Student Activity 6: Randomized Block Design, Latin Square, Repeated Latin Square, and Graeco Latin Square

Consider the “one-way treatment structure in a completely randomized design structure” experiment.

We have “*a*” treatments, each replicated *n* times (we consider the balanced case for simplicity). The appropriate means model is

$$Y_{ij} = \mu_i + \varepsilon_{ij} \qquad \begin{matrix} i = 1,2,\ldots,a \\ j = 1,2,\ldots,n \end{matrix} \qquad \text{where } \varepsilon_{ij} \sim iidN\left(0,\sigma^2\right)$$

The error terms ε_{ij} denote the plot to plot variation in the response that cannot be attributed to the treatment effect.

The variance σ^2 is a measure of this variation. If the plots are more alike (homogeneous) then σ^2 will be low. If the plots are very different from one another σ^2 will be large.

A small σ^2 enables an experimenter to attribute even small variation in the treatment sample means $\bar{Y}_{1\bullet},..,\bar{Y}_{a\bullet}$ to differences between treatment (population) means μ_{ij}. In other words, a small σ^2 results in a more powerful F-test. The reverse is true if σ^2 is large.

Thus, one of the main tasks of an experimenter is to reduce σ^2 by using homogeneous experimental units.

However, one should make sure that such homogeneity does not compromise to applicability of the results.

[e.g.: Using white males ages 21-25 in a test of a hair growing formulation will make the results inapplicable to older males and individuals of other races or females.

Another way to reduce σ^2 is by grouping experimented units that are more alike.

e.g.: 1) We have two drugs to be tested. Use identical twins, say 5 pairs. Randomly pick one twin from each pair and give drug one. The other twin gets drug two. We rely on the fact that within pair of twin variation is less than between pair of twin variation.

e.g.: 2) We need to test two types of shoe soles. Pick 20 people and randomly assign one type of sole to one foot of each person and the other type to the other foot. Here again, between foot variation within a person is less than between person variation.

e.g.: 3) In an agricultural experiment to compare the yield of 4 varieties of soybeans, divide experimental land into four blocks, each block containing 5 plots (i.e. experimental units). In each block, randomly assign each variety to a plot.

All the above are examples of "BLOCKING". In example 1), the block is a pair of twins, in example 2), the block is a person, and in example 3), the block is a piece of land consisting of 5 adjoining plots.

In all cases, plot to plot variation within a block is less than block to block variation.

THE MEANS MODEL FOR A ONE-WAY (FIXED EFFECT) TREATMENT STRUCTURE IN A RANDOMIZED BLOCK DESIGN

$$Y_{ij} = \mu_i + \beta_j + \varepsilon_{ij} \qquad i = 1,2,\ldots,a$$
$$j = 1,2,\ldots,b$$

where $\beta_i \sim iidN(o, \sigma_b^2)$

$\varepsilon_{ij} \sim iidN(0, \sigma^2)$

and $\beta_j, \varepsilon_{ij}$ are independent.

μ_i denote the population mean for the i^{th} treatment

One can consider the above model as a two-way model where the row effect is fixed but the column effect is random (so it is a mixed model). In fact, the appropriate sum of squares can be obtained by treating it as a two-way model without interaction.

The plot to plot variation within a fixed block is σ^2. Thus, the error variance of a plot selected randomly from a pre-specified block (after accounting for the block effect) is σ^2.

Thus $Var(Y_{12} - Y_{22}) = Var(\varepsilon_{12} - \varepsilon_{22}) = 2\sigma^2$. However, the variance of the response of a plot randomly picked from the totality of ab plots is not σ^2 but is $\sigma^2 + \frac{b-1}{b}\sigma_b^2 (\approx \sigma^2 + \sigma_b^2$ if b is large$)$. Note that σ_b^2 is the block variation (scaled to reflect the plot size).

If σ_b^2 is large, then blocking will enable to come up with a more "sensitive" experiment.

THE CLASSICAL MODEL

$$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij} \qquad i = 1,2,\ldots,a$$
$$j = 1,2,\ldots,b$$

$\varepsilon_{ij}, \beta_j$ defined as before.

Observe that we have no interaction term. In blocked experiments, it is assumed that there is not block by treatment interaction.

The constraints assumed are $\sum_{i=1}^{a} \alpha_i = 0$ and $\sum_{j=1}^{b} \beta_j = 0$. The second restriction is needed only if the blocking effect is considered fixed.

AN EXAMPLE OF AN ANALYSIS OF DATA FROM A RANDOMIZED COMPLETE BLOCK DESIGN

Example 1: Three different washing solutions are being compared to study their effectiveness in retarding bacteria growth in 5-gallon milk containers. The analysis is done in a laboratory, and only three trials can be run on any day. Because days could represent a potential source of variability, the experimenter decides to use a randomized block design. Observations are taken for four days, and the data are shown here. Analyze the data from this experiment and draw conclusions.

In this example, the blocking factor is the day. The treatment is “solution”. We have three types of solutions and four levels for “day.”

