HANDOUT # 12 -
Split-Plot, Repeated Measures and Crossover Designs

I. Split-Plot Designs

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b. Example

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I. Split Plot Designs

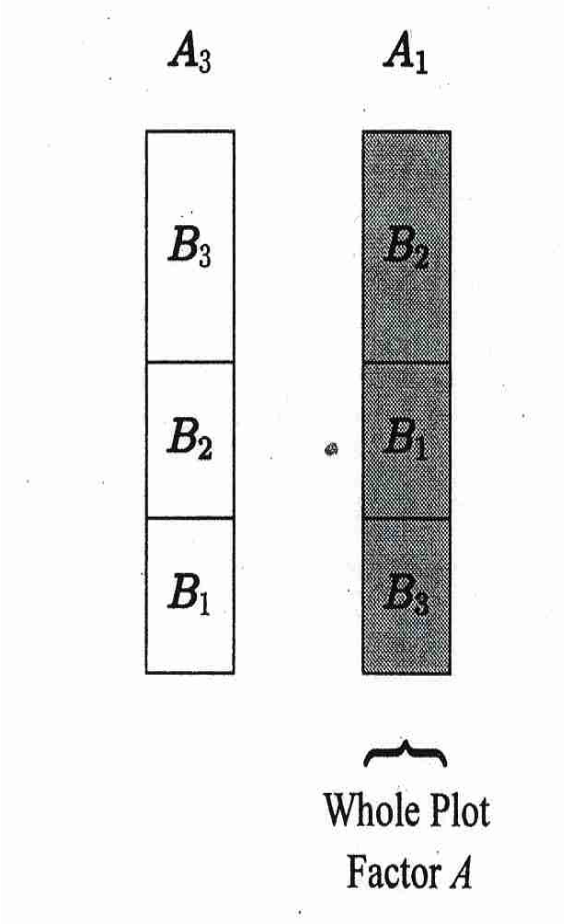
In some factorial experiments it may be impossible to completely randomize all the treatments to the EU's. This often results in a generalization of the factorial design called the **Split Plot Design**.

For example, consider a paper manufacturer who is interested in three different pulp preparation methods and four different cooking temperatures for the pulp. The process chemist wants to study the effect of pulp preparation and temperature on the tensile strength of the paper. Each replicate of a factorial experiment requires 12 observations, and the experimenter has decided to run three replicates. However, the pilot plant is only capable of making 12 runs per day, so the experimenter decides to run one replicate on each of the three days, hence days are blocks. A further complication is that it is very expensive to make individual batches of the pulp by a given preparation method. Therefore, a single batch of pulp is produced by each of the three methods each day. Each of the three batches are divided into four samples that are tested at the four temperatures. The randomization scheme is given below:

Day 1			Day 2			Day 3		
PM1	PM2	PM3	PM2	PM1	PM3	PM3	PM1	PM2
$220°F$	$275°F$	$250°F$	$220°F$	$275°F$	$220°F$	$250°F$	$225°F$	$250°F$
$225°F$	$250°F$	$225°F$	$275°F$	$220°F$	$225°F$	$220°F$	$250°F$	$220°F$
$250°F$	$225°F$	$275°F$	$225°F$	$225°F$	$250°F$	$275°F$	$275°F$	$225°F$
$275°F$	$220°F$	$220°F$	$250°F$	$250°F$	$275°F$	$225°F$	$220°F$	$275°F$

Initially, this design might have been considered as a RCBD with a 3x4 factorial treatment structure, i.e., Blocks (3-Days), 3 levels of Preparation Method and 4 levels of Temperature. In order for this to be the correct design, it would be necessary that the order of experimentation within each Block (Day) be completely randomized. That is, for each Day, randomly select a Treatment combination (a Preparation Method and a Temperature) and then obtain a tensile strength measurement. Repeat this procedure until all 12 treatments were observed in each of the three days. However, this was not how the experiment was conducted. The chemist made up a batch of pulp using a randomly selected method of preparation, divided the batch into four samples, and then randomly assigned a temperature to the samples. Because of the economics of preparing batches of pulp and the size of the batches, this was the only feasible manner to conduct the experiment. A randomized complete block design would require 36 batches of pulp (12 per day), which is not economically feasible. The split-plot design requires only 9 batches (3 batches per day). Thus, the split-plot design resulted in considerable experimental efficiency. However, as we will see in the AOV table, there is a cost in terms of statistical efficiency.

A split-plot design is essentially two experiments superimposed on each other. One experiment has the whole plot factor applied to the larger experimental units (or it is a factor whose levels are hard or expensive to change) and the other experiment has the subplot factor applied to the smaller experimental units (or it is a factor whose levels are easier or less costly to change). That is, the Whole Plot factor's levels are assigned to the whole plots in a randomized complete block design. Each whole plot is then subdivided into b subunits and the levels of subplot factor are assigned to the subunits as in a completely randomized design.



In our example, the whole plot EU is the batch and the split plot EU is the sample from the batch. The model for a randomized complete block split plot design with two fixed levels factors having r blocks, a levels of Factor A and b levels of Factor B is given here:

$$Y_{ijk} = \mu + c_k + \tau_i + d_{ik} + \gamma_j + (\tau\gamma)_{ij} + e_{ijk}, \quad \text{with}$$

1. τ_i representing the i th Whole Plot Treatment effect, $i = 1, \ldots, a$ with $\tau_a = 0$
2. γ_j representing the j th Split Plot Treatment effect, $j = 1, \ldots, b$ with $\gamma_b = 0$
3. $(\tau\gamma)_{ij}$ representing the interaction between Whole Plot Treatment and Split Plot Treatment,
4. with $(\tau\gamma)_{ib} = 0$ and $(\tau\gamma)_{aj} = 0$
5. c_k representing the block effect, $k = 1, \ldots, r$ with c_k's iid $N(0, \sigma_C^2)$ r.v.'s
6. d_{ik} representing the whole plot error term, with d_{ik}'s iid $N(0, \sigma_D^2)$ r.v.'s
7. e_{ijk} representing the split plot error term, with e_{ijk}'s iid $N(0, \sigma_e^2)$ r.v.'s
8. c_k, d_{ik}, and e_{ijk} all independent.

The AOV table for this design is given here:

ANOVA Table for RCB Split-Plot Design

Source	DF	Expected Mean Squares	F
Block	r-1	$\sigma_e^2 + b\sigma_d^2 + ab\sigma_c^2$	
A	a-1	$\sigma_e^2 + b\sigma_d^2 + brQ_A$	MS_A/MSE_{WP}
Block*A	(r-1)(a-1)	$\sigma_e^2 + b\sigma_d^2$	
B	b-1	$\sigma_e^2 + arQ_B$	MS_B/MSE_{SP}
A*B	(a-1)(b-1)	$\sigma_e^2 + rQ_{A*B}$	MS_{A*B}/MSE_{SP}
Error	a(r-1)(b-1)	σ_e^2	

From the AOV table we can observe that the degrees of freedom for testing the main effect of the Whole Plot Factor, Factor A are df = a-1,(r-1)(a-1). In a RCBD, the degrees of freedom for testing the main effect of the Whole Plot Factor, Factor A are df = a-1,(r-1)(ab-1). Thus, in order to run the experiment more economically or conveniently we have a considerable decrease in the degrees of freedom for testing the main effect of the Whole Plot Factor, Factor A. Hence, there is a potential loss in power in using the split plot design in comparison to using a crossed design in a RCBD with the same size EU's for all treatments.

Randomized Complete Block Split Plot Design Example

The experiment involves three Varieties of alfalfa (Ladak, Ranger, Cossack) and four different cutting dates (A=none, B=Sept. 1, C=Sept. 20, D=Oct. 7). There were 6 fields involved in the experiment which will be treated as blocks. Each field was divided into 3 plots and the varieties of alfalfa were randomly assigned to the plots. Each plot was then divided into 4 subplots and the Cutting Dates were randomly assigned to the subplots within each plot. The cutting date represents the date on which the 3rd cutting of the alfalfa field was made in the previous year. Thus the whole plots were the EU's for Varieties and the subplots were the EU's for the Cutting Dates.
The randomization for the plots and subplots is given below:

	Block 1			Block 2			···	Block 6		
	PLOT1	PLOT2	PLOT3	PLOT1	PLOT2	PLOT3	···	PLOT1	PLOT2	PLOT3
	Ladak	Cossack	Ranger	Cossack	Ladak	Ranger	···	Cossack	Ladak	Ranger
SUB1	NONE	S1	S20	S1	NONE	07	···	S20	O7	NONE
SUB2	07	S20	070	S20	O7	S1	···	S1	S20	07
SUB3	S20	NONE	NONE	01	S1	S20	···	O7	S1	S1
SUB4	S1	07	S1	NONE	S20	NONE	···	NONE	NONE	S20

The total yield (Tons/acre) for three cuttings in the current year are given in the following table. Do the yields vary across the factors cutting date and varieties?

		Blocks						TrT Mean
Variety	Date	1	2	3	4	5	6	$\bar{Y}_{ij.}$
Ladak	None	2.17	1.88	1.62	2.34	1.58	1.66	1.8750
	S1	1.58	1.26	1.22	1.59	1.25	0.94	1.3067
	S20	2.29	1.60	1.67	1.91	1.39	1.12	1.6633
	O7	2.23	2.01	1.82	2.10	1.66	1.10	1.8200
Cossack	None	2.33	2.01	1.70	1.78	1.42	1.35	1.7650
	S1	1.38	1.30	1.85	1.09	1.13	1.06	1.3017
	S20	1.86	1.70	1.81	1.54	1.67	0.88	1.5767
	O7	2.27	1.81	2.01	1.40	1.31	1.06	1.6433
Ranger	None	1.75	1.95	2.13	1.78	1.31	1.30	1.7033
	S1	1.52	1.47	1.80	1.37	1.01	1.31	1.4133
	S20	1.55	1.61	1.82	1.56	1.23	1.13	1.4833
	O7	1.56	1.72	1.99	1.55	1.51	1.33	1.6100

MODEL: $Y_{ijk} = \mu + c_k + \tau_i + d_{ik} + \gamma_j + (\tau\gamma)_{ij} + e_{ijk}$,

with τ_i representing the i th Variety effect, $i = 1, \ldots, a = 3$
γ_j representing the j th Date effect, $j = 1, \ldots, b = 4$
$(\tau\gamma)_{ij}$ representing the interaction between Variety and Date,
c_k representing the block effect, $k = 1, \ldots, r = 6$
d_{ik} representing the whole plot error term,
e_{ijk} representing the split plot error term.

Whole Block Analysis

Block*Variety MEANS

		Block						Variety Means
		1	2	3	4	5	6	$\bar{Y}_{i..}$
Variety	L	2.0675	1.6875	1.5825	1.9850	1.4700	1.2050	1.6663
	C	1.9600	1.7050	1.8425	1.4525	1.3845	1.0875	1.5717
	R	1.5950	1.6875	1.9350	1.5650	1.2650	1.2675	1.5525
Block Means:	$\bar{Y}_{..k}$	1.8742	1.6933	1.7867	1.6675	1.3725	1.1867	1.5968

$$SS_{TOT} = \sum_i^a \sum_j^b \sum_k^r (Y_{ijk} - \bar{Y}_{...})^2 = \sum_i^3 \sum_j^4 \sum_k^6 (Y_{ijk} - 1.5968)^2$$
$$= (2.17 - 1.5968)^2 + \cdots + (1.33 - 1.5968)^2 = 9.1218$$

$$SS_{Block} = ab \sum_{k=1}^r (\bar{Y}_{..k} - \bar{Y}_{...})^2 = 12[(1.8742 - 1.5968)^2 + \cdots + (1.1867 - 1.5968)^2] = 4.1499$$

$$SS_{Variety} = rb \sum_{i=1}^a (\bar{Y}_{i..} - \bar{Y}_{...})^2$$
$$= 24[(1.6663 - 1.5968)^2 + (1.5717 - 1.5968)^2 + (1.5525 - 1.5968)^2] = 0.1781$$

WP Error: $SSE_{WP} = SS_{B*V} = b \sum_{i=1}^a \sum_{k=1}^r (\bar{Y}_{i.k} - \bar{Y}_{...})^2 - SS_{Var} - SS_{Block}$
$$= 4[(2.0675 - 1.5968)^2 + \cdots + (1.2675 - 1.5968)^2] - SS_{Var} - SS_{Block}$$
$$= 5.6902 - 4.1499 - 0.1781 = 1.3622$$

$$SSTOT_{WP} = SS_{Block} + SS_{Variety} + SSE_{WP} = 5.6902$$

Split Plot Analysis

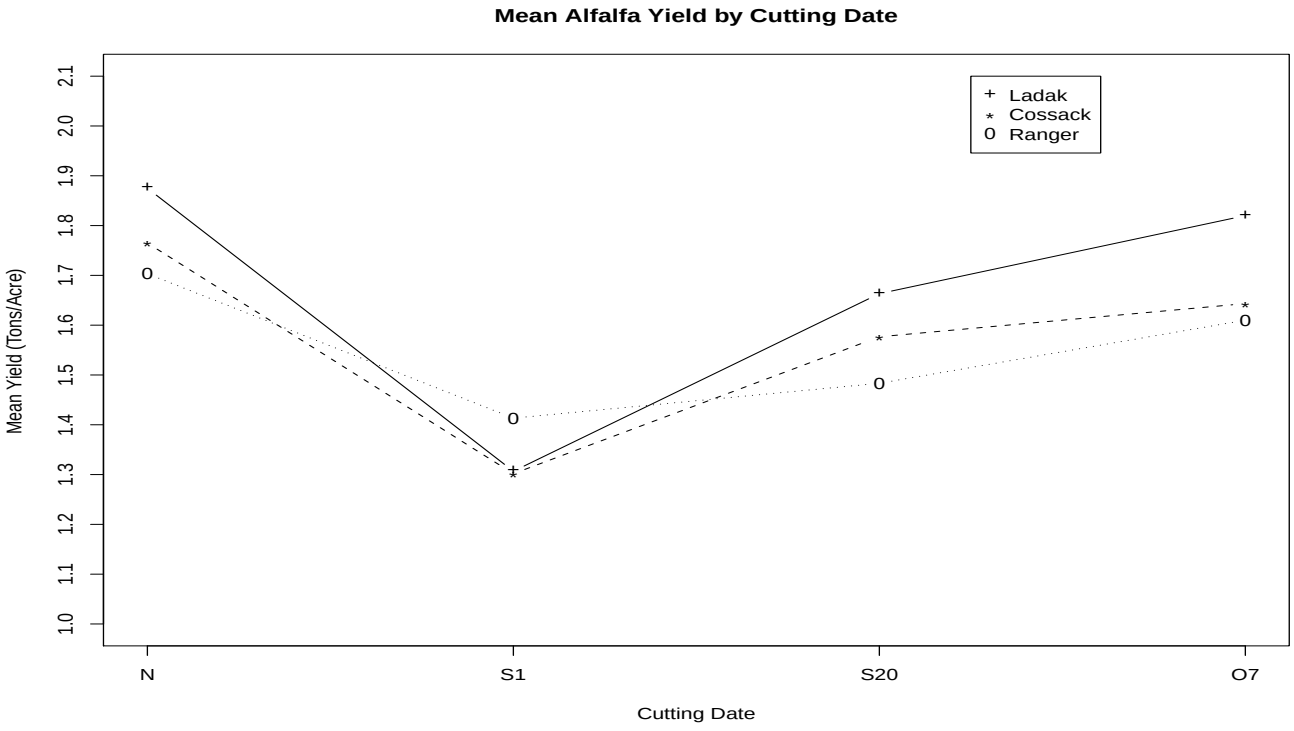
Date*Variety MEANS

		DATE				Variety Means
		None	S1	S20	O7	$\bar{Y}_{i..}$
Variety	L	1.8750	1.3067	1.6633	1.8200	1.6663
	C	1.7650	1.3017	1.5767	1.6433	1.5717
	R	1.7033	1.4133	1.4833	1.6100	1.5525
Date Means:	$\bar{Y}_{.j.}$	1.7811	1.3406	1.5744	1.6911	1.5968

$$SS_{Date} = ra\sum_{j=1}^{b}(\bar{Y}_{.j.} - \bar{Y}_{...})^2$$
$$= 18[(1.7811 - 1.5968)^2 + \cdots + (1.6911 - 1.5968)^2] = 1.9625$$

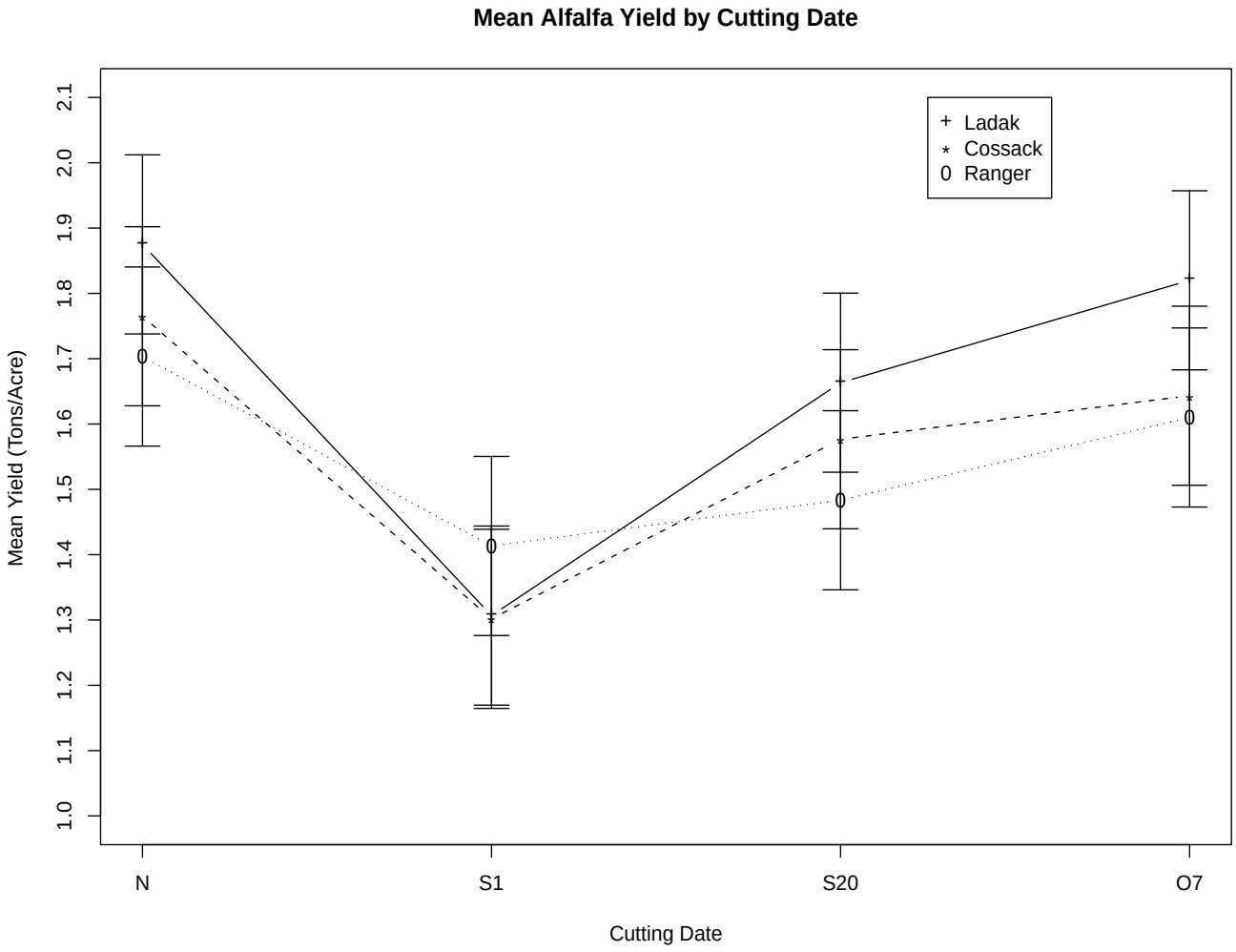
$$SS_{D*V} = r\sum_{i=1}^{a}\sum_{j=1}^{b}(\bar{Y}_{ij.} - \bar{Y}_{...})^2 - SS_{Var} - SS_{Date}$$
$$= 6[(1.8750 - 1.5968)^2 + \cdots + (1.6100 - 1.5968)^2] - SS_{Variety} - SS_{Date}$$
$$= 2.3511 - 0.1781 - 1.9625 = 0.2105$$

Split Plot Error: $SSE_{SP} = SS_{TOT} - SSTOT_{WP} - SS_{Date} - SS_{D*V}$
$= 9.1218 - 5.6902 - 1.9625 - 0.2105 = 1.2586$



ANOVA Table

Source	DF	MS	Expected Mean Squares	F	P-value
Block	5	0.8300	$\sigma_e^2 + 4\sigma_d^2 + 12\sigma_c^2$		
Variety(V)	2	0.0890	$\sigma_e^2 + 4\sigma_d^2 + 24Q_V$	.65	.5412
Block*V	10	0.1362	$\sigma_e^2 + 4\sigma_d^2$		
Date(D)	3	0.6542	$\sigma_e^2 + 18Q_D$	23.4	$3.x10^{-9}$
D*V	6	0.0351	$\sigma_e^2 + 6Q_{D*V}$	1.25	.2973
Error	45	0.0280	σ_e^2		



$$\text{Model:} \quad Y_{ijk} = \mu + c_k + \tau_i + d_{ik} + \gamma_j + (\tau\gamma)_{ij} + e_{ijk},$$

$$i = 1, \ldots, a = 3; \quad j = 1, \ldots, b = 4; \quad k = 1, \ldots, r = 6$$

Comparisons of Treatment Means: (Formulas are valid only for Balanced Designs)

Variance Components From REML:

$\hat{\sigma}_c^2 = .05781 \quad \hat{\sigma}_d^2 = .02707 \quad \hat{\sigma}_e^2 = .02797$

Variance Components From AOV:

$\hat{\sigma}_c^2 = \frac{MS_B - MS_{B*V}}{(3)(4)} = .05782 \quad \hat{\sigma}_d^2 = \frac{MS_{B*V} - MSE}{4} = .02705 \quad \hat{\sigma}_e^2 = MSE.02880$

Let μ_{ij} be the mean yield of Variety i at Date j.

