Did it have an impact or not? Using JMP® for the design and analysis of an experiment in a Vaccine product root cause investigation

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Introduction

During the investigation of an observed downward trend in the potency of a vaccine bulk material, a list of recent changes to process and/or methods showed that several had been implemented around the same timeframe. Analysis of historical data in this case does not provide conclusive information for deciding which one of these changes is the largest contributor to the issue or by how much. Use of Design of Experiments (DOE) is a better approach at quantifying the impact of a given change or set of changes. When there are hard-to-change factors, a split-plot design structure needs to be considered and appropriately analyzed to correctly assess statistical significance of the different factors. JMP statistical software is one of the few off-the-shelf packages that makes analysis of split-plot designs accessible to the industrial practitioner.

Experimental design

One set of changes under investigation involved changes in the thawing of samples for potency testing. Four factors (A: thaw time; B: thawing temperature; C: sample handling; and D: sample incoming potency) were involved, each at two-levels, with one of the factors (A) a hard-to-change factor. Blocking was also necessary due to assay logistics and physical limitations of the size of the block to accommodate all factor-level combinations and necessary replication for the hard-to-change factor. The final screening design had a split-plot structure, with a full factorial design in the three easy-to-change factors (B-D) within each whole-plot. The whole-plot factor A was replicated 6 times. Interactions with factor D were of special interest.

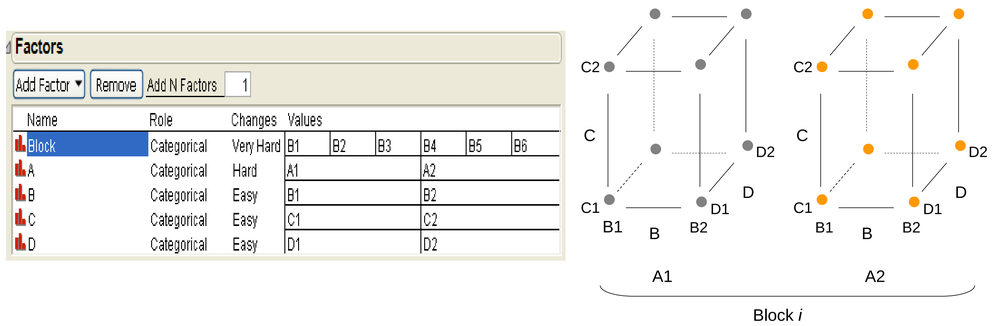


Figure 1. Defining whole-plot, sub-plot and blocking factors with the Custom Design option of the DOE platform. Cube plots showing treatment and design structures

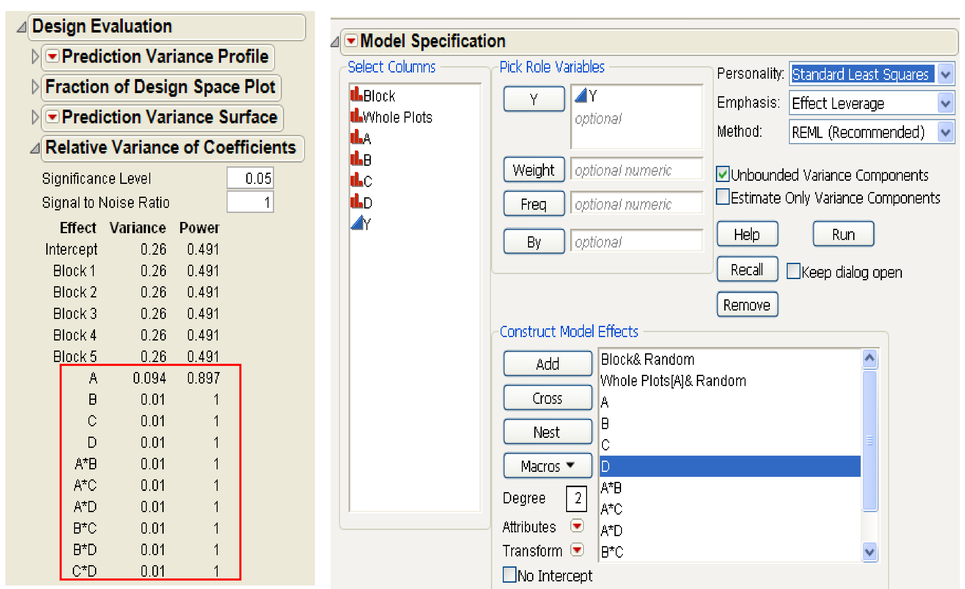


Figure 2. (left) The experimental design allowed detecting a signal to noise ratio of 1 with a power greater than 80% for all effects and interactions. (right) Setting up the correct analysis of the split-plot structure of the design

Results

REML Variance Component Estimates

Random Effect	Var Ratio	Var Component	Std Error	95% Lower	95% Upper	Pct of Total
Block	0.479617	36.8344	29.3115	-20.615	94.284	30.34
WholePlot[A]	0.101284	7.7786	11.1024	-13.982	29.539	6.41
Residual		76.7998	12.5413	57.120	108.798	63.26
Total		121.4128				100.00

