An Introduction to

The Analysis of Crossover Designs

Using SAS

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Introduction

Crossover designs form one class of commonly-used experimental designs. They are called Crossover Designs because experimental units receive first one treatment and are then typically crossed over to receive a second and perhaps a third or a fourth treatment. That is, each experimental unit is administered each treatment in a predetermined sequence.

Crossover designs, like other designs, are used to compare the effects of treatments on experimental units. Crossover designs are appropriate for experiments in which experimental units are expensive and few in number.

In a crossover design, the between-experimental-unit variation is eliminated by applying all treatments to the same experimental unit. However, one problem which may occur is that the effect of the current treatment may carry over and affect the effect of the next treatment. This problem is called the carryover effect. Carryover effects occur when the effect of the current treatment has not worn off by the time the next treatment is applied. Sometimes a carryover effect can be eliminated or minimized by inserting a rest or washout period between administrations of the treatments.

The simplest crossover design has two different treatments denoted by A and B. Half of experimental units receive A first and then crossover to B. The other half receive B first and then cross over to A. Thus, there are two different treatment sequences $A \rightarrow B$ and $B \rightarrow A$.

In this design, two treatments, A and B , are studied for two equal length periods. The basic pattern of this design is

Period	Sequence	
	1	2
1	A	B
2	B	A

Units are chosen at random and assigned at random to a sequence of treatments.

The main advantage of a crossover design is that the treatments are compared within units. That is, all treatments are observed on the same experimental unit. Therefore, every unit can provide a direct comparison of treatments. The disadvantage is possibly encountering a carryover effect.

The typical crossover design model consists of a sequence effect, a period effect, a treatment effect, carryover effects and an experimental unit error term. More details will be given when discussing examples.

A brief history

Crossover designs are used frequently in clinical trial experiments. However, the earliest applications were in agriculture field experiments. The first crossover design referred in the literature appeared in 1852. It was run by John Bennett Lawes and Baron Justus von Liebig who disagreed about the nutrition of crop plants. Lawes and J.H. Gilbert seem to be the first to have been explicitly concerned with carryover effects.

The foundation on which all ensuing work on crossover designs rested was a paper by Cochran, Antrey and Cannon in 1941. They explained 3 feeding ways carried over the period of a single lactation of eighteen Holstein cows.

Williams, in 1949, formalized the ideas of Cochran, Antrey and Cannon. In 1950, he produced a follow up paper in which designs balanced for pairs of residual effects are considered in more detail. Quenouille, in 1953, was the first to put forward the idea of a completely balanced crossover design. In 1955, Federer gave a design for three treatment, six sequences and seven periods for estimation of direct and residual effects.

In 1961, Sheehe and Bross gave a procedure which is easier than Williams for constructing designs which are balanced for preceding treatments. In 1969, Davis and Hall discussed cyclic incomplete block designs interpreted as crossover designs. Petterson, in 1973, showed how the cyclic designs of Quenouille could be extended to a design for V treatments, 2V periods and V^2 sequences . In the same year, Hall and Williams introduced cyclic superimposed design.

Berenblut and Webb, in 1974, showed that if there was an autocorrelated error structure then Williams(1949) designs minimized the generalized variance for randomized block and latin square arrangements. In 1975, Hedayat and Afsarinejad gave a summary of designs balanced with respect to sets of direct and residual effects.

This is only a brief description of some of the early uses of crossover designs. For more information of review and use of these designs see Bishop and Jones (1984) and Jones and Kenward (1989).

Definitions, Assumptions and Models

For crossover designs, we assume that t treatments are to be compared. There are s sequence groups and experimental units within each group receive t treatments in a specific sequence corresponding to that group. The n_i experimental units are randomly assigned to each sequence.

For example, three treatments (A, B and C) can be compared by using three periods. There might be six sequences of subject corresponding to the six different treatment sequences, as shown in Table 1.

Table 1 Six Sequences with Three Treatments

Sequence	Period		
	1	2	3
1	A	B	C
2	A	C	B
3	B	A	C
4	B	C	A
5	C	A	B
6	C	B	A

A model to describe the response corresponding to the k^{th} unit in period j of sequence i is

$$Y_{ijk} = \mu + S_{ik} + \pi_j + \tau_{(i,j)} + \lambda_{(i,j-1)} + e_{ijk}$$

where

μ is the grand mean.