1. To compare Variety 1 to Variety 2 (Whole Plot Treatment Comparisons): $\mu_{1.} - \mu_{2.}$

   $\bar{Y}_{1..} - \bar{Y}_{2..} = [\mu + \bar{c}_. + \tau_1 + \bar{d}_{1.} + \bar{\gamma}_. + \overline{(\tau\gamma)}_{1.} + \bar{e}_{1..}] - [\mu + \bar{c}_. + \tau_2 + \bar{d}_{2.} + \bar{\gamma}_. + \overline{(\tau\gamma)}_{2.} + \bar{e}_{2..}] \Rightarrow$

   $\text{VAR}(\bar{Y}_{1..} - \bar{Y}_{2..}) = Var(\bar{d}_{1.} - \bar{d}_{2.}) + Var(\bar{e}_{1..} - \bar{e}_{2..}) = \frac{2}{rb}(b\sigma_d^2 + \sigma_e^2) = \frac{2}{rb}(EMS_{BL*V}) \quad \Rightarrow$

   $\widehat{SE}(\bar{Y}_{1..} - \bar{Y}_{2..}) = \sqrt{\frac{2MS_{BL*V}}{rb}} = \sqrt{\frac{(2)(.1362)}{(6)(4)}} = .1066$ with $df = df_{BL*V} = 10$

2. To compare Date 1 to Date 2 (Split Plot Treatment Comparisons): $\mu_{.1} - \mu_{.2}$

   $\bar{Y}_{.1.} - \bar{Y}_{.2.} = [\mu + \bar{c}_. + \bar{\tau}_. + \bar{d}_{..} + \gamma_1 + \overline{(\tau\gamma)}_{.1} + \bar{e}_{.1.}] - [\mu + \bar{c}_. + \bar{\tau}_. + \bar{d}_{..} + \gamma_1 + \overline{(\tau\gamma)}_{.2} + \bar{e}_{.2.}] \Rightarrow$

   $\text{VAR}(\bar{Y}_{.1.} - \bar{Y}_{.2.}) = Var(\bar{e}_{.1.} - \bar{e}_{.2.}) = \frac{2\sigma_e^2}{ra} = \frac{2EMSE}{ra} \quad \Rightarrow$

   $\widehat{SE}(\bar{Y}_{1..} - \bar{Y}_{2..}) = \sqrt{\frac{2MSE}{ra}} = \sqrt{\frac{2(.0280)}{18}} = .0577$, with $df = df_E = 45$.

3. To compare Date 1 to Date 2 for Variety 1 : $\mu_{11} - \mu_{12}$

   $\bar{Y}_{11.} - \bar{Y}_{12.} = [\mu + \bar{c}_. + \bar{\tau}_1 + \bar{d}_{1.} + \gamma_1 + \overline{(\tau\gamma)}_{11} + \bar{e}_{11.}] - [\mu + \bar{c}_. + \bar{\tau}_1 + \bar{d}_{1.} + \gamma_2 + \overline{(\tau\gamma)}_{12} + \bar{e}_{12.}] \Rightarrow$

   $VAR(\bar{Y}_{11.} - \bar{Y}_{12.}) = Var(\bar{e}_{11.} - \bar{e}_{12.}) = \frac{2\sigma_e^2}{r} = \frac{2EMSE}{r} \quad \Rightarrow$

   $\widehat{SE}(\bar{Y}_{11.} - \bar{Y}_{12.}) = \sqrt{\frac{2(.0280)}{6}} = .0966$, with df=45.

$$i = 1, \ldots, a = 3; \quad j = 1, \ldots, b = 4; \quad k = 1, \ldots, r = 6$$

4. To compare Variety 1 to Variety 2 for Date 1: $\mu_{11} - \mu_{21}$

   $\bar{Y}_{11.} - \bar{Y}_{21.} = [\mu + \bar{c}_{.} + \bar{\tau}_1 + \bar{d}_{1.} + \gamma_1 + \overline{(\tau\gamma)}_{11} + \bar{e}_{11.}] - [\mu + \bar{c}_{.} + \bar{\tau}_2 + \bar{d}_{2.} + \gamma_1 + \overline{(\tau\gamma)}_{21} + \bar{e}_{21.}] \Rightarrow$

   $VAR(\bar{Y}_{11.} - \bar{Y}_{21.}) = Var(\bar{d}_{1.} - \bar{d}_{2.}) + Var(\bar{e}_{11.} - \bar{e}_{21.}) = \frac{2}{r}(\sigma_d^2 + \sigma_e^2) = \frac{2}{r}(\frac{EMS_{BL*V} + (b-1)EMSE}{b}) \Rightarrow$

   $\widehat{SE}(\bar{Y}_{11.} - \bar{Y}_{12.}) = \sqrt{\frac{2}{6}\left(\frac{MS_{BL*V} + (4-1)MSE}{4}\right)} = .1355$, with $df \approx \frac{(MS_{BL*V} + 3MSE)^2}{\frac{MS_{BL*V}^2}{10} + \frac{9(MSE)^2}{45}} = 24.1$

5. To compare Variety 1 at Date 2 to Variety 2 at Date 3: $\mu_{12} - \mu_{23}$

   $\bar{Y}_{12.} - \bar{Y}_{23.} == [\mu + \bar{c}_{.} + \bar{\tau}_1 + \bar{d}_{1.} + \gamma_2 + \overline{(\tau\gamma)}_{12} + \bar{e}_{12.}] - [\mu + \bar{c}_{.} + \bar{\tau}_2 + \bar{d}_{2.} + \gamma_3 + \overline{(\tau\gamma)}_{23} + \bar{e}_{23.}] \Rightarrow$

   $VAR(\bar{Y}_{12.} - \bar{Y}_{23.}) = Var(\bar{d}_{1.} - \bar{d}_{2.}) + Var(\bar{e}_{12.} - \bar{e}_{23.}) = \frac{2}{r}(\sigma_d^2 + \sigma_e^2) \Rightarrow$

   $\widehat{SE}(\bar{Y}_{12.} - \bar{Y}_{23.}) = .1355$, with $df \approx 24.1$

6. To estimate the ith WP Treatment Mean: $\mu_{i.}$

   $VAR(\bar{Y}_{i..}) = Var(\bar{c}_{.}) + Var(\bar{d}_{i.}) + Var(\bar{e}_{i..}) = \frac{1}{rb}(b\sigma_c^2 + b\sigma_d^2 + \sigma_e^2) =$

   $\frac{1}{rab}(EMS_{BL} + (a-1)EMS_{BL*V}) \Rightarrow$

   $\widehat{SE}(\bar{Y}_{i..}) = \sqrt{\frac{1}{72}(.83 + (3-1)(.1362))} = .1237$, with $df \approx \frac{(MS_{BL} + (3-1)MS_{BL*V})^2}{\frac{MS_{BL}^2}{5} + \frac{4(MS_{BL*V})^2}{10}} = 8.4$

7. To estimate the jth SP Treatment Mean: $\mu_{.j}$

   $VAR(\bar{Y}_{.j.}) = Var(\bar{c}_{.}) + Var(\bar{d}_{..}) + Var(\bar{e}_{.j.}) = \frac{1}{ra}(r\sigma_c^2 + \sigma_d^2 + \sigma_e^2) =$

   $\frac{1}{rab}(EMS_{BL} + (b-1)EMSE) \Rightarrow \widehat{SE}(\bar{Y}_{.j.}) = .1127$, with $df \approx \frac{(MS_{BL} + (4-1)MSE)^2}{\frac{MS_{BL}^2}{5} + \frac{9(MSE)^2}{45}} = 6.1$

8. To estimate the ijth Treatment Mean: μ_{ij}

   $VAR(\bar{Y}_{ij.}) = Var(\bar{c}_{.}) + Var(\bar{d}_{i.}) + Var(\bar{e}_{ij.}) = \frac{1}{r}(\sigma_c^2 + \sigma_d^2 + \sigma_e^2) =$

   $\frac{1}{rab}(EMS_{BL} + (a-1)EMS_{BL*V} + a(b-1)EMSE) \Rightarrow \widehat{SE}(\bar{Y}_{ij.}) = .1371$, with

   $df \approx \frac{(MS_{BL} + (3-1)MS_{BL*V} + 3(4-1)MSE)^2}{\frac{MS_{BL}^2}{5} + \frac{4MS_{BL*V}^2}{10} + \frac{81(MSE)^2}{45}} = 12.5$

```
*splitplotexample.sas;
option ls=80 ps=55 nocenter nodate formdlim='*';
title 'Split-Plot Design';

data yields;
INPUT VA $ DATE $ BLOCK $ Y @@;
cards;
 L N 1 2.17 L N 2 1.88 L N 3 1.62 L N 4 2.34 L N 5 1.58 L N 6 1.66
 L S1 1 1.58 L S1 2 1.26 L S1 3 1.22 L S1 4 1.59 L S1 5 1.25 L S1 6 0.94
 L S20 1 2.29 L S20 2 1.60 L S20 3 1.67 L S20 4 1.91 L S20 5 1.39 L S20 6 1.12
 L O7 1 2.23 L O7 2 2.01 L O7 3 1.82 L O7 4 2.10 L O7 5 1.66 L O7 6 1.10
 C N 1 2.33 C N 2 2.01 C N 3 1.70 C N 4 1.78 C N 5 1.42 C N 6 1.35
 C S1 1 1.38 C S1 2 1.30 C S1 3 1.85 C S1 4 1.09 C S1 5 1.13 C S1 6 1.06
 C S20 1 1.86 C S20 2 1.70 C S20 3 1.81 C S20 4 1.54 C S20 5 1.67 C S20 6 0.88
 C O7 1 2.27 C O7 2 1.81 C O7 3 2.01 C O7 4 1.40 C O7 5 1.31 C O7 6 1.06
 R N 1 1.75 R N 2 1.95 R N 3 2.13 R N 4 1.78 R N 5 1.31 R N 6 1.30
 R S1 1 1.52 R S1 2 1.47 R S1 3 1.80 R S1 4 1.37 R S1 5 1.01 R S1 6 1.31
 R S20 1 1.55 R S20 2 1.61 R S20 3 1.82 R S20 4 1.56 R S20 5 1.23 R S20 6 1.13
 R O7 1 1.56 R O7 2 1.72 R O7 3 1.99 R O7 4 1.55 R O7 5 1.51 R O7 6 1.33
run;
proc mixed cl alpha=.05;
class VA DATE BLOCK;
model  Y =  VA  DATE VA*DATE/ddfm=SAT;
RANDOM BLOCK BLOCK*VA;
LSMEANS VA DATE VA*DATE/ADJUST=TUKEY CL;
run;
```

OUTPUT FROM SAS PROGRAM:

Split-Plot Design

The Mixed Procedure

Class	Levels	Values
VA	3	C L R
DATE	4	N O7 S1 S20
BLOCK	6	1 2 3 4 5 6

Number of Observations Read	72
Number of Observations Used	72

Covariance Parameter Estimates

Cov Parm	Estimate	Alpha	Lower	Upper
BLOCK	0.05781	0.05	0.01960	0.6138
VA*BLOCK	0.02707	0.05	0.01140	0.1255
Residual	0.02797	0.05	0.01924	0.04437

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
VA	2	10	0.65	0.5412
DATE	3	45	23.39	<.0001
VA*DATE	6	45	1.25	0.2973

Least Squares Means

Effect	VA	DATE	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha
VA	C		1.5717	0.1237	8.37	12.70	<.0001	0.05
VA	L		1.6663	0.1237	8.37	13.47	<.0001	0.05
VA	R		1.5525	0.1237	8.37	12.55	<.0001	0.05
DATE		N	1.7811	0.1127	6.06	15.81	<.0001	0.05
DATE		O7	1.6911	0.1127	6.06	15.01	<.0001	0.05
DATE		S1	1.3406	0.1127	6.06	11.90	<.0001	0.05
DATE		S20	1.5744	0.1127	6.06	13.98	<.0001	0.05
VA*DATE	C	N	1.7650	0.1371	12.5	12.87	<.0001	0.05
VA*DATE	C	O7	1.6433	0.1371	12.5	11.98	<.0001	0.05
VA*DATE	C	S1	1.3017	0.1371	12.5	9.49	<.0001	0.05
VA*DATE	C	S20	1.5767	0.1371	12.5	11.50	<.0001	0.05
VA*DATE	L	N	1.8750	0.1371	12.5	13.67	<.0001	0.05
VA*DATE	L	O7	1.8200	0.1371	12.5	13.27	<.0001	0.05
VA*DATE	L	S1	1.3067	0.1371	12.5	9.53	<.0001	0.05
VA*DATE	L	S20	1.6633	0.1371	12.5	12.13	<.0001	0.05
VA*DATE	R	N	1.7033	0.1371	12.5	12.42	<.0001	0.05
VA*DATE	R	O7	1.6100	0.1371	12.5	11.74	<.0001	0.05
VA*DATE	R	S1	1.4133	0.1371	12.5	10.31	<.0001	0.05
VA*DATE	R	S20	1.4833	0.1371	12.5	10.82	<.0001	0.05

Differences of Least Squares Means

Effect	VA	DATE	_VA	_DATE	Estimate	Standard Error	DF	t Value	Pr > |t|
VA	C		L		-0.09458	0.1065	10	-0.89	0.3956
VA	C		R		0.01917	0.1065	10	0.18	0.8608
VA	L		R		0.1137	0.1065	10	1.07	0.3108
DATE		N		O7	0.09000	0.05575	45	1.61	0.1134
DATE		N		S1	0.4406	0.05575	45	7.90	<.0001
DATE		N		S20	0.2067	0.05575	45	3.71	0.0006
DATE		O7		S1	0.3506	0.05575	45	6.29	<.0001
DATE		O7		S20	0.1167	0.05575	45	2.09	0.0420
DATE		S1		S20	-0.2339	0.05575	45	-4.20	0.0001
VA*DATE	C	N	C	O7	0.1217	0.09655	45	1.26	0.2141
VA*DATE	C	N	C	S1	0.4633	0.09655	45	4.80	<.0001
VA*DATE	C	N	C	S20	0.1883	0.09655	45	1.95	0.0574

VA*DATE	C	N	L	N	-0.1100	0.1354	24.1	-0.81	0.4247
VA*DATE	C	N	L	O7	-0.05500	0.1354	24.1	-0.41	0.6883
VA*DATE	C	N	L	S1	0.4583	0.1354	24.1	3.38	0.0024
VA*DATE	C	N	L	S20	0.1017	0.1354	24.1	0.75	0.4602
VA*DATE	C	N	R	N	0.06167	0.1354	24.1	0.46	0.6530
VA*DATE	C	N	R	O7	0.1550	0.1354	24.1	1.14	0.2637
VA*DATE	C	N	R	S1	0.3517	0.1354	24.1	2.60	0.0158
VA*DATE	C	N	R	S20	0.2817	0.1354	24.1	2.08	0.0484
VA*DATE	C	O7	C	S1	0.3417	0.09655	45	3.54	0.0009
VA*DATE	C	O7	C	S20	0.06667	0.09655	45	0.69	0.4934
VA*DATE	C	O7	L	N	-0.2317	0.1354	24.1	-1.71	0.1000
VA*DATE	C	O7	L	O7	-0.1767	0.1354	24.1	-1.30	0.2044
VA*DATE	C	O7	L	S1	0.3367	0.1354	24.1	2.49	0.0203
VA*DATE	C	O7	L	S20	-0.02000	0.1354	24.1	-0.15	0.8838
VA*DATE	C	O7	R	N	-0.06000	0.1354	24.1	-0.44	0.6617
VA*DATE	C	O7	R	O7	0.03333	0.1354	24.1	0.25	0.8077
VA*DATE	C	O7	R	S1	0.2300	0.1354	24.1	1.70	0.1024
VA*DATE	C	O7	R	S20	0.1600	0.1354	24.1	1.18	0.2490
VA*DATE	C	S1	C	S20	-0.2750	0.09655	45	-2.85	0.0066
VA*DATE	C	S1	L	N	-0.5733	0.1354	24.1	-4.23	0.0003
VA*DATE	C	S1	L	O7	-0.5183	0.1354	24.1	-3.83	0.0008
VA*DATE	C	S1	L	S1	-0.00500	0.1354	24.1	-0.04	0.9709
VA*DATE	C	S1	L	S20	-0.3617	0.1354	24.1	-2.67	0.0134
VA*DATE	C	S1	R	N	-0.4017	0.1354	24.1	-2.97	0.0067
VA*DATE	C	S1	R	O7	-0.3083	0.1354	24.1	-2.28	0.0320
VA*DATE	C	S1	R	S1	-0.1117	0.1354	24.1	-0.82	0.4178
VA*DATE	C	S1	R	S20	-0.1817	0.1354	24.1	-1.34	0.1923
VA*DATE	C	S20	L	N	-0.2983	0.1354	24.1	-2.20	0.0374
VA*DATE	C	S20	L	O7	-0.2433	0.1354	24.1	-1.80	0.0850
VA*DATE	C	S20	L	S1	0.2700	0.1354	24.1	1.99	0.0577
VA*DATE	C	S20	L	S20	-0.08667	0.1354	24.1	-0.64	0.5283
VA*DATE	C	S20	R	N	-0.1267	0.1354	24.1	-0.94	0.3590
VA*DATE	C	S20	R	O7	-0.03333	0.1354	24.1	-0.25	0.8077
VA*DATE	C	S20	R	S1	0.1633	0.1354	24.1	1.21	0.2396
VA*DATE	C	S20	R	S20	0.09333	0.1354	24.1	0.69	0.4973
VA*DATE	L	N	L	O7	0.05500	0.09655	45	0.57	0.5718
VA*DATE	L	N	L	S1	0.5683	0.09655	45	5.89	<.0001
VA*DATE	L	N	L	S20	0.2117	0.09655	45	2.19	0.0336
VA*DATE	L	N	R	N	0.1717	0.1354	24.1	1.27	0.2171
VA*DATE	L	N	R	O7	0.2650	0.1354	24.1	1.96	0.0621
VA*DATE	L	N	R	S1	0.4617	0.1354	24.1	3.41	0.0023
VA*DATE	L	N	R	S20	0.3917	0.1354	24.1	2.89	0.0080
VA*DATE	L	O7	L	S1	0.5133	0.09655	45	5.32	<.0001
VA*DATE	L	O7	L	S20	0.1567	0.09655	45	1.62	0.1117
VA*DATE	L	O7	R	N	0.1167	0.1354	24.1	0.86	0.3975
VA*DATE	L	O7	R	O7	0.2100	0.1354	24.1	1.55	0.1341
VA*DATE	L	O7	R	S1	0.4067	0.1354	24.1	3.00	0.0062
VA*DATE	L	O7	R	S20	0.3367	0.1354	24.1	2.49	0.0203
VA*DATE	L	S1	L	S20	-0.3567	0.09655	45	-3.69	0.0006
VA*DATE	L	S1	R	N	-0.3967	0.1354	24.1	-2.93	0.0073
VA*DATE	L	S1	R	O7	-0.3033	0.1354	24.1	-2.24	0.0346
VA*DATE	L	S1	R	S1	-0.1067	0.1354	24.1	-0.79	0.4386
VA*DATE	L	S1	R	S20	-0.1767	0.1354	24.1	-1.30	0.2044