-2 LogLikelihood = 674.64499615

Covariance Matrix of Variance Component Estimates

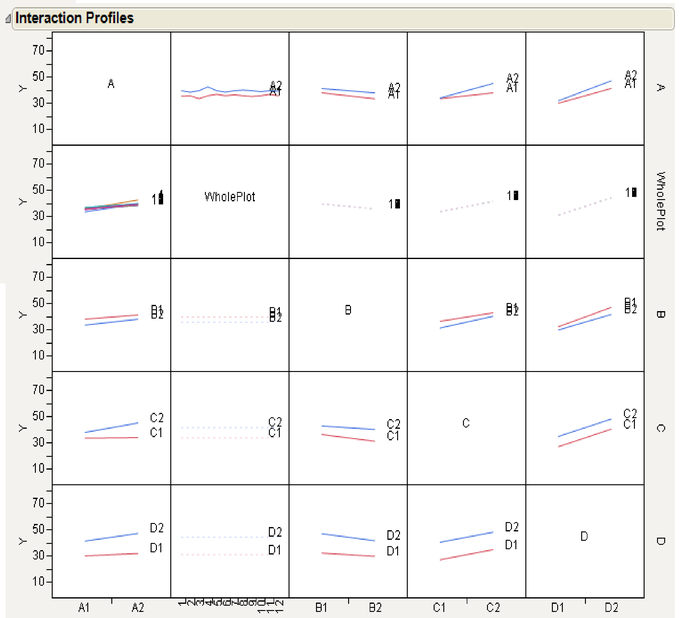
Iterations

Fixed Effect Tests

Source	Nparm	DF	DFDen	F Ratio	Prob > F
A	1	1	5	2.5879	0.1686
B	1	1	75	4.8578	0.0306*
C	1	1	75	18.8555	<.0001*
B*C	1	1	75	0.4477	0.5055
A*B	1	1	75	0.1160	0.7343
A*C	1	1	75	3.4701	0.0664
D	1	1	75	55.9122	<.0001*
D*B	1	1	75	0.6828	0.4113
D*C	1	1	75	0.0003	0.9866
D*A	1	1	75	1.2383	0.2694

•The variance component estimates suggest accounting for block effects was a good strategy as about a third of the total variability in the data is accounted for by block to block differences.

•Main effects of factors B, C and D, the sub-plot factors, were all statistically significant at the chosen significance level of α = 0.05. None of the interactions with factor D were significant.



•The largest main effect was that of factor D, the sample incoming potency (increase of 13.4 units when changing level from low to high), This effect was as expected, since more potent samples should result in larger unit counts. The purpose of including this factor was to check for interactions with the other factors.

D

Least Squares Means Table

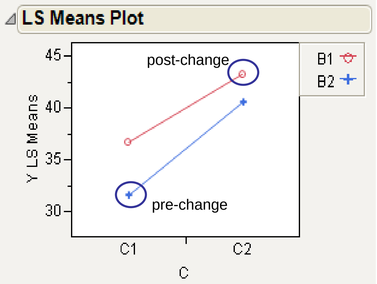
Level	Least Sq Mean	Std Error
D1	31.3510	2.8961
D2	44.7271	2.8961

B*C

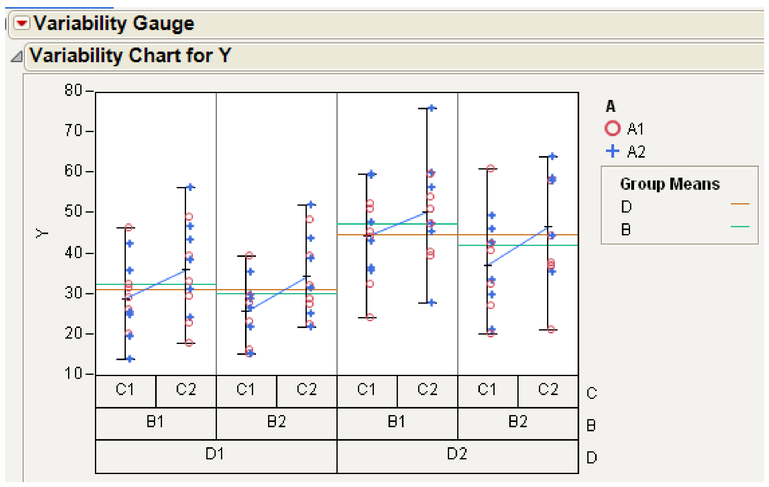
Least Squares Means Table

Level	Least Sq Mean	Std Error
B1,C1	36.7250	3.1603
B1,C2	43.2958	3.1603
B2,C1	31.5854	3.1603
B2,C2	40.5500	3.1603

LS Means Plot



•The combination of levels B2,C1 represented the pre-change thawing practice vs. the post-change practice represented by B1,C2. The fitted model predicts an average increase of 11.4 unit counts with the use of the post-change thawing practice.



•The variability plot helped in the assessment of statistical vs. practical significance of the effects by visually contrasting effects size to the residual variability.

Conclusions

•Combined effect of the implemented changes in sample thawing would cause an increase in potency rather than a decrease.

•The implemented changes in sample thawing were not a root cause for the observed downward trend in potency of the vaccine bulk material.

Acknowledgements

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