S_{ik} is the effect of k in sequence i , i = 1,2,3,...,s , k = 1,2,3,...,n_i

π_j is the effect of period j , j = 1,2,3,...,p

$\tau_{(i,j)}$ is the direct effect of the treatment administered in period j of sequence i

$\lambda_{(i,j-1)}$ is the effect of the carryover of treatment administered in period j-1 of sequence i . By definition, $\lambda_{(i,0)} = 0$.

e_{ijk} is a random error for unit k in period j in sequence i such that

$$e_{ijk} \sim N(0, \sigma^2)$$

So, for example, the model terms for the three responses observed on the k^{th} unit in each of groups 1 and 2 of our six-group example would be:

Group 1 : $Y_{11k} = \mu + S_{1k} + \pi_1 + \tau_1 + e_{11k}$

$$Y_{12k} = \mu + S_{1k} + \pi_2 + \tau_2 + \lambda_1 + e_{12k}$$

$$Y_{13k} = \mu + S_{1k} + \pi_3 + \tau_3 + \lambda_2 + e_{13k}$$

Group 2 : $Y_{21k} = \mu + S_{2k} + \pi_1 + \tau_1 + e_{21k}$

$$Y_{22k} = \mu + S_{2k} + \pi_2 + \tau_3 + \lambda_1 + e_{22k}$$

$$Y_{23k} = \mu + S_{2k} + \pi_3 + \tau_2 + \lambda_3 + e_{23k}$$

For example, if there are 5 subjects per group, the ANOVA Table is shown in Table 2:

Table 2 Analysis of Variance for six-group example

Source	d.f.
Between units	29
Within units	
Periods	2
Treatments	2
Carryover	2
Residual	54
Total	89

Two-period crossover designs

The two-period crossover design for two treatments, (with s=2 and p=2), is also called the 2×2 crossover design. There are two possible sequences. Each unit is assigned to either sequence 1 (A$\rightarrow$B) or sequence 2 (B$\rightarrow$A). The expected response for units is as follows:

Sequence	Period 1	Period 2
1	$\mu+\pi_1+\tau_1$	$\mu+\pi_2+\tau_2+\lambda_1$
2	$\mu+\pi_1+\tau_2$	$\mu+\pi_2+\tau_1+\lambda_2$

We assume the S_{ik}'s are random effects which are independent and identically distributed with mean 0 and variance σ_s^2. τ_1 and τ_2 are the direct treatment effects of treatment A and B, and λ_1 and λ_2 are the corresponding carryover effects, respectively.

The Analysis of Variance

The analysis-of-variance table for 2×2 crossover designs was first presented by Grizzle in 1965, but his results were only correct for the special case of $n_1 = n_2$. A correct table was presented by Hills and Armitage in 1979 as shown in Table 3.

Table 3 Analysis of Variance Table

Source	d.f.	SS	EMS	F
Between-subjects:				
Carry-over	1	$\frac{2n_1n_2}{(n_1+n_2)}\left(\bar{y}_{1..}-\bar{y}_{2..}\right)^2$	$\frac{2n_1n_2}{(n_1+n_2)}\left(\lambda_1-\lambda_2\right)^2+2\sigma_s^2+\sigma^2$	$\frac{Carry-over\ MS}{B-S\ residual\ MS}$
B-S residual	$\left(n_1+n_2-2\right)$	$\sum_{i=1}^{2}\sum_{k=1}^{n_i}\frac{y_{i.k}^2}{2}-\sum_{i=1}^{2}\frac{y_{i..}^2}{2n_i}$	$2\sigma_s^2+\sigma^2$	$\frac{Direct\ Treatments\ M}{W-S\ residual\ MS}$
Within-subjects:				
Direct treatments (adjusted for Periods)	1	$\frac{n_1n_2}{2(n_1+n_2)}\left(\bar{y}_{11.}-\bar{y}_{12.}-\bar{y}_{21.}+\bar{y}_{22.}\right)^2$	$\frac{2n_1n_2}{(n_1+n_2)}\left[\left(\tau_1-\tau_2\right)-\frac{\left(\lambda_1-\lambda_2\right)}{2}\right]^2+\sigma^2$	$\frac{Periods\ MS}{W-S\ residual\ MS}$
Periods (adjusted for treatments)	1	$\frac{n_1n_2}{2(n_1+n_2)}\left(\bar{y}_{11.}-\bar{y}_{12.}+\bar{y}_{21.}-\bar{y}_{22.}\right)^2$	$\frac{2n_1n_2}{(n_1+n_2)}\left(\pi_1-\pi_2\right)^2+\sigma^2$	
W-S residual	$\left(n_1+n_2-2\right)$	$\sum_{i=1}^{2}\sum_{j=1}^{2}\sum_{k=1}^{n_i}y_{ijk}^2-\sum_{i=1}^{2}\sum_{k=1}^{n_i}\frac{y_{i.k}^2}{2}-\sum_{i=1}^{2}\sum_{j=1}^{2}\frac{y_{ij.}^2}{n_i}+\sum_{i=1}^{2}\frac{y_{i..}^2}{2n_i}$	σ^2	
Total	$2\left(n_1+n_2\right)-1$	$\sum_{i=1}^{2}\sum_{j=1}^{2}\sum_{k=1}^{n_i}y_{ijk}^2-\frac{y_{...}^2}{2\left(n_1+n_2\right)}$		