VA*DATE	L	S20	R	N	-0.04000	0.1354	24.1	-0.30	0.7703
VA*DATE	L	S20	R	O7	0.05333	0.1354	24.1	0.39	0.6972
VA*DATE	L	S20	R	S1	0.2500	0.1354	24.1	1.85	0.0773
VA*DATE	L	S20	R	S20	0.1800	0.1354	24.1	1.33	0.1963
VA*DATE	R	N	R	O7	0.09333	0.09655	45	0.97	0.3389
VA*DATE	R	N	R	S1	0.2900	0.09655	45	3.00	0.0043
VA*DATE	R	N	R	S20	0.2200	0.09655	45	2.28	0.0275
VA*DATE	R	O7	R	S1	0.1967	0.09655	45	2.04	0.0476
VA*DATE	R	O7	R	S20	0.1267	0.09655	45	1.31	0.1962
VA*DATE	R	S1	R	S20	-0.07000	0.09655	45	-0.72	0.4722

Differences of Least Squares Means

Effect	VA	DATE	_VA	_DATE	Adjustment	Adj P	Alpha	Lower	Upper
VA	C		L		Tukey-Kramer	0.6598	0.05	-0.3320	0.1428
VA	C		R		Tukey-Kramer	0.9824	0.05	-0.2182	0.2566
VA	L		R		Tukey-Kramer	0.5541	0.05	-0.1237	0.3512
DATE		N		O7	Tukey-Kramer	0.3810	0.05	-0.02228	0.2023
DATE		N		S1	Tukey-Kramer	<.0001	0.05	0.3283	0.5528
DATE		N		S20	Tukey-Kramer	0.0031	0.05	0.09439	0.3189
DATE		O7		S1	Tukey-Kramer	<.0001	0.05	0.2383	0.4628
DATE		O7		S20	Tukey-Kramer	0.1710	0.05	0.004390	0.2289
DATE		S1		S20	Tukey-Kramer	0.0007	0.05	-0.3462	-0.1216
VA*DATE									

There was not significant evidence of a Variety by Date interaction. Therefore, it is not necessary to compare the Treatment means (VA*DATE). The comparisons only need to examine the Marginal means of Variety (VA) and Date.

Grouping of the Treatment Means:

DATE: $G_1 = \{N,\ O7\}$; $G_2 = \{O7,\ S20\}$; $G_3 = \{S1\}$

VARIETY: $G_1 = \{C,\ R,\ L\}$

Completely Randomized Split-Plot Design Example

The superintendent of the city schools in a large midwestern city decided to investigate the effect of three teaching methods on young student achievement in mathematics concepts:

1. Classical classroom instruction
2. Web-based instruction
3. Classical classroom instruction plus Web-based instruction

He was also concerned about the effect of the use of calculators on achievement in mathematics concepts. Because the teaching methods must naturally be applied to a block of students, the experiment was designed in the following manner. Twelve third grade classrooms were randomly selected with four classrooms randomly assigned to each of the three teaching methods. Ten students in each classroom were randomly selected and given calculators and ten students were randomly selected and were not allowed to use calculators. Prior to receiving instruction and at the end of the instruction period, each student was given a test and the students gain in mathematical achievement was recorded. This is a complete randomized split plot design with Whole Plot being the classroom and the Split Plot being the individual students. The Whole Plot treatment is method of instruction and the Split Plot treatment is calculator use. Note, this study could have been designed as a randomized block split plot design by randomly selecting four elementary schools throughout the city, then randomly selecting three fourth grade classes within each school with the Method of Instruction randomly assigned within each school. The CR-Split Plot Design has the following change in its model and AOV table:
The model for a completely randomized split plot design with two fixed levels factors having r reps, a levels of Factor A and b levels of Factor B is given here:

$$y_{ijkh} = \mu + \tau_i + d_{ik} + \gamma_j + (\tau\gamma)_{ij} + e_{ijkh},$$

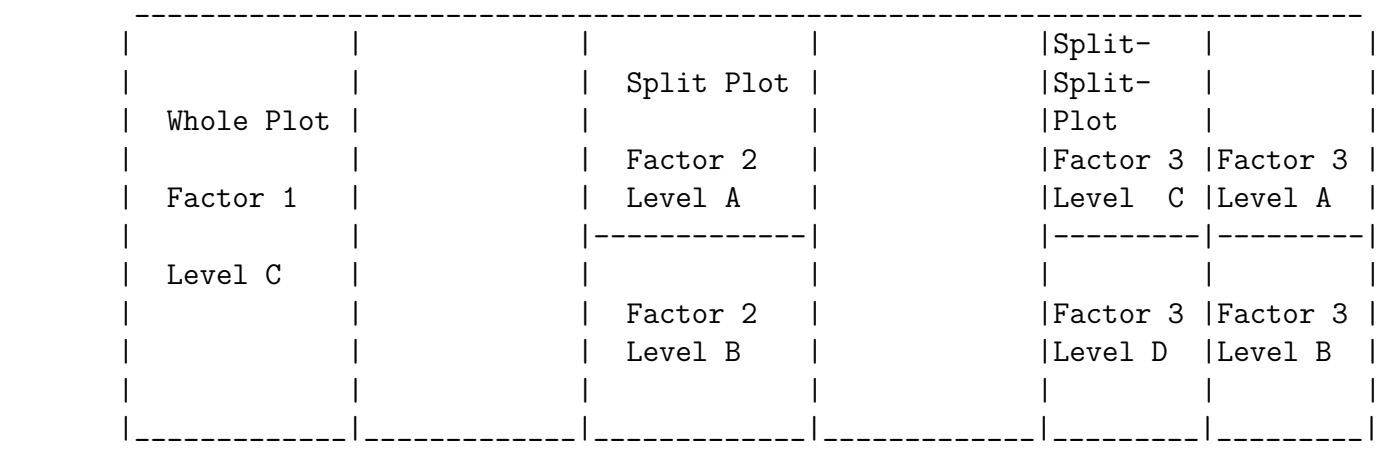
with y_{ijkh} being the score of student h in classroom r with calculator j and instruction method i
τ_i representing the i th Whole Plot Treatment (method of instruction) effect, $i = 1, \ldots, a = 3$
γ_j representing the j th Split Plot Treatment (calculator) effect, $j = 1, \ldots, b = 2$
$(\tau\gamma)_{ij}$ representing the interaction between Whole Plot Treatment and Split Plot Treatment,
d_{ik} representing the whole plot error term (classroom), $k = 1, \ldots, r = 4$
e_{ijkh} representing the split plot error term (student), $h = 1, \ldots, m = 10$.

ANOVA Table for CR Split-Plot Design

Source	DF	Expected Mean Squares	F
A	a-1	$\sigma_e^2 + bm\sigma_d^2 + brmQ_A$	MS_A/MSE_{WP}
Rep(A)	a(r-1)	$\sigma_e^2 + bm\sigma_d^2$	
B	b-1	$\sigma_e^2 + armQ_B$	MS_B/MSE_{SP}
A*B	(a-1)(b-1)	$\sigma_e^2 + rmQ_{A*B}$	MS_{A*B}/MSE_{SP}
Error	a[rbm-r-b+1]	σ_e^2	

Randomized Complete Block Split-Split-Plot Design

When we have three factors, Factor A assigned to Whole Plot EU's, Factor B assigned to Split-Plot EU's, Factor C assigned to Split-Split-Plot EU's, a subdivision of the subplots is required with all levels of Factor C randomly assigned to these new subdivisions, referred to as sub-subplots. There are r blocks containing r Whole Plot EU's each. The design is referred to as a **Randomized Complete Block Split-Split-Plot Design** and the design has three different sizes of EU's. For example, suppose Factor 1 has 5 levels, Factor 2 has 2 levels and Factor 3 has 2 levels:



The model for a randomized complete block split-split plot design with three fixed levels factors having r blocks, a levels of Factor A, b levels of Factor B, and c levels of Factor C is given here:

$$Y_{ijkl} = \mu + c_l + \tau_i + d_{il} + \gamma_j + (\tau\gamma)_{ij} + e_{ijl} + \beta_k + (\tau\beta)_{ik} + (\beta\gamma)_{jk} + (\tau\gamma\beta)_{ijk} + f_{ijkl},$$

with τ_i representing the i th Whole Plot Treatment effect, $i = 1, \ldots, a$

γ_j representing the j th Split Plot Treatment effect, $j = 1, \ldots, b$

β_k representing the k th Split-Split Plot Treatment effect, $k = 1, \ldots, c$

$(\tau\gamma)_{ij}$ representing the interaction between Whole Plot and Split Plot Treatment,

$(\tau\beta)_{ik}$ representing the interaction between Whole Plot and Split-Split Plot Treatment,

$(\gamma\beta)_{jk}$ representing the interaction between Split Plot and Split-Split Plot Treatment,

$(\tau\gamma\beta)_{ijk}$ representing the interaction between Whole Plot, Split Plot, and Split-Split Plot Treatment,

c_l representing the block effect, $l = 1, \ldots, r$

d_{il} representing the whole plot error term,

e_{ijl} representing the split plot error term.

f_{ijkl} representing the split-split plot error term.

ANOVA Table for RCB Split-Split-Plot Design

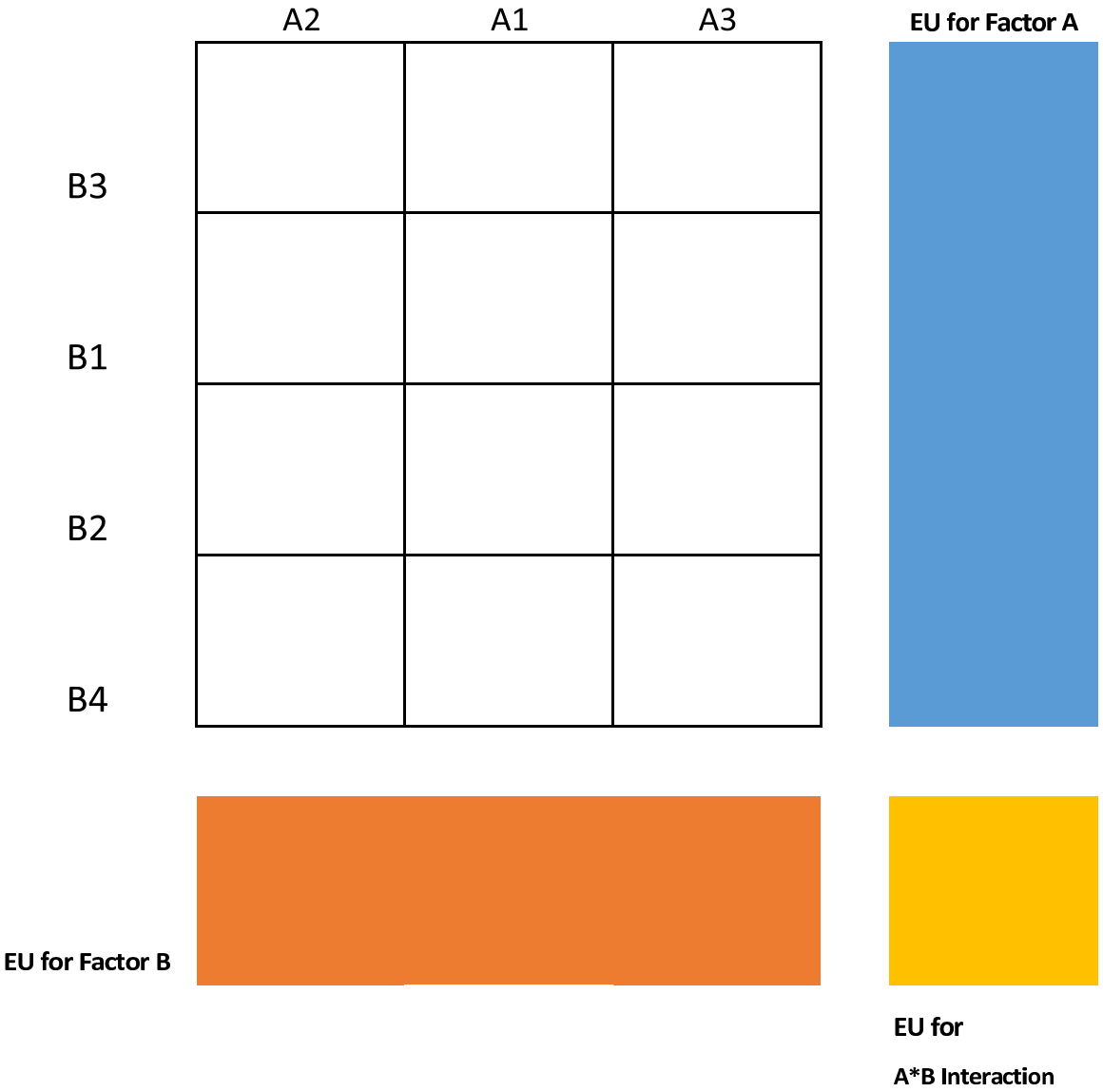
Source	DF	Expected Mean Squares	F
Block	r-1	$\sigma_f^2 + c\sigma_e^2 + bc\sigma_d^2 + abc\sigma_c^2$	
A	a-1	$\sigma_f^2 + c\sigma_e^2 + bc\sigma_d^2 + bcrQ_A$	MS_A/MSE_{WP}
Block*A	(r-1)(a-1)	$\sigma_f^2 + c\sigma_e^2 + bc\sigma_d^2$	
B	b-1	$\sigma_f^2 + c\sigma_e^2 + acrQ_B$	MS_B/MSE_{SP}
A*B	(a-1)(b-1)	$\sigma_f^2 + c\sigma_e^2 + crQ_{A*B}$	MS_{A*B}/MSE_{SP}
Block*B(A)	a(b-1)(r-1)	$\sigma_f^2 + c\sigma_e^2$	
C	c-1	$\sigma_f^2 + abrQ_C$	MS_C/MSE_{SSP}
A*C	(a-1)(c-1)	$\sigma_f^2 + brQ_{A*C}$	MS_{A*C}/MSE_{SSP}
B*C	(b-1)(c-1)	$\sigma_f^2 + arQ_{B*C}$	MS_{B*C}/MSE_{SSP}
A*B*C	(a-1)(b-1)(c-1)	$\sigma_f^2 + rQ_{A*B*C}$	MS_{A*B*C}/MSE_{SSP}
Error	ab(r-1)(c-1)	σ_f^2	

EXAMPLE In an industrial experiment, large batches of four alloys (A1, A2, A3, A4) are prepared in a furnace. After a batch is removed from the furnace, it is divided into containers for application at different cooling temperatures (T1, T2, T3). After each container of alloy is cooled to its proper temperature, the alloy is then poured into molds for application of one of five levels of an anti-oxidizing agent (O1, O2, O3, O4, O5).

Identify the factors and relevant EU's in this study. The complete experiment is repeated on each of three days. Also describe the appropriate randomization procedures.

Strip-Plot Design

Another variation on the Split-Plot design occurs when the subunit treatments occur in a strip across the wholeplot units. The Strip-plot design can be useful in agricultural field studies when two treatment factors can only be applied across large field plots. The levels of Factor A are randomly assigned to the plots in a randomized complete block design. The plots for Factor B are constructed in the same manner but are laid out perpendicular to the plots for Factor A. The levels of Factor B are then randomly assigned to this second array of plots across the same block. A diagram displaying this arrangement is given below:



The Strip-Plot design has three sizes for experimental units where the units for the main effects of Factors A and B are equivalent to whole plots, but each with a different orientation. The EU's for the A*B interaction effect is a subplot where there is an intersection of the two whole plots for the respective levels of Factors A and B. Consequently, there are three experimental

error terms used in the AOV table to test for main effects and the interaction. The model for a randomized complete block strip-plot design with two fixed levels factors having r blocks, a levels of Factor A, and b levels of Factor Bis given here:

$$Y_{ijk} = \mu + c_k + \tau_i + d_{ik} + \gamma_j + e_{jk} + (\tau\gamma)_{ij} + f_{ijk}$$

with τ_i representing the i th Whole Plot A Treatment effect, $i = 1, \ldots, a$
γ_j representing the j th Whole Plot B Treatment effect, $j = 1, \ldots, b$
$(\tau\gamma)_{ij}$ representing the interaction between Whole Plot and Split Plot Treatment,
c_k representing the k th block effect, $k = 1, \ldots, r$
d_{ik} representing the whole plot error term for Factor A,
e_{jk} representing the whole plot error term for Factor B,
f_{ijk} representing the intersection error term.

ANOVA Table for RCB Strip-Plot Design

Source	DF	Expected Mean Squares	F
Block	r-1	$\sigma_f^2 + a\sigma_e^2 + b\sigma_d^2 + ab\sigma_c^2$	
A	a-1	$\sigma_f^2 + b\sigma_d^2 + brQ_A$	MS_A/MSE_{WPA}
Block*A	(r-1)(a-1)	$\sigma_f^2 + b\sigma_d^2$	
B	b-1	$\sigma_f^2 + a\sigma_e^2 + arQ_B$	MS_B/MSE_{WPB}
Block*B	(r-1)(b-1)	$\sigma_f^2 + a\sigma_e^2$	
A*B	(a-1)(b-1)	$\sigma_f^2 + rQ_{A*B}$	MS_{A*B}/MSE_{SP}
Error	(r-1)(a-1)(b-1)	σ_f^2	

Industrial Example

An engineer wants to investigate the strength of ceramic components made with three different percentages of silicon (P1, P2, P3) and four heating temperatures (T1, T2, T3, T4). The process is to repeated on five consecutive days. The engineer has only one kiln for heating the ceramic material and the material must be heated for five hours. Therefore, she can conduct at most four heat treatments per day. On each day, a single batch of the ceramic material is made with each of the silicon percentages. The three batches are subdivided into four sub-batches and one sub-batch from each of the three silicon percentages is then placed in the kiln at one of the four temperatures. The three ceramic sub-batches are then heated for 5 hours. Thus, on a given day, three batches of ceramic material are made and the kiln is run for five hours at each of the four temperatures.

- Is this a RCBD with a 3x4 treatment structure?
- Is this a RCB split-plot Design with a 3x4 treatment structure?
- Is this a RCB split-split-plot Design with a 3x4 treatment structure?
- Is this a RCB strip-plot Design with a 3x4 treatment structure?

Comments:

There many other variation on the basic split-plot design.

The crucial elements to consider are

1. What restrictions have been placed on the randomization?
2. Are there different size EU's for the different factors?
3. To which EU's were the levels of each factor randomly assigned.
4. Is there true replication or do we have subsampling within the basic design?

Repeated Measures Designs

Repeated Measures designs are experiments or studies in which the experimental unit, EU's, have several measurements taken on them either spatially or temporally. This is in contrast to the standard design in which a single measurement is taken on the experimental unit. Examples of this type of study are subsampling or crossover designs. In a study involving subsampling there is not generally any other variable of interest associated with the repeated measurements. The goal is to evaluate the measuring device or to evaluate the variability across the surface of an EU, for example, a field of corn or silicon wafer. A further application of repeated measures is when an EU receives a treatment and the experimenter measures a response at specific time points or a specific locations on the EU. In this type of situation, the variable Time or Location is of interest to the experimenter. By using the Repeated Measures Analysis the researcher will be able to determine time trends or spatial patterns and determine if certain trends or patterns vary amongst the different treatments.

