Tackling *Life's* Biological Complexity with JMP® Software

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Introduction

Life Technologies™, a global biotechnology company, enables researchers by providing tools that accelerate scientific and medical advancements. A number of these tools are comprised of complex biological systems or isolated components used to model these systems *in vitro*. JMP® Statistical Discovery software has become an integral tool for helping us describe and control the complexity in our products. Our enriched understanding has enabled us to deliver whether designing a robust manufacturing process or improving product reliability for our customers. In this presentation we describe a project where loss of antibody was observed in recent batches and our approach to stabilize the product through reformulation. The team used a sequential approach to experimentation to identify possible process/formulation changes that would minimize the loss of antibody. Fractional factorial modeling followed by a Response Surface Method design were used to optimize the system and make practical decisions on the path to improvement. The new formulation was manufactured in pilot lots and tested to verify that the production material would be consistent with the predictive model. Finally, the new formulation was implemented and control measures put in place to monitor the effectiveness of the process.

Problem Statement

Life Technologies™ manufactures tools for the life science community and occasionally these tools need to be redeveloped in order to satisfy customer demands. Recently, a product containing an antibody, needed such a redevelopment effort. Over several manufacturing batches, it had been observed that this antibody would aggregate and potentially adsorb to the storage vessel surface resulting in significant product loss. In some cases the loss was so extreme that no measureable product remained. The team decided it was out of scope to identify the root cause of the loss, but could reformulate the solution to reduce or eliminate the occurrence. To do this, the team decided to leverage the tools that JMP® Statistical Discovery Software provides.

Methods

To attack this problem, the team employed the following methods:

- A high level process map exercise to make sure the team understood the manufacturing process and potential steps that could be impacted by the improvement effort (not shown).
- A Cause and Effect (Ishikawa Diagram, Figure 1) to focus the team on potential root causes of the antibody loss.
- A Measurement Systems Analysis (Figure 2) to verify that the measurement system that was chosen would be capable of quantifying the changes.
- A series of Designed Experiments:
    - Fractional Factorial (Figures 3 & 4) to narrow down the number of factors to optimize.
    - Response Surface Method (Figure 5) to optimize the critical factors identified.
- To confirm the model, a number of pilot batches were manufactured to verify that the formulation was effective and that the manufacturing material is being monitored using control chart methods (Figure 6).

Results

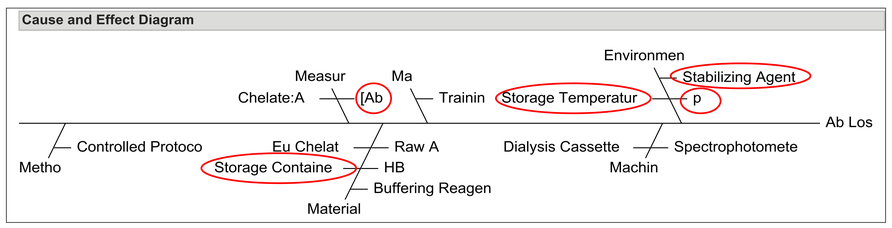
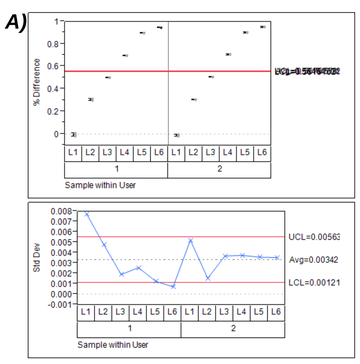


Figure 1. Cause and Effect Diagram listing out the factors associated with the 5M's and E. The red circles indicate the factors the team identified to use in experimentation.

A)



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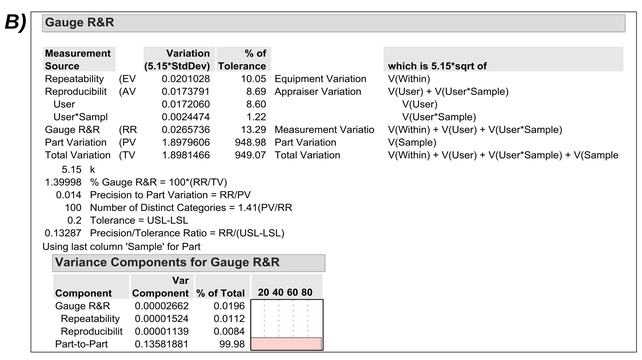


Figure 2. Spectrophotometer Measurement Systems Analysis (AIAG Method): Before any experimentation could begin, an MSA was performed to understand the Gauge. The team prepared antibody samples at 6 different concentrations. Measurements were carried out by two different operators in triplicate using a Shimadzu UV-1800 Spectrophotometer. The % difference was calculated by dividing each sample by the most concentrated sample and multiplying by 100. A) Mean and Standard Deviation plots of the data. B) Gauge R&R Summary for the data.