From the above table, it is obvious from the EMS column that it is only sensible to test the hypothesis that $\tau_1 = \tau_2$ if it can first be assumed that $\lambda_1 = \lambda_2$.

Example 1. Grizzle's Data

Table 4 Data from Grizzle's(1965) Paper

Treatment	Person 1	2	3	4	5	6	7	8
Sequence1								
A	0.2	0.0	-0.8	0.6	0.3	1.5		
B	1.0	-0.7	0.2	1.1	0.4	1.2		
Sequence2								
B	1.3	-2.3	0.0	-0.8	-0.4	-2.9	-1.9	-2.9
A	0.9	1.0	0.6	-0.3	-1.0	1.7	-0.3	0.9

Table 5 gives an analysis of variance table for the above data by using SAS whose code and output are shown in the Appendix I.

Table 5 Analysis of variance for Grizzle's

Source	d.f.	S.S	M.S	F	P-value
Within units:					
Carryover	1	4.57	4.57	4.57	0.0538
B-S residual	12	12.00	1.00		
Between units:					
Treatments	1	5.14	5.14	4.13	0.0649
Periods	1	6.24	6.24	5.01	0.0449
W-S residual	12	14.94	1.245		

The Interpretation

To test the null hypothesis that $\lambda_1 = \lambda_2$, an F-ratio is calculated as follows:

FC = Carryover MS / B-S residual MS

= 4.57 / 1.00 = 4.57

The associated p-value is 0.0538 , so there is marginally insufficient evidence to reject the null hypothesis at an $\alpha = 0.05$ level.

Therefore, we can proceed to test the null hypothesis that $\tau_1 = \tau_2$.

FT = Direct treatment MS / W-S residual

= 5.14 / 1.245 = 4.13

The associated p-value is 0.0649. There is insufficient evidence to reject the null hypothesis at an $\alpha = 0.05$ level.

To test the null hypothesis that $\pi_1 = \pi_2$, we calculate

FP = Periods MS / W-S residual MS

= 6.24 / 1.245 = 5.01

The associated p-value is 0.0449. At an $\alpha = 0.05$ level there is sufficient evidence to reject the null hypothesis.

Since we have only two treatments to compare, we can also test these hypotheses with two sample t-test.

Testing $\lambda_1 = \lambda_2$

For the null hypothesis that $\lambda_1 = \lambda_2$, the statistic

$$T_\lambda = \frac{\hat{\lambda}_d}{\left(\hat{\sigma}_T^2 m\right)^{\frac{1}{2}}}$$

has Student's t-distribution with $n_1 + n_2 - 2$ d.f. where

t_{ik} = $Y_{11k} + Y_{12k}$ for the k^{th} unit in sequence 1

t_{2k} = Y_{21k} + Y_{22k} for the kth unit in sequence 2

$$\hat{\lambda}_d = \bar{t}_{1.} - \bar{t}_{2.}$$

$$\hat{\sigma}_T^2 = \sum_{i=1}^{2}\sum_{k=1}^{n}\left(t_{ik} - \bar{t}_{i.}\right)^2 / n_1 + n_2 - 2 \quad \text{for the sample pooled variance}$$