	Days			
Solution	1	2	3	4
1	13	22	18	39
2	16	24	17	44
3	5	4	1	22

```
options ls=72 nodate;
data wash;
input solution day bacteria;
cards;
1 1 13
1 2 22
1 3 18
1 4 39
2 1 16
2 2 24
2 3 17
2 4 44
3 1 5
3 2 4
3 3 1
3 4 22
;
proc print;
title1 ' MATH 338 : Experimental Design';
title2 'Example on Randomized Complete Block Design';
title3 'List of Data';
proc glm;
title3 'analysis of variance results';
class solution day;
model bacteria = day solution / solution;
means solution / tukey;
proc glm;
title3 'analysis of variance results with lsmeans';
class solution day;
model bacteria = day solution / solution;
lsmeans solution / tdiff;
run;
```

THE SAS OUTPUT IS GIVEN BELOW

MATH 338 : Experimental Design

Example on Randomized Complete Block Design

List of Data

Obs	solution	day	bacteria
1	1	1	13
2	1	2	22
3	1	3	18
4	1	4	39
5	2	1	16
6	2	2	24
7	2	3	17
8	2	4	44
9	3	1	5
10	3	2	4
11	3	3	1
12	3	4	22

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MATH 338 : Experimental Design

Example on Randomized Complete Block Design

analysis of variance results

The GLM Procedure

Class Level Information		
Class	Levels	Values
solution	3	1 2 3
day	4	1 2 3 4

Number of Observations Read	12
Number of Observations Used	12

MATH 338 : Experimental Design

Example on Randomized Complete Block Design

analysis of variance results

The GLM Procedure

Dependent Variable: bacteria

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1810.416667	362.083333	41.91	0.0001
Error	6	51.833333	8.638889		
Corrected Total	11	1862.250000			

R-Square	Coeff Var	Root MSE	bacteria Mean
0.972166	15.67573	2.939199	18.75000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
day	3	1106.916667	368.972222	42.71	0.0002
solution	2	703.500000	351.750000	40.72	0.0003

Source	DF	Type III SS	Mean Square	F Value	Pr > F
day	3	1106.916667	368.972222	42.71	0.0002
solution	2	703.500000	351.750000	40.72	0.0003

Parameter	Estimate		Standard Error	t Value	Pr > |t|
Intercept	24.25000000	B	2.07832732	11.67	<.0001
day 1	-23.66666667	B	2.39984567	-9.86	<.0001
day 2	-18.33333333	B	2.39984567	-7.64	0.0003
day 3	-23.00000000	B	2.39984567	-9.58	<.0001
day 4	0.00000000	B	.	.	.
solution 1	15.00000000	B	2.07832732	7.22	0.0004
solution 2	17.25000000	B	2.07832732	8.30	0.0002
solution 3	0.00000000	B	.	.	.

MATH 338 : Experimental Design

Example on Randomized Complete Block Design

analysis of variance results

The GLM Procedure

Tukey's Studentized Range (HSD) Test for bacteria

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	8.638889
Critical Value of Studentized Range	4.33917
Minimum Significant Difference	6.3768

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	solution
A	25.250	4	2
A			
A	23.000	4	1
B	8.000	4	3

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MATH 338 : Experimental Design

Example on Randomized Complete Block Design

analysis of variance results with lsmeans

The GLM Procedure

Class Level Information		
Class	Levels	Values
solution	3	1 2 3
day	4	1 2 3 4

Number of Observations Read	12
Number of Observations Used	12

Example on Randomized Complete Block Design

analysis of variance results with lsmeans

The GLM Procedure

Dependent Variable: bacteria

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1810.416667	362.083333	41.91	0.0001
Error	6	51.833333	8.638889		
Corrected Total	11	1862.250000			

R-Square	Coeff Var	Root MSE	bacteria Mean
0.972166	15.67573	2.939199	18.75000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
day	3	1106.916667	368.972222	42.71	0.0002
solution	2	703.500000	351.750000	40.72	0.0003

Source	DF	Type III SS	Mean Square	F Value	Pr > F
day	3	1106.916667	368.972222	42.71	0.0002
solution	2	703.500000	351.750000	40.72	0.0003

Parameter	Estimate		Standard Error	t Value	Pr > |t|
Intercept	24.25000000	B	2.07832732	11.67	<.0001
day 1	-23.66666667	B	2.39984567	-9.86	<.0001
day 2	-18.33333333	B	2.39984567	-7.64	0.0003
day 3	-23.00000000	B	2.39984567	-9.58	<.0001
day 4	0.00000000	B	.	.	.
solution 1	15.00000000	B	2.07832732	7.22	0.0004
solution 2	17.25000000	B	2.07832732	8.30	0.0002
solution 3	0.00000000	B	.	.	.

uations. Terms whose estimates are followed by the letter 'B' are not uniquely estima

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MATH 338 : Experimental Design

Example on Randomized Complete Block Design

analysis of variance results with lsmeans

The GLM Procedure
Least Squares Means

solution	bacteria LSMEAN	LSMEAN Number
1	23.0000000	1
2	25.2500000	2
3	8.0000000	3

Least Squares Means for Effect solution
t for H0: LSMean(i)=LSMean(j) / Pr > |t|
Dependent Variable: bacteria

i/j	1	2	3
1		-1.0826	7.217342
		0.3206	0.0004
2	1.082601		8.299944
	0.3206		0.0002
3	-7.21734	-8.29994	
	0.0004	0.0002	

OTHER BLOCK DESIGNS

There are many types of block designs, with RCB being one of them. Some of the other block designs are Latin Square Designs, Greaco-Latin Square Designs, and Split-Plot Designs.

LATIN SQUARE DESIGN

In a randomized complete block design, the blocking was done to reduce variation that can be attributed to some random (and in some cases fixed) factor. For example, in an agricultural experiment, blocking may be done to remove the effect due to a fertility gradient; in a chemistry experiment blocking may be done to remove the effect of the chemists' skills. In some situations, it is possible that one wishes to remove the effect of two factors. Then blocking has to be done in two "directions", each "direction" corresponding to the "gradient' of a given factor.

e.g: An agricultural scientist wishes to study the effects of 4 different kinds of fertilizer on a certain variety of wheat. The experimental field in which the wheat is to be grown has a moisture gradient in one direction and a sunlight gradient perpendicular to it.

Hence we need to block in both directions.

Column Blocks

Row Blocks

A	*B*	*C*	*D*
B	*C*	*D*	*A*
C	*D*	*A*	*B*
D	*A*	*B*	*C*

Sun Light Gradient

Moisture Gradient

One may block as above (with 4 row blocks to take care of the sunlight gradient and 4 column blocks to take care of the moisture gradient).