For example, a drug company may be interested in knowing if their drug reduces blood pressure linearly with respect to time t and if after time t, a person's blood pressure remains constant. That is, evaluate a temporal relationship in blood pressure.

A second example would be that a wafer manufacturer may want to know if there is a different pattern in the conductivity of the wafer across the surface of the wafer depending on the type of protective coating placed on the wafer. If measurements are taken at the same locations on all the wafers then this would be investigating a spatial relationship across the surface of the wafer. If the locations where measurements are taken on the wafer are randomly selected for each wafer then the locations would be subsamples and it would not be possible to obtain a spatial relationship across the surface of the wafer.

There are numerous methods to analyze studies/experiments that involve repeated measures:

- A split-plot approach in which the repeated measures are considered as a split plot treatment with the whole plot being the EU
- An approach involving the modeling of the dependency across the repeated measures
- A multivariate response approach where repeated measures are considered as a vector response on each EU

The following example from Crowder and Hand's *Repeated Measures* book will be used to illustrate these concepts. In their study, three levels of a vitamin E supplement: Zero (Control), Low, High were given to guinea pigs. Five pigs were randomly assigned to each of the three levels of Vitamin E supplement. The weight of the pigs were recorded at 1, 2, 3, 4, 5, 6 weeks after the beginning of the study. This is a repeated measures experiment because each pig, the EU, is given only one treatment but each pig is measured at 6 specified time points. The experimenter is interested in determining if there exists a trend in the weight of the pigs over time and if the trend is different for the three treatments (treatment by time interaction).

Weight of Guinea Pigs Under 3 Levels of Vitamin E

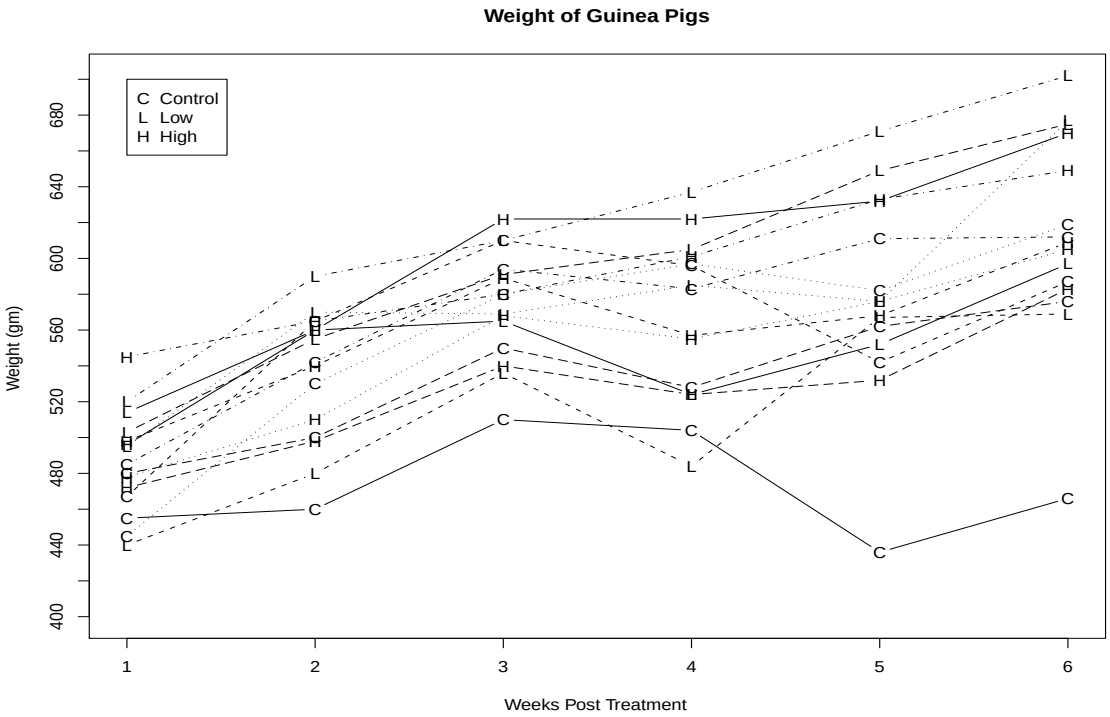
Level of E	Animal	Week1	Week2	Week3	Week4	Week5	Week6
C	1	455	460	510	504	436	466
C	2	467	565	610	596	542	587
C	3	445	530	580	597	582	619
C	4	485	542	594	583	611	612
C	5	480	500	550	528	562	576
L	6	514	560	565	524	552	597
L	7	440	480	536	484	567	569
L	8	495	570	569	585	576	677
L	9	520	590	610	637	671	702
L	10	503	555	591	605	649	675
H	11	496	560	622	622	632	670
H	12	498	540	589	557	568	609
H	13	478	510	568	555	576	605
H	14	545	565	580	601	633	649
H	15	472	498	540	524	532	583

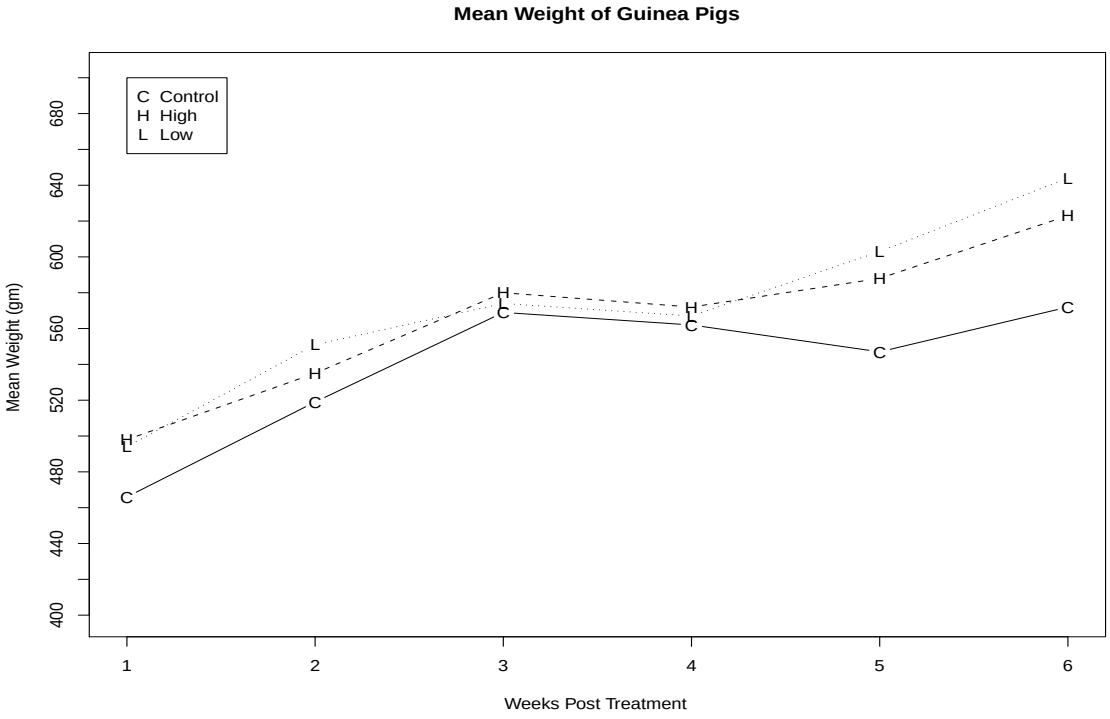
Note that there are three treatments with $r = 5$ reps per treatment for a total of 15 EU's (pigs) each of which is weighed 6 times for a total of 90 observations. In contrast, a completely randomized design with 90 observations would have 90 EU's each weighed once. Thus, 75 more pigs are required to perform the CRD. However, the gain in economy of using a repeated measures design has limitations. The inferences are being made to a population of pigs. In the repeated measures design only 15 pigs from the population are being observed. Thus, there may be greater variability in the estimation of the treatment means due to having such a small sample size per treatment. On the other hand the repeated measures design allows the researcher to observe the behavior of the individual pig over the 6 weeks and hence provides information concerning the potential differences in fluctuations in weight for the individual pigs. A plot of the data on the next page reveals widely varying patterns for the 15 pigs.

The model for the weight, y_{ijk}, the jth week's measurement of the kth pig receiving treatment i would be

$$y_{ijk} = \mu + \tau_i + d_{k(i)} + \beta_j + (\tau\beta)_{ij} + e_{ijk}$$

In the above model the error terms, e_{ijk} would be correlated across j for fixed i and k because we are taking multiple measurements across time on each of the pigs.


Weight of Guinea Pigs
C Control
L Low
H High
Weight (gm)
400
440
480
520
560
600
640
680
1
2
3
4
5
6
Weeks Post Treatment


Mean Weight of Guinea Pigs
C Control
H High
L Low
Mean Weight (gm)
400
440
480
520
560
600
640
680
1
2
3
4
5
6
Weeks Post Treatment

The analysis of a repeated measures design can, under certain conditions, be approximated by the methods used in a split plot experiment.

- Each treatment is randomly assigned to an EU.

  This is the whole plot in the split plot design.

- Each EU is then measured at p time points or p locations on the EU.

  This is considered the split plot unit.

- The major difference between split plots and repeated measures is that in a split plot design the levels of Factor A are randomly assigned to the whole plot EU's and the levels of Factor B are randomly assigned to the split plot EU's.

  In the repeated measures design, the second randomization does not occur. The treatment (Factor A) is randomly assigned to the EU's (whole plot EU's) but the levels of Factor B (time or location) are NOT randomly assigned to a subunit of the EU. Thus, there may be a strong correlation between the measurements across time (or location) for those measurements produced by the same EU.

- Therefore, the split plot analysis is an appropriate analysis for a repeated measures experiment only when the covariance matrix of the measurements satisfy a particular type of structure, called Compound Symmetry.

Covariance Structure Under Split Plot Model:

$$y_{ijk} = \mu + \tau_i + d_{k(i)} + \beta_j + (\tau\beta)_{ij} + e_{ijk}$$

$$Cov(Y_{ijk}, Y_{i'j'k'}) = \begin{cases} \sigma_d^2 + \sigma_e^2 & when \quad i = i', \quad j = j', \quad k = k' \\ \sigma_d^2 & when \quad i = i', \quad j \neq j', \quad k = k' \\ 0 & when \quad i \neq i' \quad \text{and/or} \quad k \neq k' \end{cases}$$

where Y_{ijk} is the measurement from the kth EU receiving treatment i at time j.

Thus, we have that

$$Corr(Y_{ijk}, Y_{ij'k}) = \frac{Cov(Y_{ijk}, Y_{ij'k})}{Var(Y_{ijk})} = \frac{\sigma_d^2}{\sigma_d^2 + \sigma_e^2} = \frac{1}{1+\tau} = \rho$$

that is, there is a constant correlation between observations on the same EU no matter how far apart they are taken in time or space. This may not be too realistic in many applications. One would think that observations in adjacent time periods would be more highly correlated than observations taken two or three time periods apart. In the pig example, this implies that the correlation between the weight of the pig at Week 1 and Week 2 is the same as the correlation between the weight of the pig at Week 1 and Week 5. This does not seem very likely to occur.

However, if the compound symmetry condition is satisfied, then the split plot analysis produces a relatively accurate approximation to the p-values for testing hypotheses about treatment, time, and interaction effects.

In fact, a somewhat less restrictive condition is all that is required. The Huynh-Feldt- Condition: The variance of the difference between any pair of observations on the same EU must be equal, that is,

$$\text{Huynh-Feldt- Condition:} \qquad Var(Y_{ijk} - Y_{ij'k}) = 2\lambda \ \text{ for all } \ j \neq j^{'}.$$

Note that

- Compound Symmetry Conditon **implies** the Huynh-Feldt- Condition

$$Var(Y_{ijk} - Y_{ij'k}) = Var(d_{ik} + e_{ijk} - d_{ik} - e_{ij'k}) = Var(e_{ijk}) + Var(e_{ij'k}) = 2\sigma_e^2$$

- Huynh-Feldt- Condition **does not** imply Compound Symmetry.

A test of the Huynh-Feldt- Condition, Mauchly test, is provided in SAS. However, when the sample sizes are relatively small, the Mauchly test has very low power and hence will often fail to detect that the compound symmetry is invalid. This will often result in an incorrect application of the split plot analysis of a repeated measures experiment.

If the H-F condition is valid, then the split plot analysis is an appropriate approximation. The model would then be a

$$\text{Split-Plot Model:} \qquad y_{ijk} = \mu + \tau_i + d_{ik} + \beta_j + (\tau\beta)_{ij} + e_{ijk}$$

with $i = 1, \ldots, t \ \ j = 1, \ldots, p \ \ k = 1, \ldots, r$, where

$\tau_i \ ith$ treatment effect, $\beta_j \ jth$ time effect, $(\tau\beta)_{ij}$ treatment-time interaction effect

$d_{ik} \ \ iid \ \ N(0, \sigma_d^2), \qquad e_{ijk} \ \ iid \ \ N(0, \sigma_e^2), \qquad d_{ik}$ and e_{ijk} independent

The above model yields the following variance-covariance structure if the H-F Condition is valid: assume $i \neq i^{'} \ \ j \neq j^{'} \ \ k \neq k^{'}$

$$\begin{aligned} Var(Y_{ijk}) &= \sigma_d^2 + \sigma_e^2 \\ Cov(Y_{ijk}, Y_{ij'k}) &= \sigma_d^2 \\ Cov(Y_{ijk}, Y_{i'jk}) &= 0 \\ Cov(Y_{ijk}, Y_{ijk'}) &= 0 \\ Cov(Y_{ijk}, Y_{ij'k'}) &= 0 \end{aligned}$$

An Improved Approach

In the general **Repeated Measures Design**, the measurements from the same EU would likely have a more complex correlation structure and measurements among EU's in the same treatment group may be correlated. Only measurements from EU's receiving different treatments would be uncorrelated.That is, in the model

$$y_{ijk} = \mu + \tau_i + d_{ik} + \beta_j + (\tau\beta)_{ij} + e_{ijk}$$

the conditions we had under the H-F condition

d_{ik} iid $N(0, \sigma_d^2)$, e_{ijk} iid $N(0, \sigma_e^2)$, d_{ik} and e_{ijk} independent

would change to

Conditions for Repeated Measures Model:

1. d_{ik} are correlated normal r.v's
2. e_{ijk} are correlated normal r.v.s
3. d_{ik} and e_{ijk} are independent of each other

In matrix notation, we can formulate the conditions on the r.v.'s in the two situations:
Define the random vectors,

$$\mathbf{d}_i^{'} = (d_{i1}, \ldots, d_{ir}) \qquad \text{and} \qquad \mathbf{e}_{ik}^{'} = (e_{i1k}, \ldots, e_{ipk})$$

we have the following conditions on the random variables for the two situations:

Split Plot Model:

$\mathbf{d}_i$ is distributed $N_r(0, \sigma_d^2\mathbf{I})$; $\mathbf{e}_{ik}$ is distributed $N_p(0, \sigma_e^2\mathbf{I})$; $\mathbf{d}_i$, and $\mathbf{e}_{ik}$ are independent

Repeated Measures Model:

$\mathbf{d}_i$ is distributed $N_r(0, \mathbf{U}_i)$; $\mathbf{e}_{ik}$ is distributed $N_p(0, \mathbf{V})$; $\mathbf{d}_i$, and $\mathbf{e}_{ik}$ are independent

where $\mathbf{U}_i = (u_{imn})_{m,n=1,\ldots,r}$ is the $r \times r$ covariance matrix for the p Subjects under Treatment i, that is, $u_{mn} = cov(u_{im}, u_{in})$

$\mathbf{V} = (v_{mn})_{m,n=1,\ldots,p}$ is the $p \times p$ covariance matrix for Measurements Across Time (Space), that is, $v_{mn} = cov(e_{imk}, e_{ink})$
which is the same matrix for all treatments and subjects.

In many experiments the following simplifying condition is imposed:

The between subjects covariance matrices $\mathbf{U}_i$ are all assumed to be $\sigma_d^2\mathbf{I}$

That is, the measurements from different subjects are assumed to be iid, uncorrelated with the same distribution. Under these conditions, the d_{ij}'s are assumed to be iid and only the e_{ijk}'s are assumed correlated. We thus obtain the following variances and covariances on the responses:

$Var(Y_{ijk}) = u_{kk} + v_{jj}$

$Cov(Y_{ijk}, Y_{ij'k}) = u_{kk} + v_{jj'}$ (Same subject different times)

$Cov(Y_{ijk}, Y_{ijk'}) = Cov(Y_{ijk}, Y_{ij'k'}) = u_{kk'} = 0$ (Different subjects at same time or different times)

For the **Split Plot model**, the time vectors per subject $\mathbf{Y}'_{ik} = (Y_{i1k}, \ldots, Y_{ipk})$ has covariances:

$$Cov(Y_{ijk}, Y_{ij'k}) = \sigma_d^2 + \sigma_e^2 \text{ when } j = j' \qquad Cov(Y_{ijk}, Y_{ij'k}) = \sigma_d^2 \text{ when } j \neq j'$$

which is represented by the covariance matrix

$\sigma_d^2\mathbf{J} + \sigma_e^2\mathbf{I}$, where $\mathbf{J}$ is a $p \times p$ matrix of all 1's and $\mathbf{I}$ is a $p \times p$ identity matrix.

Whereas, for the **Repeated Measures model**, the time vectors per subject has covariance $Cov(Y_{ijk}, Y_{ij'k}) = \sigma_d^2 + v_{jj'}$ which is represented by the covariance matrix

$\sigma_d^2\mathbf{J} + \mathbf{V}$.

Compound Symmetry requires that the covariance matrix, V, have the following form:

$$\mathbf{V} = \sigma_e^2\left[\mathbf{I} + \frac{\rho_e}{1-\rho_e}\mathbf{J}\right] = \frac{\sigma_e^2}{1-\rho_e}\begin{bmatrix} 1 & \rho_e & \rho_e & \ldots & \rho_e \\ \rho_e & 1 & \rho_e & \ldots & \rho_e \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ \rho_e & \rho_e & \ldots & 1 & \rho_e \\ \rho_e & \ldots & \rho_e & \rho_e & 1 \end{bmatrix}$$

and

$$\mathbf{U}_i = \sigma_d^2\left[\mathbf{I} + \frac{\rho_d}{1-\rho_d}\mathbf{J}\right]$$

Thus, we obtain

$Var(Y_{ijk}) = \frac{\sigma_d^2}{1-\rho_d} + \frac{\sigma_e^2}{1-\rho_e}$,

$Cov(Y_{ijk}, Y_{ij'k}) = \frac{\sigma_d^2}{1-\rho_d} + \frac{\sigma_e^2\rho_e}{1-\rho_e}$ $\qquad$ $Cov(Y_{ijk}, Y_{ij'k'}) = \frac{\sigma_d^2\rho_d}{1-\rho_d}$

An equivalent way to express the above structure on the covariances is given by

$$Var(d_{ij} - d_{ij'}) = 2\sigma_d^2 \qquad\qquad Var(e_{ijk} - e_{ijk'}) = 2\sigma_e^2$$

The above conditions are called the **Sphericity Condition**.

Huynh-Feld (1970) showed that the spericity condition allows for unequal variances among the random effects:

$$Var(e_{ijk}) = v_{kk} = \sigma_e^2(1 + 2\lambda_k), \quad Cov(e_{ijk}, e_{ij'k}) = v_{jj'} = \sigma_e^2(\lambda_j + \lambda_{j'}), \quad \text{with } \lambda_j \geq -0.5$$

That is, the covariance matrix for $\mathbf{e}$ is

$$\mathbf{V} = \begin{bmatrix} 1 + 2\lambda_1 & \lambda_1 + \lambda_2 & \dots & \lambda_1 + \lambda_p \\ \lambda_1 + \lambda_2 & 1 + 2\lambda_2 & \dots & \lambda_2 + \lambda_p \\ \vdots & \vdots & \vdots & \vdots \\ \lambda_1 + \lambda_p & \lambda_2 + \lambda_p & \dots & 1 + 2\lambda_p \end{bmatrix}$$

Under equal variances, $\mathbf{V}$ satifies compound symmetry with $\lambda_j = \frac{\rho_e}{2(1-\rho_e)}$.