$$m = \frac{n_1 + n_2}{n_1 n_2}$$

Using Grizzle's data, we obtain $\bar{t}_{1.} = 0.8333$, $\bar{t}_{2.} = -0.8$ and $\hat{\lambda}_d = 1.6333$. Also $\sum_{k=1}^{6} (t_{1k} - \bar{t}_{1.})^2 = 8.7976$ and $\sum_{k=1}^{8} (t_{2k} - \bar{t}_{2.})^2 = 15.22$. The pooled estimate of σ_T^2 is $\sigma_T^2 = 2.0011$ and the t-statistic is

$$T_\lambda = 1.6333 / (2.0011 * \frac{14}{48})^{1/2} = 2.1381$$

The critical value is $t_{.025,12} = 2.179$. There is insufficient evidence to reject the null hypothesis at $\alpha = .05$ level.

Testing $\tau_1 = \tau_2$ (assuming $\lambda_1 = \lambda_2$)

For the null hypothesis that $\tau_1 = \tau_2$ the statistic

$$T_\tau = \frac{\hat{\tau}_d}{\left(\hat{\sigma}_D^2 m / 4\right)^{\frac{1}{2}}}$$

follows a Student's t-distribution with $n_1 + n_2 - 2$ d.f. where

d_{1k} = Y_{11k} - Y_{12k} for the kth unit in sequence 1

d_{2k} = Y_{21k} - Y_{22k} for the kth unit in sequence 2

$$\hat{\tau}_d = \frac{1}{2}\left[\bar{d}_{1.} - \bar{d}_{2.}\right]$$

$$\hat{\sigma}_D^2 = \sum_{i=1}^{2}\sum_{k=1}^{n_i}\left(d_{ik} - \bar{d}_{i.}\right)^2 / n_1 + n_2 - 2$$

$$m = \frac{n_1 + n_2}{n_1 n_2}$$

Using Grizzle's data, we obtain $\bar{d}_{1.}$ = -0.2333, $\bar{d}_{2.}$ = -1.675 and $\hat{\lambda}_d$ = 0.7208. Also $\sum_{i=1}^{6}\left(d_{1k} - \bar{d}_{1.}\right)^2$ = 2.1534 and $\sum_{i=1}^{8}\left(d_{2k} - \bar{d}_{2.}\right)^2$ = 27.735. The pooled estimate of σ_D^2 is σ_D^2 = 2.4869 and the t-statistic is

$$T_\tau = 0.7208 / \left(\frac{2.4869}{4} \times \frac{14}{48}\right)^{1/2} = 1.6916.$$

The critical value is 2.179. There insufficient evidence to reject the null hypothesis at α = .05 level.

Testing $\pi_1 = \pi_2$ (assuming $\lambda_1 = \lambda_2$)

For the null hypothesis that $\pi_1 = \pi_2$ the statistic

$$T_\pi = \frac{\hat{\pi}_d}{\left(\hat{\sigma}_D^2 m / 4\right)^{\frac{1}{2}}}$$

has Student's t-distribution with $n_1 + n_2 - 2$ d.f. where

c_{1k} = Y_{11k} - Y_{12k} for the k^{th} unit in sequence 1

c_{2k} = Y_{22k} - Y_{21k} = - d_{2k} for the k^{th} unit in sequence 2

Table 6

Group 1 (AB)					Group 2 (BA)				
Subject	Period1	Period2	$t_{1.}$	$d_{1.}$	subject	Period1	Period2	$t_{2.}$	$d_{2.}$
1	0.2	1.0	1.2	-0.8	1	1.3	0.9	2.2	0.4
2	0.0	-0.7	-0.7	0.7	2	-2.3	1.0	-1.3	-3.3
3	-0.8	0.2	-0.6	-1.0	3	0.0	0.6	0.6	-0.6
4	0.6	1.1	1.7	-0.5	4	-0.8	-0.3	-1.1	-0.5
5	0.3	0.4	0.7	-0.1	5	-0.4	-1.0	-1.4	0.6
6	1.5	1.2	2.7	0.3	6	-2.9	1.7	-1.2	-4.6
					7	-1.9	-0.3	-2.2	-1.6
					8	-2.9	0.9	-2.0	-3.8
sum			5	-1.4	sum			-6.4	-13.4
mean			0.8333	-0.2333	mean			-0.8	-1.675

