimolarity was 1.57 (target is 0.9 – 1.1).

These observations were further enhanced with a second checkpoint using the model to predict responses based on the current process values for the input. That is, input:

- [Ab] = 2.0 mg/mL (Note: this [Ab] is outside the limits of the data used to construct the model, yet the predictions still conform to the current data, demonstrating the validity of the model even when extrapolated to this extent)
- [EDAC] = 1.0 mg/mL
- [NaCl] = 0.0 mM
- % solids = 1.0%

The model created by the analysis of the data of this first DOE predicts the following responses:

- Stability = 85% (NOT acceptable)
- Panel values = 1-3% below target (acceptable; within range)
- Equimolarity = 1.09 (*barely* acceptable)

This was the current performance of the assay (see above under "THE PLOT").

The conclusion of this first DOE analysis provides an important lesson on the utility of DOE. The four inputs studied here do not provide a means to simultaneously optimize stability, equimolarity, and panel values. To do so, you must *either* a.) accept a tradeoff in response outputs, i.e., change the design goals (which is not a good idea if those design goals have been properly formulated from customer requirements), *or* b.) entertain a new perturbation/parameter in the assay system, i.e., look at something new, a different parameter not yet evaluated, or something radically new to the entire system.

> "Eliminate all other factors, and the one which remains must be the truth."
> ·Sherlock Holmes, *The Sign of the Four*

A CRITICAL ADVANTAGE OF USING DOE

"Lack of Success" is not the same as "Failure." *One of the greatest benefits of DOE is the ability to terminate unfruitful lines of investigation using the objective evidence of the validated models generated to scientifically justify this decision.* This occurs when the model predictions have been verified (validating the model) and when those predictions show the impossibility of meeting all necessary goals simultaneously.

WHAT TO DO?

At this point, a preliminary experiment done months earlier surfaced again, suggesting a new direction to evaluate in earnest. Initially, the data were set aside as "interesting results" having no practical utility. It had been observed that if you added the monoclonal Ab (MAb) against the free marker to the solid phase (microparticle) diluent to create a pseudo-complexed marker from the free marker, all forms of the marker appeared to look alike in the "total" assay. This addresses the panel values and the equimolarity issue. The new hypothesis in the form of a question, therefore, was, can we optimize the solid phase coating for stability and then adjust the panel values and equimolarity results with this MAb in the diluent?

THE FACTORS & DESIGN, PART 2

Based on the first DOE the % solids and NaCl concentrations were fixed and dropped from this study, leaving only three factors:

TABLE 3: Design Inputs

Factor	*Low Level*	*High Level*	*Midpoint*	*Current*
[Ab], mg/mL	0.05	1.0	0.525	1.0
[EDAC], mg/mL	0.5	10.0	5.25	2.5
[Mab in diluent], μg/mL	0.10	2.0	1.05	0.0

With fewer factors to evaluate, and a stronger need to create a model that would be predictive, a *D*-optimal Response Surface Method (RSM) with 3 factors, 3 levels, and 5 duplicate reps was designed and executed.

TABLE 4: Design

Pattern	Trial #	Ab mg/mL	EDAC mg/mL	[MAb] µg/mL in diluent
-++	1	0.05	10	2.0
+0-	2	1.0	5.25	0.1
-+-	3	0.05	10	0.1
--+	4	0.05	0.5	2.0
+++	5	1.0	10	2.0
0+0	6	0.525	10	1.05
-00	7	0.05	5.25	1.05
00+	8	0.525	5.25	2.0
---	9	0.05	0.5	0.1
+-0	10	1.0	0.5	1.05
++0	11	1.0	10	1.05
0+-	12	0.525	10	0.1
0--	13	0.525	0.5	0.1
+0+	14	1.0	5.25	2.0
-0-	15	0.05	5.25	0.1
-++	1	0.05	10	2.0
+0-	2	1.0	5.25	0.1
-+-	3	0.05	10	0.1
--+	4	0.05	0.5	2.0
+++	5	1.0	10	2.0

THE RESULTS, PART 2

In this instance, in order to capture more than just two panels, the metric used to determine the panel performance was the slope of the plot of observed values versus the target values, making the goal a slope of one. Equimolarity and stability were measured as before.