Thus, $\mathbf{V}$ must be of this form in order for the Split plot analysis to provide an appropriate analysis of the repeated measures experiment.

In this case, the AOV table for the split plot analysis of a repeated measures experiment is given here:

Source	DF	Expected Mean Squares
Treatment	t-1	$\sigma_e^2(1 + 2\lambda_.) + p\sigma_d^2 + rpQ_{Trt}$
Subject(TRT)	(r-1)t	$\sigma_e^2(1 + 2\lambda_.) + p\sigma_d^2$
Time	p-1	$\sigma_e^2 + rtQ_{Time}$
Trt*Time	(t-1)(p-1)	$\sigma_e^2 + rQ_{Trt*Time}$
Error	t(p-1)(r-1)	σ_e^2
Total	tpr-1	

Correlated Errors - Spatial or Temporal Correlation

The topic of correlated errors when the repeated measurements on an experimental unit occur over space or time are topics covered in the courses STAT 647-Spatial Statistics and STAT 636-Applied Multivariate Analysis. The following provides a glimpse of the methods used in these two courses.

A model to describe the correlation in the errors that is of a very simple nature is the autoregressive model of order 1, AR(1). This model requires that the correlation between observations decreases for observations that are further apart in time or space, that is, as $|j - j^{'}|$ increases:

$Cov(e_{ijk}, e_{ij^{'}k}) = \sigma_e^2 \rho^{j-j^{'}}$, where $|\rho| \leq 1$.

The corresponding covariance matrix is

$$\mathbf{V} = \sigma_e^2 \left[\mathbf{I} + \rho_e^{j-j^{'}} \mathbf{J}\right] = \sigma_e^2 \begin{bmatrix} 1 & \rho_e & \rho_e^2 & \dots & \rho_e^{p-1} \\ \rho_e & 1 & \rho_e & \dots & \rho_e^{p-2} \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ \rho_e^{p-2} & \rho_e^{p-3} & \dots & 1 & \rho_e \\ \rho_e^{p-1} & \dots & \rho_e^2 & \rho_e & 1 \end{bmatrix}$$

and

$$\mathbf{U}_i = \sigma_d^2 \left[\mathbf{I} + \frac{\rho_d}{1-\rho_d}\mathbf{J}\right]$$

Thus, we obtain

$Var(Y_{ijk}) = \frac{\sigma_d^2}{1-\rho_d} + \frac{\sigma_e^2}{1-\rho_e}$,

$Cov(Y_{ijk}, Y_{ij^{'}k}) = \frac{\sigma_d^2}{1-\rho_d} + \frac{\sigma_e^2 \rho_e}{1-\rho_e}$

$Cov(Y_{ijk}, Y_{ij^{'}k^{'}}) = \frac{\sigma_d^2 \rho_d}{1-\rho_d}$

We will next analyze the Crowder-Hand Vitamin E data in several different ways to illustrate the previously described methodologies.

```
* repeat.sas;
*Example is from Crowder and Hand, "Repeated Measures"
Three levels of Vitamin E supplements: Control(C), Low(L), and High(H)
are to be evaluated. The experimental units are guinea pigs. Five pigs
are randomly assigned to each of the three treatments. The weight of the
pigs were recorded after 1, 2, 3, 4, 5, and 6 weeks after receiving the
treatment;

option ls=90 ps=55 nocenter nodate;
title 'ANALYSIS OF REPEATED MEASURES DESIGN';
data RAW;
INPUT ANM  TRT $ @;
DO WK = 1 TO 6;
INPUT Y @; OUTPUT; END;
LABEL WK = 'WEEK' TRT = 'LEVEL OF VITAMIN E' Y='WEIGHT';
cards;
 1 C 455 460 510 504 436 466
 2 C 467 565 610 596 542 587
 3 C 445 530 580 597 582 619
 4 C 485 542 594 583 611 612
 5 C 480 500 550 528 562 576
 1 L 514 560 565 524 552 597
 2 L 440 480 536 484 567 569
 3 L 495 570 569 585 576 677
 4 L 520 590 610 637 671 702
 5 L 503 555 591 605 649 675
 1 H 496 560 622 622 632 670
 2 H 498 540 589 557 568 609
 3 H 478 510 568 555 576 605
 4 H 545 565 580 601 633 649
 5 H 472 498 540 524 532 583
RUN;
PROC PRINT DATA=RAW;
VAR ANM TRT WK Y ;
RUN;

TITLE2 'ANALYSIS AS A SPLIT PLOT DESIGN';
PROC MIXED DATA=RAW;
 CLASS ANM TRT WK;
 MODEL Y = TRT WK TRT*WK;
 RANDOM ANM(TRT);
  CONTRAST 'T1VST2WK=1'
TRT 1 -1
TRT*WK 1 0 0 0 0 0 -1 0 0 0 0 0;
LSMEANS TRT WK/adjust=Tukey;
RUN;
```

```
TITLE2 'ANALYSIS AS A REPEATED DESIGN WITH UNSPECIFIED COVARIANCE STRUCTURE';
PROC MIXED DATA=RAW;
 CLASS ANM TRT WK;
 MODEL Y = TRT WK TRT*WK;
 REPEATED WK/SUB=ANM(TRT) TYPE=UN R RCorr;
  CONTRAST 'T1VST2WK=1'
TRT 1 -1
TRT*WK 1 0 0 0 0 0 -1 0 0 0 0 0;
LSMEANS TRT WK/adjust=Tukey;
RUN;
```

```
TITLE2 'ANALYSIS AS A REPEATED MEASURES DESIGN WITH COMPOUND SYMMETRY';
PROC MIXED DATA=RAW;
 CLASS ANM TRT WK;
 MODEL Y = TRT WK TRT*WK;
 REPEATED WK/SUB=ANM(TRT) TYPE=CS R RCorr;
 CONTRAST 'T1VST2WK=1'
TRT 1 -1
TRT*WK 1 0 0 0 0 0  -1 0 0 0 0 0;
  LSMEANS TRT WK/adjust=Tukey;
RUN;
```

```
TITLE2 'ANALYSIS AS A REPEATED MEASURES DESIGN WITH AR(1) COVARIANCE';
PROC MIXED DATA=RAW;
 CLASS ANM TRT WK;
 MODEL Y = TRT WK TRT*WK;
 REPEATED WK/SUB=ANM(TRT) TYPE=AR(1) R RCorr;
 CONTRAST 'T1VST2WK=1'
TRT 1 -1
TRT*WK 1 0 0 0 0 0  -1 0 0 0 0 0;
  LSMEANS TRT WK /adjust=Tukey;
RUN;
```

ANALYSIS OF REPEATED MEASURES DESIGN

Obs	ANM	TRT	WK	Y
1	1	C	1	455
2	1	C	2	460
3	1	C	3	510
4	1	C	4	504
5	1	C	5	436
6	1	C	6	466
7	2	C	1	467
8	2	C	2	565
9	2	C	3	610
10	2	C	4	596
11	2	C	5	542
12	2	C	6	587
13	3	C	1	445
14	3	C	2	530
15	3	C	3	580
16	3	C	4	597
17	3	C	5	582
18	3	C	6	619
19	4	C	1	485
20	4	C	2	542
21	4	C	3	594
22	4	C	4	583
23	4	C	5	611
24	4	C	6	612
25	5	C	1	480
26	5	C	2	500
27	5	C	3	550
28	5	C	4	528
29	5	C	5	562
30	5	C	6	576
31	1	L	1	514
32	1	L	2	560
.	.	.	.	.
.	.	.	.	.
.	.	.	.	.
82	4	H	4	601
83	4	H	5	633
84	4	H	6	649
85	5	H	1	472
86	5	H	2	498
87	5	H	3	540
88	5	H	4	524
89	5	H	5	532
90	5	H	6	583

ANALYSIS OF REPEATED MEASURES DESIGN
ANALYSIS AS A SPLIT PLOT DESIGN

The Mixed Procedure

Class	Levels	Values
ANM	5	1 2 3 4 5
TRT	3	C H L
WK	6	1 2 3 4 5 6

Covariance Parameter Estimates

Cov Parm	Estimate
ANM(TRT)	1373.94
Residual	542.54

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
TRT	2	12	1.06	0.3782
WK	5	60	52.55	<.0001
TRT*WK	10	60	1.80	0.0801

Contrasts

Label	Num DF	Den DF	F Value	Pr > F
T1 VS T2 WK=1	1	60	1.29	0.2613

Least Squares Means

Effect	VITAMIN E	WEEK	Estimate	Standard Error	DF	t Value	Pr > |t|
TRT	C		539.13	17.1135	12	31.50	<.0001
TRT	H		565.90	17.1135	12	33.07	<.0001
TRT	L		572.27	17.1135	12	33.44	<.0001
WK		1	486.20	11.3033	60	43.01	<.0001
WK		2	535.00	11.3033	60	47.33	<.0001
WK		3	574.27	11.3033	60	50.81	<.0001
WK		4	566.80	11.3033	60	50.14	<.0001
WK		5	579.27	11.3033	60	51.25	<.0001
WK		6	613.07	11.3033	60	54.24	<.0001

ANALYSIS OF REPEATED MEASURES DESIGN
ANALYSIS AS A SPLIT PLOT DESIGN

Differences of Least Squares Means

Effect	LEVEL OF VITAMIN E	WEEK	LEVEL OF VITAMIN E	WEEK	Estimate	Standard Error	df	Adjustment	Adj P
TRT	C		H		-26.7667	24.2022	12	Tukey	0.5286
TRT	C		L		-33.1333	24.2022	12	Tukey	0.3867
TRT	H		L		-6.3667	24.2022	12	Tukey	0.9627
WK		1		2	-48.8000	8.5052	60	Tukey-Kramer	<.0001
WK		1		3	-88.0667	8.5052	60	Tukey-Kramer	<.0001
WK		1		4	-80.6000	8.5052	60	Tukey-Kramer	<.0001
WK		1		5	-93.0667	8.5052	60	Tukey-Kramer	<.0001
WK		1		6	-126.87	8.5052	60	Tukey-Kramer	<.0001
WK		2		3	-39.2667	8.5052	60	Tukey-Kramer	0.0003
WK		2		4	-31.8000	8.5052	60	Tukey-Kramer	0.0053
WK		2		5	-44.2667	8.5052	60	Tukey-Kramer	<.0001
WK		2		6	-78.0667	8.5052	60	Tukey-Kramer	<.0001
WK		3		4	7.4667	8.5052	60	Tukey-Kramer	0.9504
WK		3		5	-5.0000	8.5052	60	Tukey-Kramer	0.9915
WK		3		6	-38.8000	8.5052	60	Tukey-Kramer	0.0004
WK		4		5	-12.4667	8.5052	60	Tukey-Kramer	0.6869
WK		4		6	-46.2667	8.5052	60	Tukey-Kramer	<.0001
WK		5		6	-33.8000	8.5052	60	Tukey-Kramer	0.0025

ANALYSIS OF REPEATED MEASURES DESIGN
ANALYSIS AS A REPEATED MEASURES-PROC MIXED TYPE=COMPOUND SYMMETRY

The Mixed Procedure

Model Information

Data Set	WORK.RAW
Dependent Variable	Y
Covariance Structure	Compound Symmetry
Subject Effect	ANM(TRT)
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Between-Within

Class Level Information

Class	Levels	Values
ANM	5	1 2 3 4 5
TRT	3	C H L
WK	6	1 2 3 4 5 6

Estimated R Matrix for ANM(TRT) 1 C

Row	Col1	Col2	Col3	Col4	Col5	Col6
1	1916.48	1373.94	1373.94	1373.94	1373.94	1373.94
2	1373.94	1916.48	1373.94	1373.94	1373.94	1373.94
3	1373.94	1373.94	1916.48	1373.94	1373.94	1373.94
4	1373.94	1373.94	1373.94	1916.48	1373.94	1373.94
5	1373.94	1373.94	1373.94	1373.94	1916.48	1373.94
6	1373.94	1373.94	1373.94	1373.94	1373.94	1916.48

Estimated R Correlation Matrix for ANM(TRT) 1 C

Row	Col1	Col2	Col3	Col4	Col5	Col6
1	1.0000	0.7169	0.7169	0.7169	0.7169	0.7169
2	0.7169	1.0000	0.7169	0.7169	0.7169	0.7169
3	0.7169	0.7169	1.0000	0.7169	0.7169	0.7169
4	0.7169	0.7169	0.7169	1.0000	0.7169	0.7169
5	0.7169	0.7169	0.7169	0.7169	1.0000	0.7169
6	0.7169	0.7169	0.7169	0.7169	0.7169	1.0000

ANALYSIS OF REPEATED MEASURES DESIGN
ANALYSIS AS A REPEATED MEASURES-PROC MIXED TYPE=COMPOUND SYMMETRY

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
CS	ANM(TRT)	1373.94
Residual		542.54

Fit Statistics

-2 Res Log Likelihood	720.0
AIC (smaller is better)	724.0
AICC (smaller is better)	724.2
BIC (smaller is better)	725.5

Null Model Likelihood Ratio Test

DF	Chi-Square	Pr > ChiSq
1	57.45	<.0001

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
TRT	2	12	1.06	0.3782
WK	5	60	52.55	<.0001
TRT*WK	10	60	1.80	0.0801

Contrasts

Label	Num DF	Den DF	F Value	Pr > F
T1VST2WK=1	1	60	1.29	0.2613

ANALYSIS OF REPEATED MEASURES DESIGN
ANALYSIS AS A REPEATED MEASURES-PROC MIXED TYPE=COMPOUND SYMMETRY

Least Squares Means

Effect	LEVEL OF VITAMIN E	WEEK	Estimate	Standard Error	DF	t Value	Pr > |t|
TRT	C		539.13	17.1135	12	31.50	<.0001
TRT	H		565.90	17.1135	12	33.07	<.0001
TRT	L		572.27	17.1135	12	33.44	<.0001
WK		1	486.20	11.3033	60	43.01	<.0001
WK		2	535.00	11.3033	60	47.33	<.0001
WK		3	574.27	11.3033	60	50.81	<.0001
WK		4	566.80	11.3033	60	50.14	<.0001
WK		5	579.27	11.3033	60	51.25	<.0001
WK		6	613.07	11.3033	60	54.24	<.0001

Differences of Least Squares Means

Effect	LEVEL OF VITAMIN E	WEEK	LEVEL OF VITAMIN E	WEEK	Estimate	Standard Error	df	Adjustment	Adj P
TRT	C		H		-26.7667	24.2022	12	Tukey	0.5286
TRT	C		L		-33.1333	24.2022	12	Tukey	0.3867
TRT	H		L		-6.3667	24.2022	12	Tukey	0.9627
WK		1		2	-48.8000	8.5052	60	Tukey-Kramer	<.0001
WK		1		3	-88.0667	8.5052	60	Tukey-Kramer	<.0001
WK		1		4	-80.6000	8.5052	60	Tukey-Kramer	<.0001
WK		1		5	-93.0667	8.5052	60	Tukey-Kramer	<.0001
WK		1		6	-126.87	8.5052	60	Tukey-Kramer	<.0001
WK		2		3	-39.2667	8.5052	60	Tukey-Kramer	0.0003
WK		2		4	-31.8000	8.5052	60	Tukey-Kramer	0.0053
WK		2		5	-44.2667	8.5052	60	Tukey-Kramer	<.0001
WK		2		6	-78.0667	8.5052	60	Tukey-Kramer	<.0001
WK		3		4	7.4667	8.5052	60	Tukey-Kramer	0.9504
WK		3		5	-5.0000	8.5052	60	Tukey-Kramer	0.9915
WK		3		6	-38.8000	8.5052	60	Tukey-Kramer	0.0004
WK		4		5	-12.4667	8.5052	60	Tukey-Kramer	0.6869
WK		4		6	-46.2667	8.5052	60	Tukey-Kramer	<.0001
WK		5		6	-33.8000	8.5052	60	Tukey-Kramer	0.0025

ANALYSIS OF REPEATED MEASURES DESIGN
ANALYSIS AS A SPLIT PLOT DESIGN-PROC MIXED TYPE=UNSTRUCTURED

The Mixed Procedure

Model Information

Data Set	WORK.RAW
Dependent Variable	Y
Covariance Structure	Unstructured
Subject Effect	ANM(TRT)
Estimation Method	REML
Residual Variance Method	None
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Between-Within

Class Level Information

Class	Levels	Values
ANM	5	1 2 3 4 5
TRT	3	C H L
WK	6	1 2 3 4 5 6

The Mixed Procedure

Estimated R Matrix for ANM(TRT) 1 C

Row	Col1	Col2	Col3	Col4	Col5	Col6
1	706.77	711.57	401.65	709.47	725.83	705.68
2	711.57	1430.87	1107.75	1623.03	1419.52	1669.62
3	401.65	1107.75	1082.70	1423.12	1440.65	1474.77
4	709.47	1623.03	1423.12	2408.83	2185.53	2385.43
5	725.83	1419.52	1440.65	2185.53	3074.83	2625.48
6	705.68	1669.62	1474.77	2385.43	2625.48	2794.90

Estimated R Correlation Matrix for ANM(TRT) 1 C

Row	Col1	Col2	Col3	Col4	Col5	Col6
1	1.0000	0.7076	0.4592	0.5437	0.4924	0.5021
2	0.7076	1.0000	0.8900	0.8742	0.6768	0.8349
3	0.4592	0.8900	1.0000	0.8812	0.7896	0.8478
4	0.5437	0.8742	0.8812	1.0000	0.8031	0.9193
5	0.4924	0.6768	0.7896	0.8031	1.0000	0.8956
6	0.5021	0.8349	0.8478	0.9193	0.8956	1.0000

ANALYSIS OF REPEATED MEASURES DESIGN
ANALYSIS AS A SPLIT PLOT DESIGN-PROC MIXED TYPE=UNSTRUCTURED

Fit Statistics

-2 Res Log Likelihood	661.4
AIC (smaller is better)	703.4
AICC (smaller is better)	721.8
BIC (smaller is better)	718.2

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
TRT	2	12	1.06	0.3782
WK	5	12	59.43	<.0001
TRT*WK	10	12	3.83	0.0157

Contrasts

Label	Num DF	Den DF	F Value	Pr > F
T1VST2WK=1	1	12	3.49	0.0864

Least Squares Means

Effect	LEVEL OF VITAMIN E	WEEK	Estimate	Standard Error	DF	t Value	Pr > |t|
TRT	C		539.13	17.1135	12	31.50	<.0001
TRT	H		565.90	17.1135	12	33.07	<.0001
TRT	L		572.27	17.1135	12	33.44	<.0001
WK		1	486.20	6.8642	12	70.83	<.0001
WK		2	535.00	9.7668	12	54.78	<.0001
WK		3	574.27	8.4959	12	67.59	<.0001
WK		4	566.80	12.6724	12	44.73	<.0001
WK		5	579.27	14.3174	12	40.46	<.0001
WK		6	613.07	13.6502	12	44.91	<.0001

ANALYSIS OF REPEATED MEASURES DESIGN
ANALYSIS AS A SPLIT PLOT DESIGN-PROC MIXED TYPE=UNSTRUCTURED

Differences of Least Squares Means

Effect	LEVEL OF VITAMIN E	WEEK	LEVEL OF VITAMIN E	WEEK	Estimate	Standard Error	df	Adjustment	Adj P
TRT	C		H		-26.7667	24.2022	12	Tukey	0.5286
TRT	C		L		-33.1333	24.2022	12	Tukey	0.3867
TRT	H		L		-6.3667	24.2022	12	Tukey	0.9627
WK		1		2	-48.8000	6.9017	12	Tukey-Kramer	0.0001
WK		1		3	-88.0667	8.1083	12	Tukey-Kramer	<.0001
WK		1		4	-80.6000	10.6354	12	Tukey-Kramer	<.0001
WK		1		5	-93.0667	12.4631	12	Tukey-Kramer	<.0001
WK		1		6	-126.87	11.8048	12	Tukey-Kramer	<.0001
WK		2		3	-39.2667	4.4577	12	Tukey-Kramer	<.0001
WK		2		4	-31.8000	6.2909	12	Tukey-Kramer	0.0030
WK		2		5	-44.2667	10.5409	12	Tukey-Kramer	0.0121
WK		2		6	-78.0667	7.6878	12	Tukey-Kramer	<.0001
WK		3		4	7.4667	6.5590	12	Tukey-Kramer	0.8563
WK		3		5	-5.0000	9.2240	12	Tukey-Kramer	0.9931
WK		3		6	-38.8000	7.8658	12	Tukey-Kramer	0.0036
WK		4		5	-12.4667	8.6124	12	Tukey-Kramer	0.7004
WK		4		6	-46.2667	5.3719	12	Tukey-Kramer	<.0001
WK		5		6	-33.8000	6.4227	12	Tukey-Kramer	0.0021