$$\hat{\lambda}_d = \overline{t_{1.}} - \overline{t_{2.}} = 1.6333$$

$$\hat{\tau}_d = \frac{1}{2}\left[\overline{d_{1.}} - \overline{d_{2.}}\right] = 0.7208$$

$$\hat{\pi}_d = \frac{1}{2}\left[\overline{c}_{1.} - \overline{c}_{2.}\right] = -0.9541$$

$$\sum_{i=1}^{6}\left(t_{1k} - \overline{t}_{1.}\right)^2 = 8.7976$$

$$\sum_{i=1}^{6}\left(d_{1k} - \overline{d}_{1.}\right)^2 = 2.1534$$

$$n_1 = 6$$

$$\sum_{i=1}^{8}\left(t_{2k} - \overline{t}_{2.}\right)^2 = 15.22$$

$$\sum_{i=1}^{8}\left(d_{2k} - \overline{d}_{2.}\right)^2 = 27.735$$

$$n_2 = 8$$

$$\hat{\sigma}_T^2 = 2.0011$$

$$\hat{\sigma}_D^2 = 2.4907$$

$$m = \frac{n_1 + n_2}{n_1 n_2} = \frac{14}{48}$$

$$\hat{\pi}_d = \frac{1}{2}[\bar{c}_{1.} - \bar{c}_{2.}]$$

Using Grizzle's data, we obtain $\bar{c}_{1.}$ = -0.2333, $\bar{c}_{2.}$ = 1.675 and $\hat{\pi}_d$ = -0.9541. Also σ_D^2 = 2.4869 and the t-statistic is

$$T_\pi = -0.9541 / \left(\frac{2.4869}{4} \times \frac{14}{48}\right)^{1/2} = 2.2391$$

The critical value is 2.179. There sufficient evidence to reject the null hypothesis at α = .05 level.

Two Treatments in a Three-period Crossover Design

There are several possible sequences that can be constructed by using three-period designs with two treatments. In this case, the four sequences were selected. There are three different four-sequence designs which can be constructed by using different pairing of the dual sequences. A dual of a sequence is obtained by interchanging the A and B treatment labels. For example, the dual of ABB is BAA. These three designs are listed below.

Table 7

Sequence	Period		
	1	2	3
1	A	B	A
2	A	B	B
3	B	A	B
4	B	A	A

Table 8

Sequence	Period		
	1	2	3
1	A	B	B
2	B	A	A
3	A	A	B
4	B	B	A

Table 9

Sequence	Period		
	1	2	3
1	A	B	A
2	B	A	B
3	A	A	B
4	B	B	A

The model can be written as

$$Y_{ijk} = \mu + S_{ik} + \pi_j + \tau_{(i,j)} + \lambda_{(i,j-1)} + e_{ijk}$$

The terms in this model are described in the previous topic.

To make the analysis easier, we define

$$x_{ik} = \begin{cases} 0 & \text{if } k = 1 \\ 1 & \text{if the treatment in period } k = 1 \text{ is } A \\ -1 & \text{if the treatment in period } k = 1 \text{ is } B \end{cases}$$

where x_{ik} is treated as a continuous variable and reparameterize the model as

$$Y_{ijk} = \mu + S_{ik} + \pi_j + \tau_{(i,j)} + \lambda x_{ik} + e_{ijk}$$

The expected responses for the different experimental units are as follows:

Period	Sequence			
	1	2	3	4
1	$\mu+\pi_1+\tau_1$	$\mu+\pi_1+\tau_1$	$\mu+\pi_1+\tau_2$	$\mu+\pi_1+\tau_2$
2	$\mu+\pi_2+\tau_2+\lambda_1$	$\mu+\pi_2+\tau_2+\lambda_1$	$\mu+\pi_2+\tau_1+\lambda_2$	$\mu+\pi_2+\tau_1+\lambda_2$
3	$\mu+\pi_3+\tau_1+\lambda_2$	$\mu+\pi_3+\tau_2+\lambda_2$	$\mu+\pi_3+\tau_2+\lambda_1$	$\mu+\pi_3+\tau_1+\lambda_1$

The Analysis of Variance

There are two estimates of $\tau_1 - \tau_2$ available. The first one is a within-experimental-unit comparison. The another is a between-experimental-unit comparison.