If you now apply the four fertilizers (i.e. treatments A,B,C,D) in such a way that each treatment occurs once (and only once) in each row and in each column, then we have what is known as a **Latin Square Design**.

Usually, the row block effects and the column block effects are random effects and it is assumed that there is no row * column, row * treatment, column * treatment and row * column * treatment interaction. In fact, it is the contrasts that estimate the above interactions that are used to estimate the error variance σ^2.

Sometimes, the row effect or the column effects are those due to a specific treatment (or both are). Thes, the rows, columns (or both) are fixed effects.

e.g.: In the agriculture example given above, suppose the experimental field is homogeneous (and hence no blocking is necessary), but the agriculturalist is interested in two other factors, namely wheat variety and time of application of fertilizer. Suppose each of these two factors also have 4 levels each.

Then, the agriculturalist could have conducted a 3-way experiment. With 2 replications for each of the 4 * 4 * 4 treatment combinations, be would need 128 experimental units (plots).

Suppose he knows that no interaction exists, so he need not Replicate because interaction contrast can be used to estimate error. Even then he needs 64 plots.

Now, if the no interaction hypothesis is true (i.e. no variety * fertilizer, variety * time, time * fertilizer, and variety * time * fertilizer interactions), then he could use the design in on this page with the varieties randomly assigned to the rows and times of fertilizing randomly assigned to the columns. This way, he needs only 16 plots!

Usually, however, such an assumption of no interaction is not reasonable and thus the agriculturalist may end up having to use 128 plots.

THE GENERAL MEANS MODEL FOR A LATIN SQUARE DESIGN

$$Y_{ijk} = \mu_i + \alpha_j + \beta_k + \varepsilon_{ijk}$$

$$\begin{aligned} i &= 1,2,\ldots,\rho \\ j &= 1,2,\ldots,\rho \qquad (\rho = \#\text{ of treatments} = \#\text{ of rows} = \#\text{ of columns}) \\ k &= 1,2,\ldots,\rho \end{aligned}$$

$$\begin{bmatrix} \text{Here } i \text{ denotes the treatment} \\ \text{Here } j \text{ denotes the row} \\ \text{Here } k \text{ denotes the column} \end{bmatrix}$$

$$* \left\{ \begin{array}{l} \text{where } \alpha_j \sim iidN\left(0, \sigma_k^2\right) \\ \qquad \beta_k \sim iidN\left(0, \sigma_c^2\right) \\ \qquad \varepsilon_{ijk} \sim iidN\left(0, \sigma^2\right) \\ \text{with } \alpha_j, \beta_k, \varepsilon_{ijk} \text{ independent} \end{array} \right\} \text{ If row and column effects are random}$$

$$\left[\begin{array}{l}\textbf{or} \\ \qquad Y_{ijk} = \mu_{ijk} + \varepsilon_{ijk} \quad , \quad \varepsilon_{ijk} \sim iidN\left(0,\sigma^2\right) \\ \qquad \text{if row \& column effects are fixed.}\end{array}\right]$$

THE GENERAL CLASSICAL MODEL FOR LATIN SQUARE DESIGN

$$Y_{ijk} = \mu + \tau_i + \alpha_j + \beta_k + \varepsilon_{ijk}$$

$$i, j, k = 1, 2, ..., \rho \quad , \quad \sum_{i=1}^{\rho} \tau_i = 0$$

(τ_i-denoting the treatment effect and μ-denoting the overall mean)

and if α_j, β_k are considered random effects. I this case * above holds.

If α_j, β_k are fixed, then $\sum_{j=1}^{\rho} \alpha_j = 0, \quad \sum_{k=1}^{\rho} \beta_k = 0$ and $\varepsilon_{ijk} \sim iidN\left(0,\sigma^2\right)$.

Note that the above model is completely additive. That is, it has no interaction terms.

ANALYSIS OF A LATIN SQUARE DESIGN

$$SS_{\text{Total}} = \sum_{i=1}^{\rho}\sum_{j=1}^{\rho}\sum_{k=1}^{\rho} Y_{ijk}^2 \quad - N\bar{Y}_{\bullet\bullet\bullet}^2$$

where $N = \rho^2$.

$$SS_{\text{Treatment}} = \sum_{i=1}^{\rho} \rho \bar{Y}_{i\bullet\bullet}^2 \quad - N\bar{Y}_{\bullet\bullet\bullet}^2$$

$$SS_{\text{Rows}} = \sum_{j=1}^{\rho} \rho \bar{Y}_{\bullet j\bullet}^2 \quad - N\bar{Y}_{\bullet\bullet\bullet}^2$$

$$SS_{\text{Columns}} = \sum_{k=1}^{\rho} \rho \bar{Y}_{\bullet\bullet k}^2 \quad - N\bar{Y}_{\bullet\bullet\bullet}^2$$

It can be shown that $SS_{\text{Treatment}}, SS_{\text{Row}}, SS_{\text{Columns}}$ are independent, and are also independent of SS_{Error} where

$$SS_{\text{Error}} = SS_{\text{Total}} - SS_{\text{Treatment}} - SS_{\text{Rows}} - SS_{\text{Columns}}$$

Further,

$$F_o = \frac{SS_{\text{Treatment}} / (\rho - 1)}{SS_{\text{Error}} / (\rho - 2)(\rho - 1)} \sim F(\rho - 1, (\rho - 2)(\rho - 1))$$

if

$H_o \quad \tau_1 = \tau_2 = .. = \tau_\rho = 0$ (otherwise, F_o has a non-central F distribution).