ANALYSIS OF REPEATED MEASURES DESIGN
ANALYSIS AS A REPEATED MEASURES-PROC MIXED TYPE=AR(1)

The Mixed Procedure

Model Information

Data Set	WORK.RAW
Dependent Variable	Y
Covariance Structure	Autoregressive
Subject Effect	ANM(TRT)
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Between-Within

Class Level Information

Class	Levels	Values
ANM	5	1 2 3 4 5
TRT	3	C H L
WK	6	1 2 3 4 5 6

The Mixed Procedure

Estimated R Matrix for ANM(TRT) 1 C

Row	Col1	Col2	Col3	Col4	Col5	Col6
1	1849.94	1513.00	1237.44	1012.06	827.73	676.97
2	1513.00	1849.94	1513.00	1237.44	1012.06	827.73
3	1237.44	1513.00	1849.94	1513.00	1237.44	1012.06
4	1012.06	1237.44	1513.00	1849.94	1513.00	1237.44
5	827.73	1012.06	1237.44	1513.00	1849.94	1513.00
6	676.97	827.73	1012.06	1237.44	1513.00	1849.94

Estimated R Correlation Matrix for ANM(TRT) 1 C

Row	Col1	Col2	Col3	Col4	Col5	Col6
1	1.0000	0.8179	0.6689	0.5471	0.4474	0.3659
2	0.8179	1.0000	0.8179	0.6689	0.5471	0.4474
3	0.6689	0.8179	1.0000	0.8179	0.6689	0.5471
4	0.5471	0.6689	0.8179	1.0000	0.8179	0.6689
5	0.4474	0.5471	0.6689	0.8179	1.0000	0.8179
6	0.3659	0.4474	0.5471	0.6689	0.8179	1.0000

ANALYSIS OF REPEATED MEASURES DESIGN
ANALYSIS AS A REPEATED MEASURES-PROC MIXED TYPE=AR(1)

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
AR(1)	ANM(TRT)	0.8179
Residual		1849.94

Fit Statistics

-2 Res Log Likelihood	708.6
AIC (smaller is better)	712.6
AICC (smaller is better)	712.8
BIC (smaller is better)	714.0

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
TRT	2	12	1.19	0.3385
WK	5	60	28.61	<.0001
TRT*WK	10	60	1.51	0.1590

Contrasts

Label	Num DF	Den DF	F Value	Pr > F
T1VST2WK=1	1	60	1.33	0.2530

Least Squares Means

Effect	LEVEL OF VITAMIN E	WEEK	Estimate	Standard Error	DF	t Value	Pr > |t|
TRT	C		539.13	16.1359	12	33.41	<.0001
TRT	H		565.90	16.1359	12	35.07	<.0001
TRT	L		572.27	16.1359	12	35.47	<.0001
WK		1	486.20	11.1054	60	43.78	<.0001
WK		2	535.00	11.1054	60	48.17	<.0001
WK		3	574.27	11.1054	60	51.71	<.0001
WK		4	566.80	11.1054	60	51.04	<.0001
WK		5	579.27	11.1054	60	52.16	<.0001
WK		6	613.07	11.1054	60	55.20	<.0001

ANALYSIS OF REPEATED MEASURES DESIGN
ANALYSIS AS A REPEATED MEASURES-PROC MIXED TYPE=AR(1)

Differences of Least Squares Means

Effect	LEVEL OF VITAMIN E	WEEK	LEVEL OF VITAMIN E	WEEK	Estimate	Standard Error	df	Adjustment	Adj P
TRT	C		H		-26.7667	22.8197	12	Tukey	0.4905
TRT	C		L		-33.1333	22.8197	12	Tukey	0.3470
TRT	H		L		-6.3667	22.8197	12	Tukey	0.9581
WK		1		2	-48.8000	6.7026	60	Tukey-Kramer	<.0001
WK		1		3	-88.0667	9.0370	60	Tukey-Kramer	<.0001
WK		1		4	-80.6000	10.5696	60	Tukey-Kramer	<.0001
WK		1		5	-93.0667	11.6745	60	Tukey-Kramer	<.0001
WK		1		6	126.8700	12.5058	60	Tukey-Kramer	<.0001
WK		2		3	-39.2667	6.7026	60	Tukey-Kramer	<.0001
WK		2		4	-31.8000	9.0370	60	Tukey-Kramer	0.0103
WK		2		5	-44.2667	10.5696	60	Tukey-Kramer	0.0013
WK		2		6	-78.0667	11.6745	60	Tukey-Kramer	<.0001
WK		3		4	7.4667	6.7026	60	Tukey-Kramer	0.8737
WK		3		5	-5.0000	9.0370	60	Tukey-Kramer	0.9936
WK		3		6	-38.8000	10.5696	60	Tukey-Kramer	0.0065
WK		4		5	-12.4667	6.7026	60	Tukey-Kramer	0.4364
WK		4		6	-46.2667	9.0370	60	Tukey-Kramer	<.0001
WK		5		6	-33.8000	6.7026	60	Tukey-Kramer	<.0001

Comparison of 3 models in Repeated Measures Design

The following are the Estimated Correlation Matrices for the p observations on an EU, that is, $Corr(Y_{ijk},\ Y_{ij'k})$ for $j,\ j' = 1, 2, \ldots, p = 6$

Estimated Correlation Matrix with No restrictions on Correlations:

Row	Col1	Col2	Col3	Col4	Col5	Col6
1	1.0000	0.7076	0.4592	0.5437	0.4924	0.5021
2	0.7076	1.0000	0.8900	0.8742	0.6768	0.8349
3	0.4592	0.8900	1.0000	0.8812	0.7896	0.8478
4	0.5437	0.8742	0.8812	1.0000	0.8031	0.9193
5	0.4924	0.6768	0.7896	0.8031	1.0000	0.8956
6	0.5021	0.8349	0.8478	0.9193	0.8956	1.0000

Estimated Correlation Matrix with Compound Symmetry Constraint:

Row	Col1	Col2	Col3	Col4	Col5	Col6
1	1.0000	0.7169	0.7169	0.7169	0.7169	0.7169
2	0.7169	1.0000	0.7169	0.7169	0.7169	0.7169
3	0.7169	0.7169	1.0000	0.7169	0.7169	0.7169
4	0.7169	0.7169	0.7169	1.0000	0.7169	0.7169
5	0.7169	0.7169	0.7169	0.7169	1.0000	0.7169
6	0.7169	0.7169	0.7169	0.7169	0.7169	1.0000

Estimated Correlation Matrix with AR(1) Constraint:

Row	Col1	Col2	Col3	Col4	Col5	Col6
1	1.0000	0.8179	0.6689	0.5471	0.4474	0.3659
2	0.8179	1.0000	0.8179	0.6689	0.5471	0.4474
3	0.6689	0.8179	1.0000	0.8179	0.6689	0.5471
4	0.5471	0.6689	0.8179	1.0000	0.8179	0.6689
5	0.4474	0.5471	0.6689	0.8179	1.0000	0.8179
6	0.3659	0.4474	0.5471	0.6689	0.8179	1.0000

Comparison of Results from a Repeated Measures Design - 2 models

ANALYSIS AS A REPEATED MEASURES-PROC MIXED TYPE=COMPOUND SYMMETRY

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
TRT	2	12	1.06	0.3782
WK	5	60	52.55	<.0001
TRT*WK	10	60	1.80	0.0801

Least Squares Means

Effect	VITAMIN E	WEEK	Estimate	Standard Error	DF	t Value	Pr > |t|
TRT	C		539.13	17.1135	12	31.50	<.0001
TRT	H		565.90	17.1135	12	33.07	<.0001
TRT	L		572.27	17.1135	12	33.44	<.0001
WK		1	486.20	11.3033	60	43.01	<.0001
WK		2	535.00	11.3033	60	47.33	<.0001
WK		3	574.27	11.3033	60	50.81	<.0001
WK		4	566.80	11.3033	60	50.14	<.0001
WK		5	579.27	11.3033	60	51.25	<.0001
WK		6	613.07	11.3033	60	54.24	<.0001

ANALYSIS AS A REPEATED MEASURES-PROC MIXED TYPE=AR(1)

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
TRT	2	12	1.19	0.3385
WK	5	60	28.61	<.0001
TRT*WK	10	60	1.51	0.1590

Least Squares Means

Effect	VITAMIN E	WEEK	Estimate	Standard Error	DF	t Value	Pr > |t|
TRT	C		539.13	16.1359	12	33.41	<.0001
TRT	H		565.90	16.1359	12	35.07	<.0001
TRT	L		572.27	16.1359	12	35.47	<.0001
WK		1	486.20	11.1054	60	43.78	<.0001
WK		2	535.00	11.1054	60	48.17	<.0001
WK		3	574.27	11.1054	60	51.71	<.0001
WK		4	566.80	11.1054	60	51.04	<.0001
WK		5	579.27	11.1054	60	52.16	<.0001
WK		6	613.07	11.1054	60	55.20	<.0001

The SAS output provides the estimated partial correlation coefficients of the responses over the 6 weeks. The compound symmetry condition does not appear to hold. The Mauchly's test for spericity has a p-value of .0093 which implies that the split plot analysis is probably not valid. Therefore, we need to consider several alternatives:

1. The Greenhouse-Geisser and Huynh-Feldt adjustments (see textbook page 505)

   These tests of the Within Subjects effects of Week by Treatment interaction effect and Week effect use the Greenhouse-Geisser and Huynh-Feldt adjustments to the Split plot F-tests when the sphericity condition may be violated. These adjustments result in very conservative F-tests, with reduced power and hence increase chance of making a Type II error.

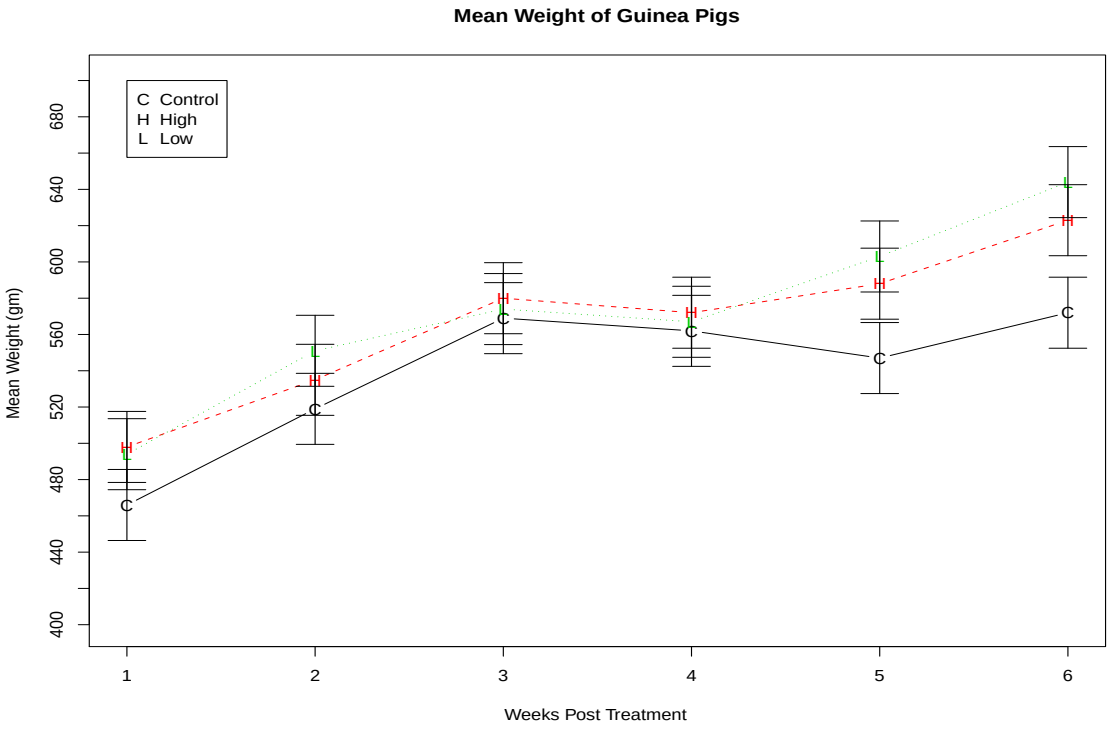
2. An unstructured covariance structure

3. An AR(1) covariance structure

	Compound Symmetry	Compound Symmetry-Adj.		Unstructured	AR(1)
		G-G	H-F		
TRT	.3782	.3782	.3782	.3782	.3385
WK	$< .0001$	$< .0001$	$< .0001$	$< .0001$	$< .0001$
TRT*WK	.0801	.1457	.1103	.0157	.1590

Thus, our conclusions may depend on the selection of the technique.

From four of the five methods of analysis, we find that there is not significant evidence (p-value=.0801 to p-value=.1590) of an interaction between the Treatment and Time factors. However, the Unstructured covariance matrix method had p-value=.0157 for the test of an interaction between treatment and week. The profile plot supports the conclusion of no interaction after taking into account the size of the standard error of the treatment by time sample mean: $\widehat{SE}(\bar{Y}_{ij.}) = 19.5780$



Since the interaction was not significant, the main effects of Treatment and Time can be analyzed separately. The p-values for Treatment differences ranged from $p-value = 0.3385$ to $p-value = 0.3782$. The p-values for a Time differences were all $p - value < .0001$. The mean weights of the pigs varies across the six weeks but there is not significant evidence of a difference in the mean weights for the three levels of Vitamin E feed supplements. Therefore, the two levels of Vitamin E supplement do not appear to provide an increase in the mean weight of the pigs in comparison to the control, which was a zero level of Vitamin E supplement. The mean weights appear to follow a cubic relationship with time during the six weeks (the p-values for the linear, quadratic, and cubic trends were $< .0001, .0016, < .0001$, respectively). The conclusions are all conditional on whether there is significant evidence of a deviation from Compound Symmetry or if not which covariance structure seems to best fit the data.

A Multivariate Analysis of Repeated Measures Experiment: MANOVA

The most complete method of analyzing a repeated measures design with observations at p time points is consider the observation as a p-dimensional variable. The model

$$Y_{ijk} = \mu_{ij} + e_{ijk}, \quad \text{for} \quad i = 1, \ldots, t; \quad j = 1, \ldots, p; \quad k = 1, \ldots, r;$$

where Y_{ijk} is the observation from the kth EU receiving treatment i during the jth time period, is written as

$$\mathbf{Y}_{ik} = \boldsymbol{\mu}_i + \mathbf{e}_{ik}, \quad \text{with} \qquad \mathbf{Y}_{ik} = \begin{pmatrix} Y_{i1k} \\ Y_{i2k} \\ \vdots \\ Y_{ipk} \end{pmatrix} \qquad \boldsymbol{\mu}_i = \begin{pmatrix} \mu_{i1} \\ \mu_{i2} \\ \vdots \\ \mu_{ip} \end{pmatrix} \qquad \mathbf{e}_{ik} = \begin{pmatrix} e_{i1k} \\ e_{i2k} \\ \vdots \\ e_{ipk} \end{pmatrix}$$

where $\mathbf{e}_{ik}$ is distributed p-dimensional normal, $N_p(0, \boldsymbol{\Sigma})$ with $\boldsymbol{\Sigma}$ a pxp variance-covariance matrix. The hypothesis of no treatment difference becomes:

$$H_o : \begin{pmatrix} \mu_{11} \\ \mu_{12} \\ \vdots \\ \mu_{1p} \end{pmatrix} = \begin{pmatrix} \mu_{21} \\ \mu_{22} \\ \vdots \\ \mu_{2p} \end{pmatrix} = \cdots = \begin{pmatrix} \mu_{t1} \\ \mu_{t2} \\ \vdots \\ \mu_{tp} \end{pmatrix}.$$

Under the null hypothesis, the t treatments have the same mean at all p time points. The hypothesis is tested using the Wilk's $\boldsymbol{\Lambda}$ statistic, with $\boldsymbol{\Lambda} = \frac{|E|}{|E+H|}$, where E is the matrix of pooled sums of squares and cross products within treatment groups and H is the matrix of sums of squares and cross products between treatment groups. The null hypothesis, H_o is rejected when $\boldsymbol{\Lambda} < \boldsymbol{\Lambda}_\alpha$. There are several other variations on this test based on the eigenvalues of the matrix $E^{-1}H$. Let $\boldsymbol{\lambda}' = (\lambda_1, \lambda_2, \ldots, \lambda_p)$, then four popular test statistics for testing H_o are:

- Wilk's $\boldsymbol{\Lambda}$: $\Lambda = \frac{|E|}{|E+H|} = \prod_{j=1}^{p} \frac{1}{1+\lambda_j}$
- Lawley-Hotelling Trace: $U = \sum_{j=1}^{p} \lambda_j$
- Pillai's Trace: $V = \sum_{j=1}^{p} \frac{\lambda_j}{1+\lambda_j}$
- Roy's Largest Root: $\Theta = \frac{\lambda_{(p)}}{1+\lambda_{(p)}}$, where $\lambda_{(}p) = max(\lambda_1, \lambda_2, \ldots, \lambda_p)$

A discussion of the various aspects of these statistics is in any multivariate book (see STAT 636 or STAT 616). Wilk's $\boldsymbol{\Lambda}$ is the most widely used of these statistics.

A rejection of the null hypothesis requires further investigation of the nature of the difference in the treatment means across the p time points. First test for an interaction between the treatment and time. That is, test

$$H_o : \mu_{i,j} - \mu_{i,j-1} = \mu_{i',j} - \mu_{i',j-1} \quad \text{for} \quad i \neq i' = 1, \ldots = t, \quad \text{and} \quad j = 2, \ldots, p \quad \text{vs}$$

$$H_a : \mu_{i,j} - \mu_{i,j-1} \neq \mu_{i',j} - \mu_{i',j-1} \quad \text{for at least one} \quad i \neq i'$$

These hypotheses can be written in matrix form as

$$H_o : \mathbf{C}\boldsymbol{\mu}_1 = \mathbf{C}\boldsymbol{\mu}_2 = \cdots = \mathbf{C}\boldsymbol{\mu}_t \quad \text{vs} \quad H_a : \quad \text{not all equalities hold}$$

where

$$\mathbf{C} = \begin{bmatrix} 1 & -1 & 0 & 0 & \cdots & 0 \\ 0 & 1 & -1 & 0 & \cdots & 0 \\ \vdots & \vdots & \vdots & \vdots & \cdots & \vdots \\ 0 & 0 & \cdots & 0 & 1 & -1 \end{bmatrix}$$

The above hypotheses are testing the "parallelism" of the treatment by time interaction profiles. This set of hypotheses can be tested using Wilks $\mathbf{\Lambda}$ with test statistics: $\mathbf{\Lambda}_1 = \frac{|CEC'|}{|C(E+H)C'|}$.

Case 1: If H_o is rejected, then there is substantial evidence in the data of a treatment by time interaction effect. That is, the size of the differences in any specific pair of treatments depends on the time period.

Case 2: If

$$H_o : \mu_{i,j} - \mu_{i,j-1} = \mu_{i',j} - \mu_{i',j-1} \quad \text{for} \quad i \neq i' = 1, \ldots = t, \quad \text{and} \quad j = 2, \ldots, p$$

is not rejected but

$$H_o : \begin{pmatrix} \mu_{11} \\ \mu_{12} \\ \vdots \\ \mu_{1p} \end{pmatrix} = \begin{pmatrix} \mu_{21} \\ \mu_{22} \\ \vdots \\ \mu_{2p} \end{pmatrix} = \cdots = \begin{pmatrix} \mu_{t1} \\ \mu_{t2} \\ \vdots \\ \mu_{tp} \end{pmatrix}.$$

is rejected then the treatment profiles are parallel but at least two of the treatments differ by a specific amount.