Table 10 Within-Experimental-Unit ANOVA Table

Source	d.f
Experimental units	n-1
Treatment	1
Carryover	1
Period	2
W-S residual	pn-n-4

Table 11 Between-Experimental-Unit ANOVA Table

Source	d.f.
Treatment	1
B-S residual	n-2

To get a single estimate of $\tau_1 - \tau_2$, the two estimates are combined using the method for combining two estimates of the same parameter with different variances. The combined within-between experimental unit estimate of $\tau_1 - \tau_2$ is

$$\left(\hat{\tau_1 - \tau_2}\right)_c = \frac{\left(1/\hat{\sigma}_b^2\right)\left(\hat{\tau_1 - \tau_2}\right)_b + \left(1/\hat{\sigma}_w^2\right)\left(\hat{\tau_1 - \tau_2}\right)_w}{\left(1/\hat{\sigma}_b^2\right) + \left(1/\hat{\sigma}_w^2\right)}.$$

The large sample variance of the combined estimate is

$$Var\left[\left(\hat{\tau_1 - \tau_2}\right)_c\right] = \frac{\sigma_b^2 \sigma_w^2}{\sigma_b^2 + \sigma_w^2}$$

and can be estimated by

$$\hat{\sigma}_C^2 = \frac{\hat{\sigma}_b^2 \hat{\sigma}_w^2}{\hat{\sigma}_b^2 + \hat{\sigma}_w^2}.$$

A confidence interval of $\tau_1 - \tau_2$ can be constructed by using an approximate t-value as

$$t^*_{.025} = \frac{\left(1/\hat{\sigma}_b^2\right) t_{\alpha/2,u} + \left(1/\hat{\sigma}_w^2\right) t_{\alpha/2,v}}{\left(1/\hat{\sigma}_b^2\right) + \left(1/\hat{\sigma}_w^2\right)}.$$

where

u is the d.f. of the between error mean square

v is the d.f. of the within error mean square.

Example 2. Data from Milliken and Johnson (1992) :

Table 12 Data of a crossover design with two treatments in three periods

Sequence	Period	Treatment	Person 1	2	3	4	5
1	1	A	25.1	22.0	25.3		
	2	B	27.6	24.3	27.7		
	3	A	24.5	21.6	25.7		
2	1	A	26.9	20.3	25.9	25.2	
	2	B	28.7	24.0	28.7	26.6	
	3	B	28.1	25.0	28.0	28.5	
3	1	B	25.5	27.4	26.2		
	2	A	23.7	27.9	27.1		
	3	B	24.9	24.6	25.0		
4	1	B	20.3	25.1	22.2	25.8	22.5
	2	A	22.2	26.2	25.0	26.5	23.6
	3	A	20.6	25.7	22.9	24.5	20.9

Table 13 and Table 14 give an analysis of variance table for the above data by using SAS whose code and output are shown in the Appendix II.

Table 13 Within-Experimental-Unit ANOVA Table for Data in Table 12

Source	d.f.	S.S	M.S	F
Experimental units	14	178.49		
Treatment	1	12.37	12.37	13.07
Carryover	1	4.78	4.78	5.09
Period	2	22.14	11.07	11.78
W-S residual	26	24.43	0.94	

Table 14 Between-Experimental-Unit ANOVA Table for Data in Table 12

Source	d.f.	S.S	M.S	F
Treatment	1	15.38	15.38	4.53
B-S residual	13	44.12	3.39	

From output in Appendix II,

$$\left(\tau_1 \hat{-} \tau_2\right)_w = -1.189 \qquad \hat{\sigma}_w = 0.3277$$

$$\left(\tau_1 \hat{-} \tau_2\right)_b = -6.089 \qquad \hat{\sigma}_b = 2.8602$$

So, we get for the combined within-between experimental unit estimate of $\tau_1 - \tau_2$

$$\left(\tau_1 \hat{-} \tau_2\right)_c = -1.252 \,,$$

$$t^*_{.025} = 2.057 \,,$$

and $\hat{\sigma}^2_c = 0.1060$.