THE ANOVA TABLE

Source	d.f.	SS	MS	F
Treatments	$\rho - 1$	$SS_{\text{Treatment}}$	$MS_{\text{Treatment}}$	$F_o = \frac{MS_{\text{Treatment}}}{MS_{\text{Error}}}$
Rows	$\rho - 1$	SS_{Rows}	MS_{Rows}	
Columns	$\rho - 1$	SS_{Columns}	MS_{Columns}	
Error	$(\rho - 2)(\rho - 1)$	SS_{Error}	MS_{Error}	
Total	$\rho^2 - 1$	SS_{Total}		

Then analysis using SAS can be done as follows:

```
proc glm data=yourdata;
class row col treatment;
model y = row col treatment;
means treatment/lsd tukey;
run;
```

Example 2: Consider an experiment to investigate the effect of 4 diets on milk production. There are 4 cows. Each lactation period the cows receive a different diet. Assume there is a washout period so previous diet does not affect future results.

```
options nocenter ls=75;
data milk;
input cow period trt resp @@;
cards;
1 1 1 38 1 2 2 32 1 3 3 35 1 4 4 33
2 1 2 39 2 2 3 37 2 3 4 36 2 4 1 30
3 1 3 45 3 2 4 38 3 3 1 37 3 4 2 35
4 1 4 41 4 2 1 30 4 3 2 32 4 4 3 33
;
proc glm;
class cow trt period;
model resp=trt period cow;
means trt/lsd tukey;
means period cow;
```

```
output out=new r=res p=pred;
symbol1 v=circle;
proc gplot;
plot res*pred;
proc univariate noprint normal;
histogram res/normal (L=1 mu=0 sigma=est) kernel (L=2);
qqplot res/normal (L=1 MU=0 sigma=est);
run;
```

The GLM Procedure

Class Level Information		
Class	Levels	Values
cow	4	1 2 3 4
trt	4	1 2 3 4
period	4	1 2 3 4

Number of Observations Read	16
Number of Observations Used	16

The GLM Procedure

Dependent Variable: resp

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	242.5625000	26.9513889	33.17	0.0002
Error	6	4.8750000	0.8125000		
Corrected Total	15	247.4375000			

R-Square	Coeff Var	Root MSE	resp Mean
0.980298	2.525780	0.901388	35.68750

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	3	40.6875000	13.5625000	16.69	0.0026
period	3	147.1875000	49.0625000	60.38	<.0001
cow	3	54.6875000	18.2291667	22.44	0.0012

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	3	40.6875000	13.5625000	16.69	0.0026
period	3	147.1875000	49.0625000	60.38	<.0001
cow	3	54.6875000	18.2291667	22.44	0.0012

The GLM Procedure

t Tests (LSD) for resp

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	0.8125
Critical Value of t	2.44691
Least Significant Difference	1.5596

Means with the same letter are not significantly different.			
t Grouping	Mean	N	trt
A	37.5000	4	3
A			
A	37.0000	4	4
B	34.5000	4	2
B			
B	33.7500	4	1

The GLM Procedure

Tukey's Studentized Range (HSD) Test for resp

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	0.8125
Critical Value of Studentized Range	4.89559
Minimum Significant Difference	2.2064

Means with the same letter are not significantly different.			
Tukey Grouping	**Mean**	**N**	**trt**
A	37.5000	4	3
A			
A	37.0000	4	4
B	34.5000	4	2
B			
B	33.7500	4	1

The GLM Procedure

Level of period	N	resp Mean	resp Std Dev
1	4	40.7500000	3.09569594
2	4	34.2500000	3.86221008
3	4	35.0000000	2.16024690
4	4	32.7500000	2.06155281

Level of cow	N	resp Mean	resp Std Dev
1	4	34.5000000	2.64575131
2	4	35.5000000	3.87298335
3	4	38.7500000	4.34932945
4	4	34.0000000	4.83045892

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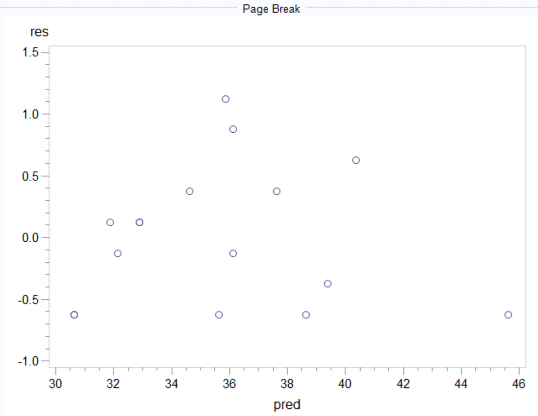

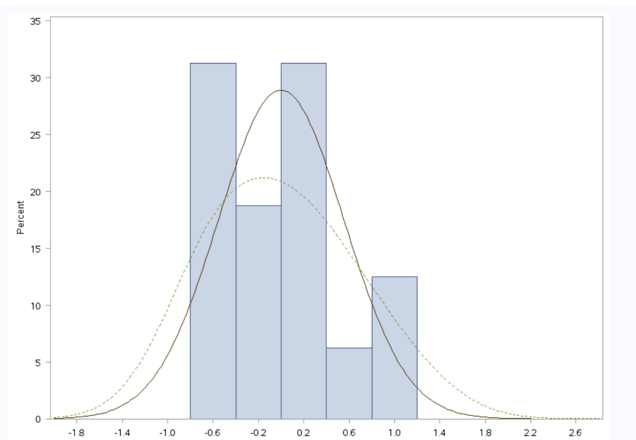

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The UNIVARIATE Procedure
Fitted Normal Distribution for res

Parameters for Normal Distribution		
Parameter	**Symbol**	**Estimate**
Mean	Mu	0
Std Dev	Sigma	0.551985

Goodness-of-Fit Tests for Normal Distribution				
Test		**Statistic**	**p Value**	
Cramer-von Mises	**W-Sq**	0.07262919	**Pr > W-Sq**	>0.250
Anderson-Darling	**A-Sq**	0.54792384	**Pr > A-Sq**	>0.250