Case 3: If both null hypotheses are rejected, then it is possible to conclude that there is substantial evidence of a time effect. However, the time effect varies depending on which treatment is used.

Case 3a: If

$$H_o : \mu_{i,j} - \mu_{i,j-1} = \mu_{i',j} - \mu_{i',j-1} \quad \text{for} \quad i \neq i' = 1, \ldots = t, \quad \text{and} \quad j = 2, \ldots, p$$

is not rejected or

Case 3b: If

$$H_o : \mu_{i,j} - \mu_{i,j-1} = \mu_{i',j} - \mu_{i',j-1} \quad \text{for} \quad i \neq i' = 1, \ldots = t, \quad \text{and} \quad j = 2, \ldots, p$$

is rejected but

$$H_o : \begin{pmatrix} \mu_{11} \\ \mu_{12} \\ \vdots \\ \mu_{1p} \end{pmatrix} = \begin{pmatrix} \mu_{21} \\ \mu_{22} \\ \vdots \\ \mu_{2p} \end{pmatrix} = \cdots = \begin{pmatrix} \mu_{t1} \\ \mu_{t2} \\ \vdots \\ \mu_{tp} \end{pmatrix}.$$

is not rejected

then we can conclude there is substantial evidence in the data that the t treatment means $\boldsymbol{\mu}_i$ are equal or are parallel planes in p dimensional space.

Case 4: To test for a time trend, when there is not a treatment by time interaction, we can test for trends in the $\mu_{\cdot j}$'s :

$$H_o : \mathbf{C}\boldsymbol{\mu}_{\cdot} = 0 \quad \text{vs} \quad H_a : \mathbf{C}\boldsymbol{\mu}_{\cdot} \neq 0$$

where $\mathbf{C}$ is the (p-1)xp matrix of trend coefficients for testing for polynomial trends in the levels of the time factor and $\boldsymbol{\mu}_{\cdot}^{'} = (\mu_{\cdot 1}, \mu_{\cdot 1}, \ldots, \mu_{\cdot p})$ is the vector of mean responses at time j averaged over all t treatments.

Case 4a: If the time factor has equal spacings then we can use the coefficients given in the textbook

Case 4b: If the time factor has unequal spacings then we would need to obtain them manually.

The Hotelling's t-statistic can be used to test this set of hypotheses:

$$T^2 = \mathbf{t}r \left[C\bar{Y}_{\cdot\cdot}\right]' \left[\frac{CEC'}{k(r-1)}\right]^{-1} \left[C\bar{Y}_{\cdot\cdot}\right].$$

The null hypothesis is rejected when $T^2 \geq T_\alpha^2$.

The MANOVA analysis of a repeated measures experiment has the major advantage that it provides an exact test of the various hypotheses without having to impose an restrictions on the variance-covariance structure in the experiment as was required in the split plot analysis.

The major disadvantage of MANOVA is that the variance-covariance matrix $\boldsymbol{\Sigma}$ must be estimated from the data. Because $\boldsymbol{\Sigma}$ is a symmetric p x p matrix there are p(p+1)/2 parameters that must be estimated from the data. Thus, the experimenter must have enough data to obtain reasonable estimates of these parameters, i.e., r must be reasonably large. The SAS output for the MANOVA analysis of the pig experiment is given here:

SAS program for a Multivariate Analysis:

```
option ls=90 ps=55 nocenter nodate;
title 'ANALYSIS OF REPEATED MEASURES DESIGN';
data RAW;
INPUT ANM  TRT $ @;
DO WK = 1 TO 6;
INPUT Y @; OUTPUT; END;
LABEL WK = 'WEEK' TRT = 'LEVEL OF VITAMIN E' Y='WEIGHT';
cards;
 1 C 455 460 510 504 436 466
 2 C 467 565 610 596 542 587
 3 C 445 530 580 597 582 619
 4 C 485 542 594 583 611 612
 5 C 480 500 550 528 562 576
 1 L 514 560 565 524 552 597
 2 L 440 480 536 484 567 569
 3 L 495 570 569 585 576 677
 4 L 520 590 610 637 671 702
 5 L 503 555 591 605 649 675
 1 H 496 560 622 622 632 670
 2 H 498 540 589 557 568 609
 3 H 478 510 568 555 576 605
 4 H 545 565 580 601 633 649
 5 H 472 498 540 524 532 583
RUN;
TITLE2 'ANALYSIS AS A MULTIVARIATE DESIGN-PROC GLM';
*Need to reconfigure data set:;
DATA MULTDATA; SET RAW;
 IF WK=1 THEN DO; W1=Y;
    RETAIN W1; END;
 IF WK=2 THEN DO; W2=Y;
    RETAIN W2; END;
 IF WK=3 THEN DO; W3=Y;
    RETAIN W3; END;
 IF WK=4 THEN DO; W4=Y;
    RETAIN W4; END;
 IF WK=5 THEN DO; W5=Y;
    RETAIN W5; END;
 IF WK=6 THEN DO; W6=Y;
    RETAIN W6; END;
 DROP OBS W;
 IF WK=6;
 RUN;
PROC PRINT DATA=MULTDATA;
VAR ANM TRT W1-W6;
PROC GLM DATA=MULTDATA;
 CLASS ANM TRT WK;
 MODEL W1-W6 = TRT / NOUNI;
 MANOVA H=TRT;
 REPEATED WK 6 (1 2 3 4 5 6 ) POLYNOMIAL / PRINTE SUMMARY;
 LSMEANS TRT/STDERR PDIFF ADJUST=TUKEY;
RUN;
```

ANALYSIS OF REPEATED MEASURES DESIGN
ANALYSIS AS A MULTIVARIATE DESIGN-PROC GLM

Obs	ANM	TRT	W1	W2	W3	W4	W5	W6
1	1	C	455	460	510	504	436	466
2	2	C	467	565	610	596	542	587
3	3	C	445	530	580	597	582	619
4	4	C	485	542	594	583	611	612
5	5	C	480	500	550	528	562	576
6	1	L	514	560	565	524	552	597
7	2	L	440	480	536	484	567	569
8	3	L	495	570	569	585	576	677
9	4	L	520	590	610	637	671	702
10	5	L	503	555	591	605	649	675
11	1	H	496	560	622	622	632	670
12	2	H	498	540	589	557	568	609
13	3	H	478	510	568	555	576	605
14	4	H	545	565	580	601	633	649
15	5	H	472	498	540	524	532	583

The GLM Procedure

Class Level Information

Class	Levels	Values
ANM	5	1 2 3 4 5
TRT	3	C H L
WK	1	6

Number of Observations Read 15
Number of Observations Used 15

Multivariate Analysis of Variance

MANOVA Test Criteria and F Approximations for the Hypothesis of No Overall TRT Effect
H = Type III SSCP Matrix for TRT
E = Error SSCP Matrix

S=2 M=1.5 N=2.5

Statistic	Value	F Value	Num DF	Den DF	Pr > F
Wilks' Lambda	0.08793025	2.77	12	14	0.0363
Pillai's Trace	1.40330988	3.14	12	16	0.0176
Hotelling-Lawley Trace	4.78594837	2.63	12	8.2712	0.0852
Roy's Greatest Root	2.76663572	3.69	6	8	0.0464

NOTE: F Statistic for Roy's Greatest Root is an upper bound.
NOTE: F Statistic for Wilks' Lambda is exact.

Repeated Measures Analysis of Variance

Repeated Measures Level Information

Dependent Variable	W1	W2	W3	W4	W5	W6
Level of WK	1	2	3	4	5	6

Partial Correlation Coefficients from the Error SSCP Matrix / Prob > |r|

DF = 12	W1	W2	W3	W4	W5	W6
W1	1.000000	0.707584	0.459151	0.543739	0.492366	0.502098
		0.0068	0.1145	0.0548	0.0874	0.0804
W2	0.707584	1.000000	0.889996	0.874228	0.676753	0.834899
	0.0068		<.0001	<.0001	0.0111	0.0004
W3	0.459151	0.889996	1.000000	0.881217	0.789575	0.847786
	0.1145	<.0001		<.0001	0.0013	0.0003
W4	0.543739	0.874228	0.881217	1.000000	0.803051	0.919350
	0.0548	<.0001	<.0001		0.0009	<.0001
W5	0.492366	0.676753	0.789575	0.803051	1.000000	0.895603
	0.0874	0.0111	0.0013	0.0009		<.0001
W6	0.502098	0.834899	0.847786	0.919350	0.895603	1.000000
	0.0804	0.0004	0.0003	<.0001	<.0001	

E = Error SSCP Matrix

WK_N represents the nth degree polynomial contrast for WK

	WK_1	WK_2	WK_3	WK_4	WK_5
WK_1	17752.61	-2920.74	-1877.33	-3078.25	693.41
WK_2	-2920.74	3750.41	-1225.09	-600.08	-516.46
WK_3	-1877.33	-1225.09	4472.04	1673.70	1639.19
WK_4	-3078.25	-600.08	1673.70	2388.59	1538.65
WK_5	693.41	-516.46	1639.19	1538.65	4188.95

Sphericity Tests

Variables	DF	Mauchly's Criterion	Chi-Square	Pr > ChiSq
Transformed Variates	14	0.0544835	29.389556	0.0093
Orthogonal Components	14	0.0544835	29.389556	0.0093

MANOVA Test Criteria and Exact F Statistics for the Hypothesis of no WK Effect
H = Type III SSCP Matrix for WK
E = Error SSCP Matrix

S=1 M=1.5 N=3

Statistic	Value	F Value	Num DF	Den DF	Pr > F
Wilks' Lambda	0.03881848	39.62	5	8	<.0001
Pillai's Trace	0.96118152	39.62	5	8	<.0001
Hotelling-Lawley Trace	24.76092347	39.62	5	8	<.0001
Roy's Greatest Root	24.76092347	39.62	5	8	<.0001

MANOVA Test Criteria and F Approximations for the Hypothesis of no WK*TRT Effect
H = Type III SSCP Matrix for WK*TRT
E = Error SSCP Matrix

S=2 M=1 N=3

Statistic	Value	F Value	Num DF	Den DF	Pr > F
Wilks' Lambda	0.17905151	2.18	10	16	0.0793
Pillai's Trace	1.07058517	2.07	10	18	0.0856
Hotelling-Lawley Trace	3.19076786	2.42	10	9.6	0.0937
Roy's Greatest Root	2.66824588	4.80	5	9	0.0205

NOTE: F Statistic for Roy's Greatest Root is an upper bound.
NOTE: F Statistic for Wilks' Lambda is exact.

Tests of Hypotheses for Between Subjects Effects

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	18548.0667	9274.0333	1.06	0.3782
Error	12	105434.2000	8786.1833		

Univariate Tests of Hypotheses for Within Subject Effects

Source	DF	Type III SS	Mean Square	F Value	Pr > F	Adj Pr > F G - G	Adj Pr > F H - F
WK	5	142554.5000	28510.9000	52.55	<.0001	<.0001	<.0001
WK*TRT	10	9762.7333	976.2733	1.80	0.0801	0.1457	0.1103
Error(WK)	60	32552.6000	542.5433				

Greenhouse-Geisser Epsilon	0.4856
Huynh-Feldt Epsilon	0.7191

Analysis of Variance of Contrast Variables

WK_N represents the nth degree polynomial contrast for WK

Contrast Variable: WK_1

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Mean	1	123662.8810	123662.8810	83.59	<.0001
TRT	2	3138.8362	1569.4181	1.06	0.3765
Error	12	17752.6114	1479.3843		

Contrast Variable: WK_2

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Mean	1	5928.007143	5928.007143	18.97	0.0009
TRT	2	4348.376190	2174.188095	6.96	0.0099
Error	12	3750.414286	312.534524		

Contrast Variable: WK_3

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Mean	1	10462.67593	10462.67593	28.07	0.0002
TRT	2	237.19185	118.59593	0.32	0.7334
Error	12	4472.03778	372.66981		

Contrast Variable: WK_4

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Mean	1	798.192857	798.192857	4.01	0.0684
TRT	2	1889.757143	944.878571	4.75	0.0303
Error	12	2388.585714	199.048810		

Contrast Variable: WK_5

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Mean	1	1702.743122	1702.743122	4.88	0.0474
TRT	2	148.571958	74.285979	0.21	0.8113
Error	12	4188.950794	349.079233		

Least Squares Means
Adjustment for Multiple Comparisons: Tukey

TRT	W1 LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
C	466.400000	11.889211	<.0001	1
H	497.800000	11.889211	<.0001	2
L	494.400000	11.889211	<.0001	3

Least Squares Means for effect TRT

Dependent Variable: W1

i/j	1	2	3
1		0.1904	0.2578
2	0.1904		0.9778
3	0.2578	0.9778	

TRT	W2 LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
C	519.400000	16.916658	<.0001	1
H	534.600000	16.916658	<.0001	2
L	551.000000	16.916658	<.0001	3

Least Squares Means for effect TRT

Dependent Variable: W2

i/j	1	2	3
1		0.8039	0.4110
2	0.8039		0.7762
3	0.4110	0.7762	

TRT	W3 LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
C	568.800000	14.715298	<.0001	1
H	579.800000	14.715298	<.0001	2
L	574.200000	14.715298	<.0001	3

Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: W3

i/j	1	2	3
1		0.8590	0.9637
2	0.8590		0.9610
3	0.9637	0.9610	

TRT	W4 LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
C	561.600000	21.949184	<.0001	1
H	571.800000	21.949184	<.0001	2
L	567.000000	21.949184	<.0001	3

Least Squares Means for effect TRT
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: W4

i/j	1	2	3
1		0.9425	0.9835
2	0.9425		0.9869
3	0.9835	0.9869	

TRT	W5 LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
C	546.600000	24.798521	<.0001	1
H	588.200000	24.798521	<.0001	2
L	603.000000	24.798521	<.0001	3

Dependent Variable: W5

i/j	1	2	3
1		0.4831	0.2798
2	0.4831		0.9072
3	0.2798	0.9072	

TRT	W6 LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
C	572.000000	23.642758	<.0001	1
H	623.200000	23.642758	<.0001	2
L	644.000000	23.642758	<.0001	3

Least Squares Means for effect TRT
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: W6

i/j	1	2	3
1		0.3117	0.1205
2	0.3117		0.8110
3	0.1205	0.8110	

From the output for MANOVA:

The Wilk's $\mathbf{\Lambda}$ test of a Week by Treatment interaction effect has a p-value=.0793 and the Wilk's $\mathbf{\Lambda}$ test of a Week effect has a p-value< .0001.

An evaluation of the trends in the weeks can be performed using contrasts in the Week variable. Because there are 6 equally spaced time points, 5 orthogonal polynomials are fit to the 6 time periods:
WK.1 = Linear Trend, WK.2 = Quadratic Trend, WK.3 = Cubic Trend,

WK.4 = Quartic Trend, WK.5 = Quintic Trend,

These 5 contrasts summarize the responses across the repeated factor (Time Period). The five trends are now considered as 5 separated responses, each of which can be analyzed in a CRD with $t = 3$ treatments and $r = 5$ replications. The results are summarized below:

1. No significant evidence of a difference in the Linear Trends (p-value=.3765)
2. Significant evidence of a difference in the Quadratic Trends (p-value=.0099)
3. No significant evidence of a difference in the Cubic Trends (p-value=.7334)
4. No significant evidence of a difference in the Quartic Trends (p-value=.0303)
5. No significant evidence of a difference in the Quintic Trends (p-value=.8113)

The p-values would be compared to $\alpha_{pc} = \frac{.05}{5} = .01$ using the Bonferroni multiple testing procedure. These conclusions are confirmed by a **careful** examination of the plot on Page 49 of this handout.

A CROSS-OVER DESIGN

In a Repeated Measures Experiment, the EU receives a Treatment and then the EU has multiple observations or measurements made on it over time or space. The EU **Does Not** receive a New Treatment between Measurements.

In a Crossover Design, each EU is observed under each of the t Treatments during t observation times. That is, every EU has multiple treatments applied to it and then a new measurement or observation is obtained.

Because the Treatments are compared on the Same EU's, the between EU variation is greatly reduced. The individual EU's serve as blocks in order to reduce the experimental variation (reduced SSE) and hence increase the efficiency of the estimation of the treatment means.

When comparing treatments, the effect of the Time Period in which the treatment was applied comes into the analysis. Differences in observations may be due to treatment differences and/or time period differences. CrossOver Designs are constructed to avoid confounding the Time Period Effects with the Treatment Effects:

Suppose we have 3 treatments: A_1, A_2, A_3 with each treatment applied to each of 12 patients during 3 time periods: P_1, P_2, P_3. The drugs were applied in the same order to all 12 patients:

	Time Period		
Patients	1	2	3
1	A_1	A_2	A_3
2	A_1	A_2	A_3
3	A_1	A_2	A_3
4	A_1	A_2	A_3
5	A_1	A_2	A_3
6	A_1	A_2	A_3
7	A_1	A_2	A_3
8	A_1	A_2	A_3
9	A_1	A_2	A_3
10	A_1	A_2	A_3
11	A_1	A_2	A_3
12	A_1	A_2	A_3

Let Y_{ijk} be the response of Patient k under Treatment i during Time Period k

Treatment Means : $\bar{Y}_{1..}$, $\bar{Y}_{2..}$, $\bar{Y}_{3..}$,

Time Period Means : $\bar{Y}_{.1.}$, $\bar{Y}_{.2.}$, $\bar{Y}_{.3.}$,

From the data, a large difference was observed in the treatment means: $\bar{y}_{1..}, \bar{y}_{2..}, \bar{y}_{3..}$. Was this difference due to Treatment Differences or Time Period Differences? With the above design, it would be impossible to determine. The sample mean responses for evaluation the differences in the effect of the 3 Time Periods are identical to the 3 Treatment Means. That is, with this design, the effects of Treatment and Time Period are Confounded.

Thus, it is necessary to consider multiple Sequences in which the Treatments, A_1, A_2, A_3 are administered to the EU's: There are $3! = 6$ possible sequences in which the three treatments could be administered to the 12 subjects during the three treatment periods.

	Time Period		
Sequence	1	2	3
1	A_1	A_2	A_3
2	A_2	A_3	A_1
3	A_3	A_1	A_2
4	A_2	A_1	A_3
5	A_3	A_2	A_1
6	A_1	A_3	A_2

The experimenter could then randomly assign 2 patients to each of the 6 sequences. Then, there would not be confounding between the effects due to treatments, sequences, and time period. Every treatment is observed in every sequences and in every time period.

EXAMPLE: Duration Effect of Three Formulations of a Drug

Twelve males volunteered to participate in a study to compare the effect of three formulations of a drug product: Formulation 1 was a 5-mg tablet, Formulation 2 was a 100-mg tablet, and Formulation 3 was a sustained-release capsule.

The experimenter selected the first 3 of the 6 sequences and randomly assigned 4 subjects to each sequence. The following model describes the study:

$$Y_{ijk} = \mu + \alpha_i + b_{j(i)} + \gamma_k + \tau_{d(i,k)} + e_{ijk},$$

where

α_i, $i = 1, 2, 3$ - Sequence effect;

$b_{j(i)}$, $j = 1, 2, 3, 4$ - Patient within Sequence effect;

γ_k, $k = 1, 2, 3$; - Time Period effect;

$\tau_{d(i,k)}$ - Direct effect of the Treatment in the ith Sequence during Time Period k;

e_{ijk} - experimental error effect

On each treatment day, volunteers were given their assigned formulation and were observed to determine the duration of effect (blood pressure lowering). The experimental data is given here.