Thus, a 95% confidence interval of $\tau_1 - \tau_2$ is

$-1.252 \pm 2.057(0.1060)^{1/2}$ or

(-1.922 , -0.582)

At an $\alpha = .05$ level , there is sufficient evidence to reject the null hypothesis, $\tau_1 - \tau_2$. Subjects receiving treatment B tend to have a higher response than subjects receiving treatment A .

Three Treatments in a Three-Period Crossover Design.

There are many possible sequences that can be constructed by using three period designs with three treatments. In this case, the six sequences were selected because we assume each treatment occurs in each sequence.

Table 15 Sequence of three treatments with three period

Period	Sequence 1	2	3	4	5	6
1	A	A	B	B	C	C
2	B	C	A	C	A	B
3	C	B	C	A	B	A

The model can be written as

$$Y_{ijk} = \mu + S_{ik} + \pi_j + \tau_{(i,j)} + \lambda x_{ik} + e_{ijk}$$

The expected responses for the different experimental units are as follows:

Table 16 The expected response for unit

Sequence	Period 1	2	3
1	$\mu+\pi_1+\tau_1$	$\mu+\pi_2+\tau_2+\lambda_1$	$\mu+\pi_3+\tau_3+\lambda_2$
2	$\mu+\pi_1+\tau_1$	$\mu+\pi_2+\tau_3+\lambda_1$	$\mu+\pi_3+\tau_2+\lambda_3$
3	$\mu+\pi_1+\tau_2$	$\mu+\pi_2+\tau_1+\lambda_2$	$\mu+\pi_3+\tau_3+\lambda_1$
4	$\mu+\pi_1+\tau_2$	$\mu+\pi_2+\tau_3+\lambda_2$	$\mu+\pi_3+\tau_1+\lambda_3$
5	$\mu+\pi_1+\tau_3$	$\mu+\pi_2+\tau_1+\lambda_3$	$\mu+\pi_3+\tau_2+\lambda_1$
6	$\mu+\pi_1+\tau_3$	$\mu+\pi_2+\tau_2+\lambda_3$	$\mu+\pi_3+\tau_1+\lambda_2$

We define a new carryover parameter as

$$\lambda_{i(k-1)} = \lambda_1 x_{1_{i(k-1)}} + \lambda_2 x_{2_i(k-1)} + \lambda_3 x_{3_i(k-1)}$$

where

$$x_{1_{i(k-1)}} = \begin{cases} 1 \textit{ if treatment A occurred in period } k-1 \textit{ of sequence } i \\ 0 \textit{ if otherwise} \end{cases}$$

Similarly, $x_{2_i(k-1)}$ and $x_{3_i(k-1)}$ are defined. Then the model can be reparameterized as :

$$Y_{ijk} = \mu + S_{ik} + \pi_j + \tau_{(i,j)} + \lambda_1 x_{1_{i(k-1)}} + \lambda_2 x_{2_i(k-1)} + \lambda_3 x_{3_i(k-1)} + e_{ijk}$$

The Analysis of Variance

In this case, the between-experimental unit comparisons consists of information about the carryover effect.

Table 17 Within-Experimental-Unit ANOVA Table

Source	d.f
Experimental units	n-1
Treatment	2
Carryover	2
Period	2
W-S residual	pn-n-6

Table 18 Between-Experimental-Unit ANOVA Table

Source	d.f
Carryover	2
B-S residual	n-3

Example 3. Data from Milliken and Johnson (1992) :

Table 19 Data from a Three-period Crossover Design with Three treatments

Sequence	Period	Treatment	Experimental unit 1	2	3	4	5	6
1	1	A	20.1	23.3	23.4	19.7	19.2	22.2
	2	B	20.3	24.8	24.8	21.3	20.9	22.0
	3	C	25.6	28.7	28.3	25.7	25.9	26.2
2	1	A	24.7	23.8	23.6	20.2	19.8	21.5
	2	C	29.4	28.7	26.4	26.2	23.7	25.5
	3	B	27.5	24.1	25.0	21.4	23.3	20.8
3	1	B	24.3	26.4	19.9	23.9	20.5	21.8
	2	A	23.2	26.4	23.7	26.8	23.2	23.6
	3	C	30.1	32.3	25.5	30.8	26.3	29.1
4	1	B	20.9	21.9	22.0	23.3	18.8	24.6
	2	C	27.5	28.6	27.4	30.7	27.9	29.8
	3	A	24.3	23.1	24.5	26.6	24.6	26.6
5	1	C	24.0	25.9	25.5	27.9	25.3	25.7
	2	A	21.8	23.7	22.0	25.4	26.4	24.7
	3	B	21.6	23.9	23.4	24.4	25.8	24.9
6	1	C	23.2	23.9	28.0	24.6	27.7	21.5
	2	B	18.9	21.5	25.3	22.7	23.5	18.1
	3	A	23.8	25.4	28.1	23.8	25.6	22.8