Quantiles for Normal Distribution		
	Quantile	
Percent	**Observed**	**Estimated**
1.0	-0.62500	-1.28411
5.0	-0.62500	-0.90793
10.0	-0.62500	-0.70740
25.0	-0.62500	-0.37231
50.0	0.00000	0.00000
75.0	0.37500	0.37231
90.0	0.87500	0.70740
95.0	1.12500	0.90793
99.0	1.12500	1.28411

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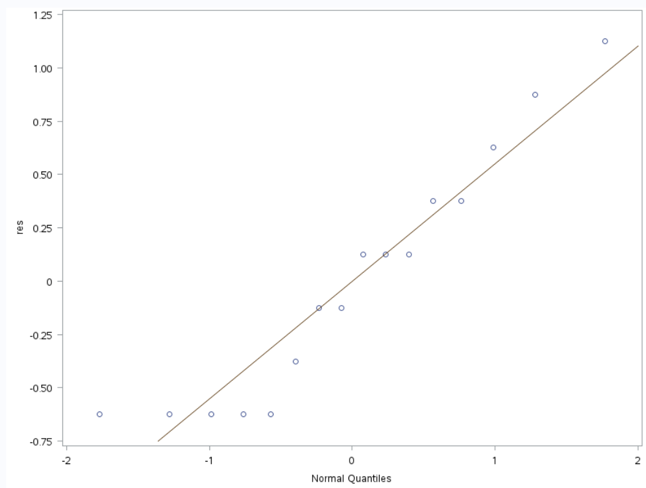
The UNIVARIATE Procedure



REPEATED LATIN SQUARES

One disadvantage of a Latin Square Design is that smaller squares yield low d.f. for error (e.g. A 3 x 3 design has only 2 d.f. for error; a 5 x 5 design has only 12 d.f. for error). To overcome this problem, one may replicate the Latin Square n times $(n > 1)$.

Case 1 Latin Squares replicated with same blocks. (Use the same col & row in each replicate)

The classical model is:

$$\underset{\substack{\uparrow \\ \text{Replication}}}{Y_{ijk\ell}} = \mu + \tau_i + \alpha_j + \beta_k + \theta_\ell + \varepsilon_{ijk}$$

i, j, k as before, $\ell = 1, 2, \ldots, n$, where θ_ℓ denote the effect of the ℓ^{th} square (which is also a block effect).

$$SS_{\text{Treatment}} = \sum_{i=1}^{\rho} n\rho \ \bar{Y}_{i\bullet\bullet}^2 \quad - N \ \bar{Y}_{\bullet\bullet\bullet}^2 \quad \text{where } N = n\rho^2.$$

$$SS_{\text{Rows}} = \sum_{j=1}^{\rho} n\rho \ \bar{Y}^2_{\bullet j \bullet\bullet} - N \ \bar{Y}^2_{\bullet\bullet\bullet\bullet}$$

$$SS_{\text{Columns}} = \sum_{k=1}^{\rho} n\rho \ \bar{Y}^2_{\bullet\bullet k \bullet} - N \ \bar{Y}^2_{\bullet\bullet\bullet\bullet}$$

$$SS_{\text{Replication}} = \sum_{\ell=1}^{n} \rho^2 \ \bar{Y}^2_{\bullet\bullet\bullet \ell} - N \ \bar{Y}^2_{\bullet\bullet\bullet\bullet}$$

$$\left(= SS_{\text{Squares}}\right)$$

$$SS_{\text{Total}} = \sum_i \sum_j \sum_k \sum_\ell Y^2_{ijk\ell} - N \ \bar{Y}^2_{\bullet\bullet\bullet\bullet}$$

$$SS_{\text{Error}} = SS_{\text{Total}} - SS_{\text{Rows}} - SS_{\text{Columns}} - SS_{\text{Rep.}}$$

ANOVA

Source	d.f.	SS	MS	F
Treatments	$\rho - 1$	$SS_{\text{Treatment}}$	$MS_{\text{Treatment}}$	$F_o = \dfrac{MS_{\text{Trt}}}{MS_{\text{Error}}}$
Rows	$\rho - 1$	SS_{Rows}	MS_{Rows}	
Columns	$\rho - 1$	SS_{Columns}	MS_{Columns}	
Replicates	$n - 1$	$SS_{\text{Replicates}}$	$MS_{\text{Replicates}}$	
Error	$(\rho-1)\left[n(\rho+1)-3\right]$	SS_{Error}	MS_{Error}	
Total	$n\rho^2 - 1$	SS_{Total}		

Example 3 (Case 1): Same rows and same columns in additional squares

	1	2	3		response	
1	A	B	C	7	8	9
2	B	C	A	4	5	6
3	C	A	B	6	3	4

	1	2	3			
1	C	B	A	8	4	7
2	B	A	C	6	3	6
3	A	C	B	5	8	7

	1	2	3			
1	B	A	C	9	6	8
2	A	C	B	5	7	6
3	C	B	A	9	3	7

```
data case1;
input rep row col trt resp;
datalines;
1 1 1 1 7
1 1 2 2 8
1 1 3 3 9
1 2 1 2 4
1 2 2 3 5
1 2 3 1 6
1 3 1 3 6
1 3 2 1 3
1 3 3 2 4

2 1 1 3 8
2 1 2 2 4
2 1 3 1 7
2 2 1 2 6
2 2 2 1 3
2 2 3 3 6
2 3 1 1 5
2 3 3 2 7

3 1 1 2 9
3 1 2 1 6
3 1 3 3 8
3 2 1 1 5
3 2 2 3 7
3 2 3 2 6
3 3 1 3 9
```

```
3 3 2 2 3
3 3 3 1 7
;
proc glm data=case1;
class rep row col trt;
model resp=rep row col trt;
run;
quit;
```