		Time Period			P(S)	Sequence
Sequence	Patient(Seq)	1	2	3	$\bar{Y}_{ij.}$	$\bar{Y}_{i..}$
	1	2.5	3.2	4.4	$3.3\bar{6}$	
1	2	2.0	2.6	3.1	$2.5\bar{6}$	$2.38\bar{3}$
$(A_1A_2A_3)$	3	1.6	2.7	3.2	2.5	
	4	0.1	1.3	1.9	1.1	
	5	2.5	3.5	1.9	$2.6\bar{3}$	
2	6	3.8	4.1	2.5	$3.4\bar{6}$	2.55
$(A_2A_3A_1)$	7	2.7	2.9	2.4	$2.6\bar{6}$	
	8	1.4	1.6	1.3	$1.4\bar{3}$	
	9	3.3	1.9	2.7	$2.6\bar{3}$	
3	10	2.1	0.6	1.5	1.40	$2.558\bar{3}$
$(A_3A_1A_2)$	11	4.6	3.3	3.2	3.70	
	12	3.0	2.5	2.0	2.50	
Time	$\bar{Y}_{..k}$	$2.4\bar{6}$	$2.51\bar{6}$	$2.508\bar{3}$		
Formulation	$\bar{Y}_{d}$	$1.88\bar{3}$	$2.4\bar{6}$	$3.141\bar{6}$		$\bar{Y}_{...} = 2.497$

The following Sum of Squares are computed from the data:(Seq=Sequence, P=Patient, F=Formulation, T=Time Period)

$$
\begin{aligned}
SS_{TOT} &= \sum_{i=1}^{n}\sum_{j=1}^{r_i}\sum_{k=1}^{p}(Y_{ijk}-\bar{Y}_{...})^2 \\
&= \sum_{i=1}^{3}\sum_{j-1}^{4}\sum_{k=1}^{3}(Y_{ijk}-2.497)^2 = 34.7497 \\
SS_{Seq} &= p\sum_{i=1}^{n} r_i(\bar{Y}_{i..}-\bar{Y}_{...})^2 \\
&= 3\sum_{i=1}^{n} 4(\bar{Y}_{i..}-\bar{Y}_{...})^2 = 12[(2.3833-2.4972)^2+(2.55-2.4972)^2+(2.5583-2.4972)^2] \\
&= 0.2339 \\
SS_{P(S)} &= p\sum_{i=1}^{n}\sum_{j=1}^{r_i}(\bar{Y}_{ij.}-\bar{Y}_{i..})^2 \\
&= 3\sum_{i=1}^{3}\sum_{j=1}^{4}(\bar{Y}_{ij.}-\bar{Y}_{i..})^2 = 22.2692 \\
SS_F &= r_{.}\sum_{d=1}^{t}(\bar{Y}_{d}-\bar{Y}_{...})^2 \\
&= 12\sum_{d=1}^{3}(\bar{Y}_{d}-\bar{Y}_{...})^2 = 12[(1.8833-2.4972)^2+(2.4667-2.4972)^2+(3.1417-2.4972)^2] \\
&= 9.5172 \\
SS_T &= r_{.}\sum_{k=1}^{p}(\bar{Y}_{..k}-\bar{Y}_{...})^2 \\
&= 12\sum_{k=1}^{3}(\bar{Y}_{..k}-\bar{Y}_{...})^2 \\
&= 12[(2.4667-2.4972)^2+(2.5167-2.4972)^2+(2.5083-2.4972)^2] = 0.0172 \\
SS_E &= SS_{TOT}-SS_{Seq}-SS_{P(S)}-SS_F-SS_T = 2.7122
\end{aligned}
$$

The corresponding AOV table is given next in which we have Sequence, Time Period, Formulations are Fixed Effects and Patient in Sequence is a Random Effect:

Source	DF	Sum Squares	Mean Squares	F	p-value
Seq	2	0.2339	0.1169	0.05	0.9541
P(Seq)	9	22.2692	2.4744		
Form	2	9.5172	4.7586	35.1	< 0.0001
Time	2	0.0172	0.0086	0.06	0.9387
Error	20	2.7122	0.1356		
Total	35	34.7497			

We can confirm these calculations with the following SAS code.

```
*CROSSOVER,WITHCARRY.SAS;
OPTIONS LS=100 PS=57 NOCENTER NODATE;
DATA DRUG;
INPUT S $ P $ D $ T $ Y C$@@;
LABEL S='SEQUENCE' P='PATIENT' D='DRUG' T='TIME' C='CARRYOVER';
CARDS;
1 1 A1 1 2.5 N    1 1 A2 2 3.2 A1    1 1 A3 3 4.4 A2
1 2 A1 1 2.0 N    1 2 A2 2 2.6 A1    1 2 A3 3 3.1 A2
1 3 A1 1 1.6 N    1 3 A2 2 2.7 A1    1 3 A3 3 3.2 A2
1 4 A1 1 0.1 N    1 4 A2 2 1.3 A1    1 4 A3 3 1.9 A2
2 1 A2 1 2.5 N    2 1 A3 2 3.5 A2    2 1 A1 3 1.9 A3
2 2 A2 1 3.8 N    2 2 A3 2 4.1 A2    2 2 A1 3 2.5 A3
2 3 A2 1 2.7 N    2 3 A3 2 2.9 A2    2 3 A1 3 2.4 A3
2 4 A2 1 1.4 N    2 4 A3 2 1.6 A2    2 4 A1 3 1.3 A3
3 1 A3 1 3.3 N    3 1 A1 2 1.9 A3    3 1 A2 3 2.7 A1
3 2 A3 1 2.1 N    3 2 A1 2 0.6 A3    3 2 A2 3 1.5 A1
3 3 A3 1 4.6 N    3 3 A1 2 3.3 A3    3 3 A2 3 3.2 A1
3 4 A3 1 3.0 N    3 4 A1 2 2.5 A3    3 4 A2 3 2.0 A1
RUN;

TITLE 'CROSSOVER DESIGN WITH TEST FOR W/O CARRYOVER-GLM' ;
PROC GLM;
CLASS S P D T;
MODEL Y = S P(S) D T;
RANDOM P(S)/TEST;
LSMEANS D/PDIFF ADJUST = TUKEY;
RUN;

TITLE 'CROSSOVER DESIGN WITH TEST FOR W/O CARRYOVER-MIXED' ;
PROC MIXED;
CLASS S P D T;
MODEL Y = S  T D/RESIDUALS;
RANDOM P(S);
LSMEANS D/ADJUST = TUKEY;
RUN;
```

CROSSOVER DESIGN WITH TEST FOR W/O CARRYOVER-GLM

The GLM Procedure

Class	Levels	Values
S	3	1 2 3
P	4	1 2 3 4
D	3	A1 A2 A3
T	3	1 2 3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	15	32.03750000	2.13583333	15.75	<.0001
Error	20	2.71222222	0.13561111		
Corrected Total	35	34.74972222			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
S	2	0.23388889	0.11694444	0.86	0.4373
P(S)	9	22.26916667	2.47435185	18.25	<.0001
D	2	9.51722222	4.75861111	35.09	<.0001
T	2	0.01722222	0.00861111	0.06	0.9387

Source	Type III Expected Mean Square
S	Var(Error) + 3 Var(P(S)) + Q(S)
P(S)	Var(Error) + 3 Var(P(S))
D	Var(Error) + Q(D)
T	Var(Error) + Q(T)

Tests of Hypotheses for Mixed Model Analysis of Variance

Source	DF	Type III SS	Mean Square	F Value	Pr > F
S	2	0.233889	0.116944	0.05	0.9541
Error: MS(P(S))	9	22.269167	2.474352		

Source	DF	Type III SS	Mean Square	F Value	Pr > F
P(S)	9	22.269167	2.474352	18.25	<.0001
D	2	9.517222	4.758611	35.09	<.0001
T	2	0.017222	0.008611	0.06	0.9387
Error: MS(Error)	20	2.712222	0.135611		

Least Squares Means Adjustment for Multiple Comparisons: Tukey

D	Y LSMEAN	LSMEAN Number
A1	1.88333333	1
A2	2.46666667	2
A3	3.14166667	3

Dependent Variable: Y

i/j	1	2	3
1		0.0026	<.0001
2	0.0026		0.0006
3	<.0001	0.0006	

CROSSOVER DESIGN WITH TEST FOR W/O CARRYOVER-MIXED

The Mixed Procedure

Class Level Information

Class	Levels	Values
S	3	1 2 3
P	4	1 2 3 4
D	3	A1 A2 A3
T	3	1 2 3

Covariance Parameter Estimates

Cov Parm	Estimate
P(S)	0.7796
Residual	0.1356

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
S	2	9	0.05	0.9541
T	2	20	0.06	0.9387
D	2	20	35.09	<.0001

Least Squares Means

Effect	DRUG	Estimate	Standard Error	DF	t Value	Pr > |t|
D	A1	1.8833	0.2762	20	6.82	<.0001
D	A2	2.4667	0.2762	20	8.93	<.0001
D	A3	3.1417	0.2762	20	11.38	<.0001

Differences of Least Squares Means

Effect	DRUG	DRUG	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
D	A1	A2	-0.5833	0.1503	20	-3.88	0.0009	Tukey-Kramer	0.0026
D	A1	A3	-1.2583	0.1503	20	-8.37	<.0001	Tukey-Kramer	<.0001
D	A2	A3	-0.6750	0.1503	20	-4.49	0.0002	Tukey-Kramer	0.0006

Crossover Design with Evaluation of Carryover Effect

The general setting of a crossover design will now be described.

Blocking on Subject

Suppose we have t treatments which are to be compared with respect to their mean responses. In the experiment we have either very heterogeneous EU's or a limited number of EU's and decide that each EU will be observed under all t treatments. The EU's serve as blocks and thus control the variation in response from EU to EU for a given treatment.

Order in Which Treatments are Applied

An obvious question of concern is whether or not the order in which the EU receives the treatments has an effect on the responses. There are $t!$ possible sequences in which the t treatments may be applied. Generally only a subset of the $t!$ possible sequences will be used in the study. The experimenter decides on n sequences which are of greatest interest. There will be r_i EU's randomly assigned to the ith treatment sequence which will be observed during p time periods.

Blocking on Time Period

Every treatment will be observed in every time period and on every subject. Therefore, we have two blocking variables Subjects and Time Periods.

There is generally a time delay between administering the treatments and when the response is measured on the EU. Furthermore, after the measurements are taken, there will be a further delay before the next treatment is applied in order that the effect of the previously administered treatment not have a *carryover effect* on the EU during the administering of the next treatment. This is called the *washout period*.

The following model would be applicable:

$$Y_{ijk} = \mu + \alpha_i + b_{j(i)} + \gamma_k + \tau_{d(i,k)} + \lambda_{c(i,k-1)} + e_{ijk}$$

where

- $i = 1, \ldots, n; \quad j = 1, \ldots, r_i; \quad k = 1, \ldots, p$
- μ is the overall mean response,
- α_i is the effect of the ith sequence,
- $b_{j(i)}$ is the random effect for the jth EU in the ith sequence,
- γ_k is the kth time period effect,
- $\tau_{d(i,k)}$ is the direct effect of the Treatment applied in Sequence i during Time Period k
- $\lambda_{c(i,k-1)}$ is the carryover effect of the Treatment applied in Sequence i during Time Period $k-1$

Note that there is randomization of the subjects to the sequences. Furthermore, there are two sizes of EU's. The **EU for Sequence is "Subject"** and the **EU for Treatment is "Time Period"**. The Sequence effect measures some form of the Time Period by Treatment Interaction and may be an indication of a Carryover Effect and/or Correlation in the measurements over Time Periods.

When the Carryover effect is highly significant, only the data from the **First Time Period** is used in testing for Treatment effects.

A particular unique characteristic of the Crossover Design is that each Subject receives all t Treatments. A degree of balance is obtained in the crossover design by having each treatment follow every other treatment the same number of times in the study, each treatment occurs the same number of times in each time period, and each treatment is observed only once on each EU. These characteristics create some particular advantages and disadvantages for the Crossover Design:

Advantages:

1. Reduction in the Between EU variation (Subject is serving as a blocking variable
2. Increases the precision in comparing treatment means
3. Reduction in experimental cost when EU's are expensive and/or difficult to recruit for study and/or difficult/expensive to maintain during study.

Disadvantages:

1. May be a carryover effect which will invalidate much of the study
2. Reduced information/coverage of the population of EU's

There is a further complication with the above model besides the potential of the carryover effect. There are t observations on each EU under the t different treatments. Thus, we have a multivariate response on each EU, not a single response. Under special conditions, which were discussed in the Repeated Measures section of this course, we can validly analyze the data as an univariate experiment. This would result because the condition of Compound Symmetry may be a reasonable condition in Crossover Designs due to the Washout Time between treatment applications to the experimental units.

Furthermore, if there was not a carryover effect then we could analyze the experiment as a Latin Square Design with Blocking Variables: Sequence and Time Period. In the previous example, we did not consider the carryover effect in the model. In order to include this term in the model it is necessary to run several models and determine the change in sum of squares for error due to excluding particular terms from the model. In order to accomplish this we must run PROC GLM in order to obtain all the pertinent sums of squares:

```
*CROSSEX,WITHCARRY.SAS;
OPTIONS LS=100 PS=57 NOCENTER NODATE;
DATA DRUG;
INPUT S $ P $ D $ T $ Y C$@@;
LABEL S='SEQUENCE' P='PATIENT' D='DRUG' T='TIME' C='CARRYOVER';
CARDS;


1 1 A1 1 2.5 N    1 1 A2 2 3.2 A1    1 1 A3 3 4.4 A2
1 2 A1 1 2.0 N    1 2 A2 2 2.6 A1    1 2 A3 3 3.1 A2
1 3 A1 1 1.6 N    1 3 A2 2 2.7 A1    1 3 A3 3 3.2 A2
1 4 A1 1 0.1 N    1 4 A2 2 1.3 A1    1 4 A3 3 1.9 A2

2 1 A2 1 2.5 N    2 1 A3 2 3.5 A2    2 1 A1 3 1.9 A3
2 2 A2 1 3.8 N    2 2 A3 2 4.1 A2    2 2 A1 3 2.5 A3
2 3 A2 1 2.7 N    2 3 A3 2 2.9 A2    2 3 A1 3 2.4 A3
2 4 A2 1 1.4 N    2 4 A3 2 1.6 A2    2 4 A1 3 1.3 A3

3 1 A3 1 3.3 N    3 1 A1 2 1.9 A3    3 1 A2 3 2.7 A1
3 2 A3 1 2.1 N    3 2 A1 2 0.6 A3    3 2 A2 3 1.5 A1
3 3 A3 1 4.6 N    3 3 A1 2 3.3 A3    3 3 A2 3 3.2 A1
3 4 A3 1 3.0 N    3 4 A1 2 2.5 A3    3 4 A2 3 2.0 A1


RUN;



TITLE 'CROSSOVER DESIGN WITH TEST FOR CARRYOVER-GLM' ;
PROC GLM;
CLASS S P D T C;
MODEL Y = S P(S) D T C;
RANDOM P(S)/TEST;
MEANS P(S) S D T;
LSMEANS D/PDIFF ADJUST = TUKEY;
RUN;



TITLE 'CROSSOVER DESIGN WITH TEST FOR CARRYOVER-MIXED' ;
PROC MIXED;
CLASS S P D T C;
MODEL Y = S  T D C/RESIDUALS;
RANDOM P(S);
LSMEANS D/ADJUST = TUKEY;
RUN;
```

CROSSOVER DESIGN WITH TEST FOR CARRYOVER - PROC GLM

The GLM Procedure

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	17	32.68638889	1.92272876	16.77	<.0001
Error	18	2.06333333	0.11462963		
Corrected Total	35	34.74972222			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
S	2	0.05583333	0.02791667	0.24	0.7864
P(S)	9	22.26916667	2.47435185	21.59	<.0001
D	2	3.98433333	1.99216667	17.38	<.0001
T	1	0.00041667	0.00041667	0.00	0.9526
C	2	0.64888889	0.32444444	2.83	0.0853

Source	Type III Expected Mean Square
S	Var(Error) + 1.5 Var(P(S)) + Q(S)
P(S)	Var(Error) + 3 Var(P(S))
D	Var(Error) + Q(D)
T	Var(Error) + Q(T)
C	Var(Error) + Q(C)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
S	2	0.055833	0.027917	0.02	0.9787
Error	9.8426	12.741209	1.294491		

Error: 0.5*MS(P(S)) + 0.5*MS(Error)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
P(S)	9	22.269167	2.474352	21.59	<.0001
D	2	3.984333	1.992167	17.38	<.0001
T	1	0.000417	0.000417	0.00	0.9526
C	2	0.648889	0.324444	2.83	0.0853
Error: MS(Error)	18	2.063333	0.114630		

Least Squares Means
Adjustment for Multiple Comparisons: Tukey

D	Y LSMEAN	LSMEAN Number
A1	Non-est	1
A2	Non-est	2
A3	Non-est	3

CROSSOVER DESIGN WITH TEST FOR CARRYOVER - PROC MIXED

Class	Levels	Values
S	3	1 2 3
P	4	1 2 3 4
D	3	A1 A2 A3
T	3	1 2 3
C	4	A1 A2 A3 N

Covariance Parameter Estimates

Cov Parm	Estimate
P(S)	0.7866
Residual	0.1146

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
S	2	9	0.02	0.9787
T	1	18	0.00	0.9526
D	2	18	17.38	<.0001
C	2	18	2.83	0.0853

Least Squares Means

Effect	DRUG	Estimate	Standard Error	DF	t Value	Pr > |t|
D	A1	Non-est	.	.	.	.
D	A2	Non-est	.	.	.	.
D	A3	Non-est	.	.	.	.

From the above printout we have that

The F-tests for Carryover effect is then given by

$$F = \frac{.6489/2}{2.0633/18} = 2.83 \;\; \text{with} df = 2, 18 \Rightarrow p - value = .0853$$

The F-tests for Formulation effect is then given by

$$F = \frac{3.9844/2}{2.0633/18} = 17.38 \;\; \text{with} df = 2, 18 \Rightarrow p - value < .0001$$

Because there was not a significant Carryover Effect, we can thus conclude that there is significant evidence of a difference in the mean responses from the three drugs.

Note, that in the output from the model containing the carryover effect the Least Squares Means for the three Drugs are Non-Estimable. Therefore, we would use the output from the model without the carryover effect to evaluate differences in the three drug means:

MODEL Y = S T D / RESIDUALS;

Least Squares Means from PROC MIXED

Effect	DRUG	Estimate	Standard Error	DF	t Value	Pr > |t|
D	A1	1.8833	0.2762	20	6.82	<.0001
D	A2	2.4667	0.2762	20	8.93	<.0001
D	A3	3.1417	0.2762	20	11.38	<.0001

Differences of Least Squares Means

Effect	DRUG	DRUG	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
D	A1	A2	-0.5833	0.1503	20	-3.88	0.0009	Tukey-Kramer	0.0026
D	A1	A3	-1.2583	0.1503	20	-8.37	<.0001	Tukey-Kramer	<.0001
D	A2	A3	-0.6750	0.1503	20	-4.49	0.0002	Tukey-Kramer	0.0006

A Balanced Row-Column Design for a Crossover Experiment

Suppose we have 3 Treatments which will be administered to 12 EU's in one of 6 Sequences of Treatments. That is, The six possible sequences of treatments will be randomly assigned to the 12 EU's. However, the order of treatment application to each of the EU's was not randomized. Rather, each of the six sequences of the three treatments must be present with equal frequency to avoid confounding the period effects with the treatment effects and to have a design balanced for carryover effects.

	Sequence					
Period	1	2	3	4	5	6
I	T_1	T_2	T_3	T_1	T_2	T_3
	$\downarrow$	$\downarrow$	$\downarrow$	$\downarrow$	$\downarrow$	$\downarrow$
II	T_2	T_3	T_1	T_3	T_1	T_2
	$\downarrow$	$\downarrow$	$\downarrow$	$\downarrow$	$\downarrow$	$\downarrow$
III	T_3	T_1	T_2	T_2	T_3	T_1

The above design has 6 sequences of 3 treatments occurring in 3 time periods. There are 2 EU's randomly assigned to each of the 6 sequences. This design achieves balance in several ways:

1. Each Treatment follows every other treatment twice within each of the 6 sequences
   (a) $T_1 \rightarrow T_2$ in Sequences 1 and 3
   (b) $T_1 \rightarrow T_3$ in Sequences 4 and 5
   (c) $T_2 \rightarrow T_1$ in Sequences 5 and 6
   (d) $T_2 \rightarrow T_3$ in Sequences 1 and 2
   (e) $T_3 \rightarrow T_1$ in Sequences 2 and 3
   (f) $T_3 \rightarrow T_2$ in Sequences 4 and 6
2. Every treatment appears in every sequence
3. Treatments appear equally often in each time period (twice)
4. Each EU is observed under every treatment and in every time period