Figures 11-13 show considerably better R square values for this data and target values (arrows) are on the y axis:

Figure 11: Panel Slope Predicted vs. Actual

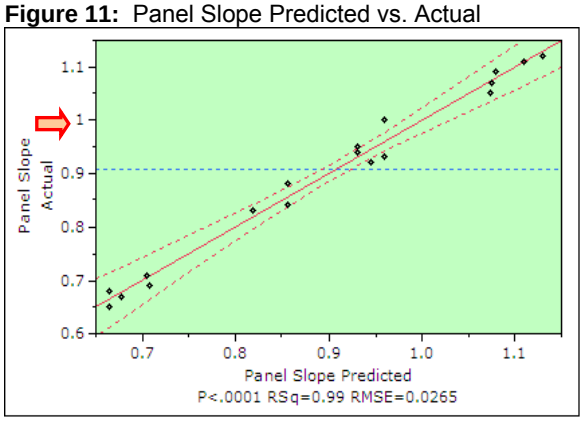


Figure 12: Equimolarity Predicted vs. Actual

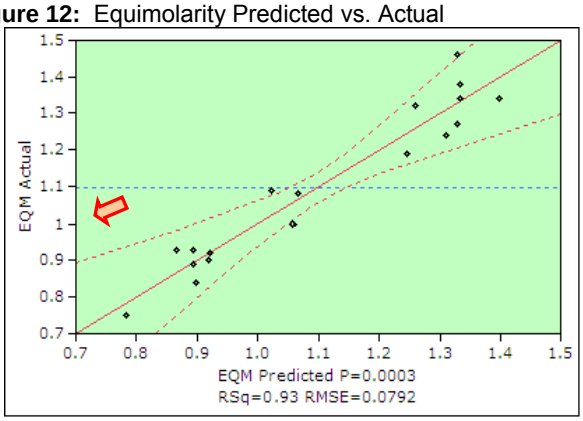
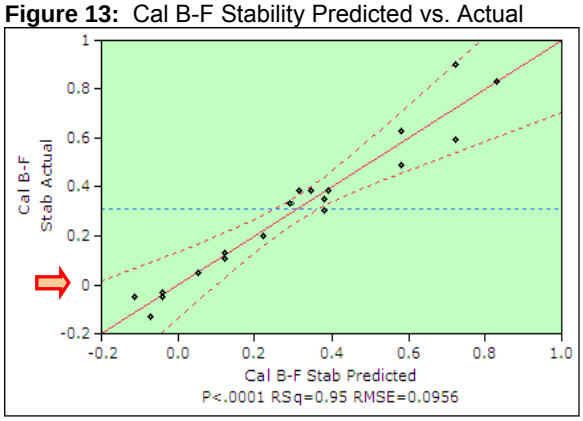


Figure 13: Cal B-F Stability Predicted vs. Actual



Another JMP output that is informative is the sorted parameter estimates. When sorted, the factor having the greatest influence on the response is on the top. Table 5 shows the results for the Panel Slope, and as expected from our hypothesis, the most important factor is the concentration of the MAb in the microparticle diluent.

Similarly, Table 6 shows that the same input factor is the primary driver of the equimolarity response.

Table 7, however, shows a different story, but one expected based on the theory of our process. The concentration of the coupling reagent plays the most important role in the generation of that response.

Seeing such results that agree with the known the chemistry of the process enhances the confidence that the models created are describing reality.

TABLE 5: Panel Slope

Sorted Parameter Estimates

Term	Estimate	Std Error	t Ratio	t Ratio	Prob>|t|
[MAb] ug/mL in diluent	0.1764243	0.008012	22.02		<.0001
(EDAC mg/mL-6)*([MAb] ug/mL in diluent-1)	0.0118634	0.002058	5.76		0.0003
Ab mg/mL	0.0876801	0.016448	5.33		0.0005
(Ab mg/mL-0.5)*(EDAC mg/mL-6)	-0.021259	0.004288	-4.96		0.0008
(Ab mg/mL-0.5)*([MAb] ug/mL in diluent-1)	-0.057951	0.019001	-3.05		0.0138
([MAb] ug/mL in diluent-1)*([MAb] ug/mL in diluent-1)	-0.050856	0.017105	-2.97		0.0156
EDAC mg/mL	-0.004822	0.001845	-2.61		0.0281
(EDAC mg/mL-6)*(EDAC mg/mL-6)	-0.000842	0.000616	-1.37		0.2048
(Ab mg/mL-0.5)*(Ab mg/mL-0.5)	0.0592606	0.067526	0.88		0.4030