With a result of 1.4% for the Gauge R&R and 13.2% for a Precision to Tolerance Ratio, the spectrophotometer appeared to be adequate for the process improvement project.

A)

Factors	Type	Levels
pH	Continuous	6.5 – 8.0
[Antibody]	Continuous	0.1 – 0.4
Additive 1	Continuous	-1 – +1
Additive 2	Continuous	-1 – +1
Temperature	Categorical	-20°, 4° C
Storage Vessel	Categorical	Glass, Plastic

B)

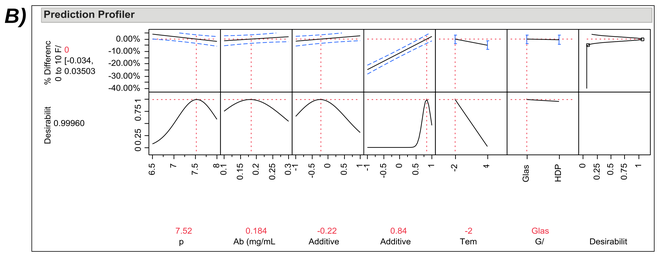
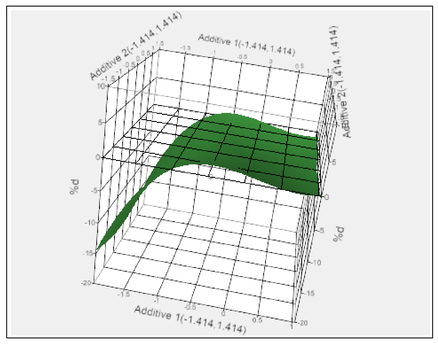


Figure 3. Fractional Factorial DOE: 6 factors tested and Prediction Profiler from the experiment. A) The table summarizes the six factors the team decided to focus on and the levels used in the experimentation. A ½ Fractional Design with Center points was used and samples were measured in triplicate. B) Prediction Profiler obtained from the experimental results set to the On Target Desirability of 0% Difference.

Factors	Predicted Optimum	Current	What will we do?
pH	7.52	7.5	**No change** – risk to long term stability.
Antibody (mg/mL)	0.18	0.25	**No Change** – [Antibody] impacts customer.
Additive 1	-0.22	0	**Study Further**
Additive 2	0.84	0	**Study Further**
Glass/Plastic	Glass	Glass	**Change** –impact low; plastic is less fragile.
Temp	-20	-20	**No Change**

Figure 4. Table Summary from Fractional Factorial DOE. The team used the information obtained from the Prediction Profile predicted optimum settings for the different factors to determine which ones required further investigation. The two stabilizing agents (Additive 1 & Additive 2) were studied in a Central Composite Design DOE to determine the optimal concentrations for the formulation.

A)



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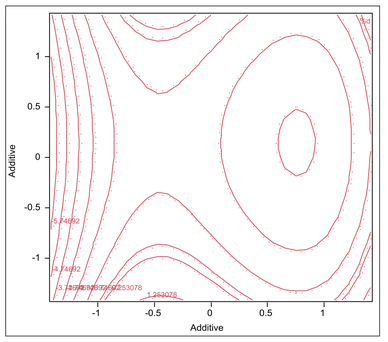

Figure 5. Central Composite Design. A) Surface Profiler illustrating the response surface of the experimental results. The grid line indicates 0 % Difference (antibody loss) showing a relatively flat region to set formulation within. B) the Contour Plot illustrates a similar trend.

From this data, factor settings were selected to prepare pilot batch formulations and verify the model was predictive and improved the product performance.

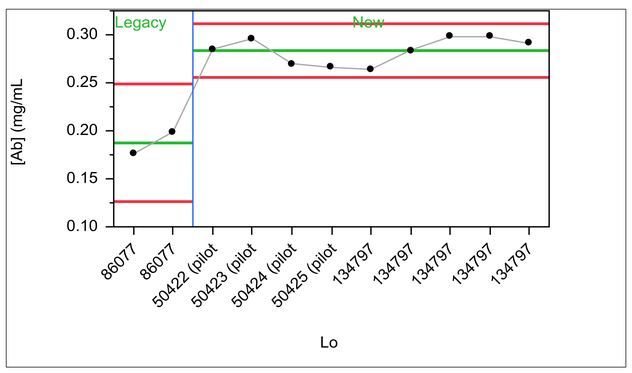

Figure 6. Control Chart. Antibody concentration results of 6 different batches of the antibody phased by Legacy and New process indicating the New process has reduced the loss of the antibody.

Discussion and Conclusions

Leveraging the tools that JMP® Statistical Discovery Software provides have enabled Life Technologies scientists to improve product designs and production processes on a number of different projects.

In this case study, the team leveraged:

- Cause and Effect Diagram to narrow down what factors to test.
- MSA to verify the Gauge was adequate.
- DOE methodology to determine important factors and optimize factor settings.
- Monitor the process through control charting methods.