The GLM Procedure

Class Level Information		
Class	Levels	Values
rep	3	1 2 3
row	3	1 2 3
col	3	1 2 3
trt	3	1 2 3

Number of Observations Read	26
Number of Observations Used	26

The GLM Procedure

Dependent Variable: resp

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	57.64245014	7.20530627	4.34	0.0053
Error	17	28.20370370	1.65904139		
Corrected Total	25	85.84615385			

R-Square	Coeff Var	Root MSE	resp Mean
0.671462	21.19556	1.288038	6.076923

Source	DF	Type I SS	Mean Square	F Value	Pr > F
rep	2	4.79059829	2.39529915	1.44	0.2636
row	2	22.24747475	11.12373737	6.70	0.0071
col	2	19.40252525	9.70126263	5.85	0.0117
trt	2	11.20185185	5.60092593	3.38	0.0583

Source	DF	Type III SS	Mean Square	F Value	Pr > F
rep	2	5.33518519	2.66759259	1.61	0.2293
row	2	22.44629630	11.22314815	6.76	0.0069
col	2	16.41851852	8.20925926	4.95	0.0202
trt	2	11.20185185	5.60092593	3.38	0.0583

Case 2 Replicated by introducing additional versions of one blocking factor but using the same blocks for the other blocking factor. (use different rows but same columns in each replicate)

$w.\ell.o.g.$ assume that columns blocks are repeated but row blocks have additional versions.

The classical model is:

$$Y_{ijk\ell} = \mu + \tau_i + \alpha_{j\ell} + \beta_k + \theta_\ell + \varepsilon_{jk\ell}$$
$$\ell = 1, 2, \ldots, n$$

$$SS_{\text{Total}} = \sum_i \sum_j \sum_k \sum_\ell Y_{ijk\ell}^2 - n\ \bar{Y}_{\bullet\bullet\bullet\bullet}^2$$

where $N = n\rho^2$

$$SS_{\text{Treatment}} = \sum_{i=1}^{\rho} n\rho\ \bar{Y}_{i\bullet\bullet\bullet}^2 - N\ \bar{Y}_{\bullet\bullet\bullet\bullet}^2$$

$$SS_{\text{Rows}} = \sum_{i=1}^{\rho}\sum_{\ell=1}^{n} \rho Y_{\bullet j\bullet\ell}^2 - \sum_{\ell=1}^{n}\rho^2\bar{Y}_{\bullet\bullet\bullet\ell}^2$$

$$SS_{\text{Columns}} = \sum_{k=1}^{n} n\rho\bar{Y}_{\bullet\bullet k\bullet}^2 - N\ \bar{Y}_{\bullet\bullet\bullet\bullet}^2$$

$$SS_{\text{Replicates}} = \sum_{\ell-1}^{n}\rho^2\bar{Y}_{\bullet\bullet\bullet\ell}^2 - N\ \bar{Y}_{\bullet\bullet\bullet\bullet}^2$$

$$SS_{\text{Error}} = SS_{\text{Total}} - SS_{\text{Trt}} - SS_{\text{Rows}} - SS_{\text{Col}} - SS_{\text{Rep}}$$

ANOVA

Source	d.f.	SS	MS	F
Treatments	$\rho-1$	$SS_{\text{Treatment}}$	$MS_{\text{Treatment}}$	$F_o = \frac{MS_{\text{Trt}}}{MS_{\text{Error}}}$
Rows	$n(\rho-1)$	SS_{Rows}	MS_{Rows}	
Columns	$\rho-1$	SS_{Columns}	MS_{Columns}	
Replicates	$n-1$	$SS_{\text{Replicates}}$	$MS_{\text{Replicates}}$	
Error	$n(\rho-1)(\rho-1)$	SS_{Error}	MS_{Error}	
Total	$n\rho^2-1$	SS_{Total}		

Example (Case 2): New (different) rows and same columns

	1	2	3	response		
1	A	B	C	7	8	9
2	B	C	A	4	5	6
3	C	A	B	6	3	4

	1	2	3			
4	C	B	A	8	4	7
5	B	A	C	6	3	6
6	A	C	B	5	8	7

	1	2	3			
7	B	A	C	9	6	8
8	A	C	B	5	7	6
9	C	B	A	9	3	7

Case 3 Latin Squares replicated by introducing additional versions of both blocking variables. (use different row & col in each replicate)

The model is:

$$Y_{ijk\ell} = \mu + \tau_i + \alpha_{j\ell} + \beta_{k\ell} + \theta_\ell + \varepsilon_{ijk\ell}$$

SS_{Total} computed as before

$SS_{\text{Treatment}}$ computed as before

SS_{Rows} computed as before

$$SS_{\text{Columns}} = \sum_{k=1}^{\rho}\sum_{\ell=1}^{n} \rho \; \bar{Y}_{\bullet\bullet k\ell}^2 - \sum_{\ell=1}^{n} \rho^2 \bar{Y}_{\bullet\bullet\bullet\ell}^2$$

$$SS_{\text{Replicates}} = \sum_{\ell=1}^{n} \rho^2 \bar{Y}_{\bullet\bullet\bullet\ell}^2 - N \; \bar{Y}_{\bullet\bullet\bullet}^2 \text{ where } N = n\rho^2$$

SS_{Error} obtained by subtraction.

The ANOVA table as in Case 2, except SS_{Column} computed differently and has $n(\rho-1)$ d.f. and SS_{Error} has $(\rho-1)\left[n(\rho-1)-1\right]$ d.f.