TABLE 6: Equimolarity

Sorted Parameter Estimates

Term	Estimate	Std Error	t Ratio	t Ratio	Prob>|t|
[MAb] ug/mL in diluent	-0.250386	0.023948	-10.46		<.0001
([MAb] ug/mL in diluent-1)*([MAb] ug/mL in diluent-1)	0.1138585	0.051129	2.23		0.0530
Ab mg/mL	0.1009066	0.049166	2.05		0.0703
(EDAC mg/mL-6)*([MAb] ug/mL in diluent-1)	-0.011816	0.006152	-1.92		0.0870
(EDAC mg/mL-6)*(EDAC mg/mL-6)	0.0022738	0.00184	1.24		0.2479
(Ab mg/mL-0.5)*(Ab mg/mL-0.5)	-0.071951	0.201843	-0.36		0.7297
EDAC mg/mL	0.0019068	0.005514	0.35		0.7374
(Ab mg/mL-0.5)*(EDAC mg/mL-6)	0.0026686	0.012818	0.21		0.8397
(Ab mg/mL-0.5)*([MAb] ug/mL in diluent-1)	0.0070362	0.056798	0.12		0.9041

TABLE 7: Cal B-F Stability

Sorted Parameter Estimates

Term	Estimate	Std Error	t Ratio	t Ratio	Prob>|t|
EDAC mg/mL	0.0634739	0.006657	9.54		<.0001
Ab mg/mL	-0.236815	0.05936	-3.99		0.0032
(Ab mg/mL-0.5)*(EDAC mg/mL-6)	-0.03898	0.015475	-2.52		0.0328
(Ab mg/mL-0.5)*(Ab mg/mL-0.5)	0.5580778	0.243692	2.29		0.0478
[MAb] ug/mL in diluent	0.0488119	0.028913	1.69		0.1256
([MAb] ug/mL in diluent-1)*([MAb] ug/mL in diluent-1)	0.0978185	0.06173	1.58		0.1475
(EDAC mg/mL-6)*([MAb] ug/mL in diluent-1)	0.0112314	0.007428	1.51		0.1648
(Ab mg/mL-0.5)*([MAb] ug/mL in diluent-1)	0.1006162	0.068574	1.47		0.1764
(EDAC mg/mL-6)*(EDAC mg/mL-6)	0.0004016	0.002222	0.18		0.8606

Turning then to the simultaneous optimization of all three responses:

- Optimum conditions *predicted* from the model analysis:
  - [Ab] = 0.75 mg/mL
  - [EDAC] = 2.5 mg/mL
  - [MAb in diluent] = 2.0 µg/mL

- Observed responses in confirmation runs at these levels (checkpoints):
  - Stability = 94-96% (acceptable)
  - Panels = within 3% of target (acceptable)
  - Equimolarity = 0.94 – 0.97 (acceptable)

Conclusion: dancing in the halls might commence!

UNEXPECTED CHECKPOINT ACCURACY

Remember that I mentioned preliminary experiments that suggested the viability of this strategy as the basis for trying the approach. The scary part is how accurately the RSM model generated months later predicts the results of that preliminary experiment. In this case, panel values were monitored by the slope of the regression line between observed panel values versus known panel values (target therefore is one, with a permissible range of 0.95-1.05).

Output	*[MAb in diluent] µg/mL*	PREDICTIONS FROM THIS MODEL ***Predicted Target***	*Predicted 95% CI*	***OBSERVED Previously***
Panel Slope	0.10	**0.71**	0.64 - 0.79	**0.71**
	0.50	**0.80**	0.72 - 0.87	**0.78**
	1.00	**0.88**	0.81 - 0.96	**0.93**
Equimolarity	0.10	**1.25**	1.04 - 1.45	**1.23**
	0.50	**1.10**	0.89 - 1.31	**1.08**
	1.00	**0.97**	0.76 - 1.18	**0.99**

CONCLUSION OF THE MATTER

When used properly, DOE is a powerful tool in an experimenter's toolbox that will amply reward its mastery.

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AUTHOR BIO

Steve received his Bachelor's (neurobiology) from Cornell University, a Master's (chemistry) from Northern Illinois University, and was finally ejected with a Ph.D. (biochemistry) from Florida State University in 1984. After a two year post-doc at Los Alamos National Laboratory, he escaped to Abbott Laboratories in Chicagoland, where he's been hiding ever since, developing automated *in vitro* diagnostic immunoassays. Having traumatized his Ph.D. major advisor by consistently yet unsystematically changing multiple variables simultaneously between experiments, Steve became proficient over the ensuing years in a methodology that actually requires him to do so. Meanwhile, having acquired enough statistics to be dangerous, Steve persists in trying to share that knowledge with the world at large.

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JMP version 7 was used to create the JMP figures and analysis in the above.