SAS can be used to analyze repeated Latin Squares as follows:

Example 4 (Case 3): different rows and new columns

	1	2	3	response		
1	A	B	C	7	8	9
2	B	C	A	4	5	6
3	C	A	B	6	3	4

	4	5	6			
4	C	B	A	8	4	7
5	B	A	C	6	3	6
6	A	C	B	5	8	7

	7	8	9			
7	B	A	C	9	6	8
8	A	C	B	5	7	6
9	C	B	A	9	3	7

Case 1 (rows and columns crossed w/reps)

```
proc glm data=yourdata;
class rep row col treatment;
model y = rep row col treatment;
run;
```

Case 2 (row nested, columns crossed w/reps)

```
proc glm data=yourdata;
class rep row col treatment;
model y = rep row(rep) col treatment;
run;
```

Case 3 (rows and columns nested w/reps)

```
proc glm data=yourdata;
class rep row col treatment;
model y = rep row(rep) col(rep) treatment;
run;
```

Note: ROW (REP) gives SS due to rows within replication. (Similarly for COLUMN (REP)).

GRAECO-LATIN SQUARES

Def.ⁿ Let a $\rho x \rho$ Latin square consists of Latin letters and another $\rho x \rho$ Latin square consist of Greek letters. Suppose they have the property that when superimposed, each Latin letter coincides exactly once with each Greek letter. Then the two squares are said to be orthogonal.

A collection of n pxp Latin squares are said to be a mutually orthogonal set of Latin squares if each letter in one square coincides with each combination of the letters in the other squares exactly once.

Def.ⁿ A pair $\rho x \rho$ Latin, Greek, Greek squares that are orthogonal form a Graeco-Latin square.

Using a Graeco-Latin square, one may block in a 3rd direction or analyze a 2nd treatment.

An example of a Graeco-Latin Square

$$
\begin{array}{ccccc}
A\alpha & B\beta & C\partial & D\delta & E\varepsilon \\
B\partial & C\delta & D\varepsilon & E\alpha & A\beta \\
C\varepsilon & D\alpha & E\beta & A\partial & B\delta \\
D\beta & E\partial & A\delta & B\varepsilon & C\alpha \\
E\delta & A\varepsilon & B\alpha & C\beta & D\partial
\end{array}
$$

Note: When more than two orthogonal Latin squares are superimposed, we obtain a Hyper-Graeco-Latin square.

Analysis of Graeco-:Latin Squares

Model is:

$$Y_{ijk\ell} = \mu + \underset{\substack{\uparrow \\ \text{Latin} \\ \text{TRT}}}{\tau_i} + \underset{\substack{\uparrow \\ \text{Greek} \\ \text{Letter}}}{w_j} + \underset{\substack{\uparrow \\ \text{Row}}}{\alpha_k} + \underset{\substack{\uparrow \\ \text{Column}}}{\beta_\ell} + \varepsilon_{ijk\ell}$$

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$$SS_{\text{Total}} = \sum_i \sum_j \sum_k \sum_\ell Y_{ijk\ell}^2 - N \ \bar{Y}_{\bullet\bullet\bullet}^2 \quad \text{where } N = \rho \ .$$

d.**f**.)

$$SS_{\text{Trt}} = SS_{\text{Latin}} = \sum_{i=1}^{\rho} \rho \bar{Y}_{i\bullet\bullet}^2 - N \ \bar{Y}_{\bullet\bullet\bullet}^2 \qquad (\rho - 1)$$

$$SS_{\text{Greek}} = \sum_{j=1}^{\rho} \rho \bar{Y}_{\bullet j \bullet}^2 - \bar{Y}_{\bullet\bullet\bullet}^2 \qquad (\rho - 1)$$

$$SS_{\text{Rows}} = \sum_{k=1}^{\rho} \rho \bar{Y}^2_{\bullet\bullet k \bullet} - \bar{Y}^2_{\bullet\bullet\bullet\bullet} \qquad (\rho - 1)$$

$$SS_{\text{Columns}} = \sum_{\ell=1}^{\rho} \rho \bar{Y}^2_{\bullet\bullet\bullet \ell} - \bar{Y}^2_{\bullet\bullet\bullet\bullet} \qquad (\rho - 1)$$

SS_{Error} obtained by subtraction $\qquad ((\rho-3)(\rho-1))$

SAS can be utilized as follows:

```
proc glm data=yourdata;
class greek row col tx;
model y = row col greek tx;
run;
```

Example 5: Graeco-Latin Square
An experiment is conducted to compare four gasoline additives by testing them on four cards with four drivers over four days. Only four runs can be conducted in each day. The response is the amount of automobile emission.
Treatment factor: gasoline additive, denoted by A, B, C, and D
Block factor 1: driver, denoted by 1,2,3,4
Block factor 2: day, denoted by 1,2,3,4
Block factor 3: car, denoted by α, β, γ, δ

	days			
drivers	1	2	3	4
1	$A\alpha = 32$	$B\beta = 25$	$C\gamma = 31$	$D\delta = 27$
2	$B\delta = 24$	$A\gamma = 36$	$D\beta = 20$	$C\alpha = 25$
3	$C\beta = 28$	$D\alpha = 30$	$A\delta = 23$	$B\gamma = 31$
4	$D\gamma = 34$	$C\delta = 35$	$B\alpha = 29$	$A\beta = 33$

Graeco-Latin Square Design Matrix:

driver	day	additive	car
1	1	A	α
1	2	B	β
1	3	C	γ
1	4	D	δ
$\vdots$	$\vdots$	$\vdots$	$\vdots$
4	1	D	γ
4	2	C	δ
4	3	B	α
4	4	A	β

SAS codes:

```
data additives;
input row col trt greek resp @@;
datalines;
1 1 1 1 32 1 2 2 2 25
1 3 3 3 31 1 4 4 4 27
2 1 2 4 24 2 2 1 3 36
2 3 4 2 20 2 4 3 1 25
3 1 3 2 28 3 2 4 1 30
3 3 1 4 23 3 4 2 3 31
4 1 4 3 34 4 2 3 4 35
4 3 2 1 29 4 4 1 2 33
;
proc glm data=additives;
class row col trt greek;
model resp=row col trt greek;
run;
```

Multiple comparisons can be carried out using similar methods.

SAS outputs:

The GLM Procedure

Class Level Information		
Class	Levels	Values
row	4	1 2 3 4
col	4	1 2 3 4
trt	4	1 2 3 4
greek	4	1 2 3 4

Number of Observations Read	16
Number of Observations Used	16

Page Break

The GLM Procedure

Dependent Variable: resp

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	12	296.7500000	24.7291667	2.83	0.2122
Error	3	26.1875000	8.7291667		
Corrected Total	15	322.9375000			

R-Square	Coeff Var	Root MSE	resp Mean
0.918908	10.20999	2.954516	28.93750

Source	DF	Type I SS	Mean Square	F Value	Pr > F
row	3	90.6875000	30.2291667	3.46	0.1674
col	3	68.1875000	22.7291667	2.60	0.2263
trt	3	36.6875000	12.2291667	1.40	0.3942
greek	3	101.1875000	33.7291667	3.86	0.1481

Source	DF	Type III SS	Mean Square	F Value	Pr > F
row	3	90.6875000	30.2291667	3.46	0.1674
col	3	68.1875000	22.7291667	2.60	0.2263
trt	3	36.6875000	12.2291667	1.40	0.3942
greek	3	101.1875000	33.7291667	3.86	0.1481

Practice:

1. Run the repeated Latin square design case 2 and 3. Interpret your result.
2. Interpret the result for Example 1-4
3. Draw conclusion for Example 5. Include the analysis of multiple comparisons.

Assignments:

1. Lew (2007) presents the data from an experiment to determine whether cultured cells respond to two drugs. The experiment was conducted using a stable cell line plated onto Petri dishes, with each experimental run involving assays of responses in three Petri dishes: one treated with drug 1, one treated with drug 2, and one untreated serving as a control. The data are shown in the table below:

	Control	Drug 1	Drug 2
Experiment 1	1147	1169	1009
Experiment 2	1273	1323	1260
Experiment 3	1216	1276	1143
Experiment 4	1046	1240	1099
Experiment 5	1108	1432	1385
Experiment 6	1265	1562	1164

(a) Analyze the data as if it came from a completely randomized design (CRD). Write down the classical effect model for CRD and the five steps for hypothesis testing. Is there a significant difference between the treatment groups?
(b) Analyze the data as complete randomized block design (CRBD). What is the treatment? What is the blocking factor? Write down the classical effect model for CRD and the five steps for hypothesis testing. Is there a significant difference between the treatment groups?
(c) Is there any difference in the results you obtain in (a) and (b)? If so, explain what may be the cause of the difference in the results and which method would you recommend?

2. Le Riche and Csima (1964) evaluated four hypnotic drugs and a placebo to determine their effect on quality of sleep in elderly patients. The treatment levels were labeled (A=Placebo, E=Ethchlorvynol, C=Glutethimide, D=Chloral hydrate and E=Secobarbitol sodium). Elderly patients were given one of the capsules for five nights in succession and their quality of sleep was rated by a trained nurse on a four-point scale (0=poor to 3=excellent) each night. An average score was calculated for each patient over the five nights in a week. Each patient received all five treatments in successive weeks. The design and the response (mean quality of sleep rating) are shown in the table below:

	Week									
Patient	1		2		3		4		5	
1	B	2.92	E	2.43	A	2.19	C	2.71	D	2.71
2	D	2.86	A	1.64	E	3.02	B	3.03	C	3.03
3	E	1.97	B	2.5	C	2.47	D	2.65	A	1.89
4	A	1.99	C	2.39	D	2.37	E	2.33	B	2.71
5	C	2.64	D	2.31	B	2.44	A	1.89	E	2.78

(a) What are the nuisance factors in this problem? What is the appropriate model for this data?
(b) Write down the classical effect model for this design and determine if there are any significant differences among the treatments.

(c) Use an appropriate method to determine if there is a significant difference between the placebo and other four drugs?
(d) Use an appropriate method to determine which drug/drugs has/have the highest rating?
(e) Use residual plots to check the assumption for the model you fit.

3. A manufacturing firm investigated the breaking strengths of components made from raw materials purchased from 4 supplies (A, B, C, D). Data was collected from 2 replicates of a 4X4 Latin square design. The blocking factors were days and operators.

(a) The same four operators were used in both replicates. Each replicate was also run on the same four days with replicated values taken during the morning and afternoons of these four days. Write down the statistical model for this data. Is there any significant difference among the different supplies?

Replicate 1	Days 1	Days 2	Days 3	Days 4
Operator 1	B 810	C 1080	A 700	D 910
2	C 1100	D 880	B 780	A 600
3	D 840	A 540	C 1055	B 830
4	A 650	B 740	D 1025	C 900

Replicate 2	Days 1	Days 2	Days 3	Days 4
Operator 1	D 840	C 1050	A 775	B 805
2	A 670	D 930	B 720	C 1035
3	C 980	B 700	D 810	A 610
4	B 860	A 730	C 970	D 900

(b) Eight operators were used with four operators randomly assigned to each replicate. The two replicates were run over 8 days with the first 4 days assigned to replicate 1 and the second four days assigned to replicate 2. Write down the statistical model for this data. Is there any significant difference among the different supplies?

Replicate 1	Days 1	Days 2	Days 3	Days 4
Operator 1	B 810	C 1080	A 700	D 910
2	C 1100	D 880	B 780	A 600
3	D 840	A 540	C 1055	B 830
4	A 650	B 740	D 1025	C 900

Replicate 2	Days 1	Days 2	Days 3	Days 4
Operator 5	D 840	C 1050	A 775	B 805
6	A 670	D 930	B 720	C 1035
7	C 980	B 700	D 810	A 610
8	B 860	A 730	C 970	D 900