Table 20 and Table 21 give an analysis of variance table for the above data by using SAS whose code and output are shown in the Appendix III.

Table 20 Within-Experimental-Unit ANOVA Table for data in table 19

Source	d.f	S.S	M.S	F
Experimental units	35	360.97		
Treatment	2	249.72	124.86	124.86
Carryover	2	4.45	2.23	2.23
Period	2	106.64	53.32	53.32
W-S residual	66	66.19	1.00	

Table 21 Between-Experimental-Unit ANOVA Table for data in table 19

Source	d.f	S.S	M.S	F
Carryover	2	0.0615	0.0307	0.0100
B-S residual	33	120.2609	3.64432	

From output in Appendix III, we get

Table 22 Analysis of Treatment Differences for Within-Experimental-Unit

Parameter	Estimate	Standard Error
$\tau_1 - \tau_2$	0.83	0.264
$\tau_1 - \tau_3$	-3.12	0.264
$\tau_2 - \tau_3$	-3.95	0.264
$\lambda_1 - \lambda_2$	-0.27	0.354
$\lambda_1 - \lambda_3$	0.46	0.354
$\lambda_2 - \lambda_3$	0.73	0.354

Table 23 Analysis of Treatment Differences for Between-Experimental-Unit

Parameter	Estimate	Standard Error
$\lambda_1 - \lambda_2$	-0.30	2.34
$\lambda_1 - \lambda_3$	-0.11	2.34
$\lambda_2 - \lambda_3$	0.19	2.34

At an $\alpha = 0.05$ level, there is sufficient evidence to reject the null hypothesis, $\tau_1 = \tau_2 = \tau_3$. That is, at least one of those treatments affect the response differently than the other treatments.

Comments

The difference between crossover designs from other designs is that measurements on different treatments are obtained from each unit. Each experimental unit is administered each treatment in a predetermined sequence. There are many possible crossover designs, but each design depends on the number of treatments and the number of periods and sequence chosen. Thus, there are also many models involving those effects. Therefore, the model and ANOVA table can not be written in general form.

In this writing project, the purpose is to analyze the basic crossover designs using SAS. Several examples were used to develop a better understanding. All three examples showed the important methods for analyzing crossover designs including SAS code and output. However, there are other crossover designs which are not discussed in this project. In addition, the real experimental method may be more complicated than these examples. On the other hand, the methods discussed in this project could be a foundation of analysis of these more complicated crossover designs.

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APPENDIX I

SAS CODE:

```
dm 'log;clear;out;clear;';
 data Grizzle;
 infile 'Grizzle.dat';
 input seq $ per $ treat $ rep $ unit $ resp;
 proc glm;
  class seq unit per treat ;
  model resp = seq unit treat per/ss1;
  test h=seq e=unit;
 proc glm;
  class seq unit per treat ;
  model resp = seq unit treat per/ss3;
 run;
```

SAS OUTPUT:

General Linear Models Procedure

Dependent Variable: RESP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	15	27.9658333	1.8643889	1.50	0.2435
Error	12	14.9441667	1.2453472		
Corrected Total	27	42.9100000			

R-Square	C.V.	Root MSE	RESP Mean
0.651732	-2231.903	1.11595	-0.05000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEQ	1	4.5733333	4.5733333	3.67	0.0794
UNIT	12	12.0066667	1.0005556	0.80	0.6446
TREAT	1	5.1428571	5.1428571	4.13	0.0649
PER	1	6.2429762	6.2429762	5.01	0.0449

Tests of Hypotheses using the Type I MS for UNIT as an error term

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEQ	1	4.57333333	4.57333333	4.57	0.0538

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SEQ	0	0.0000000	.	.	.
UNIT	12	12.0066667	1.0005556	0.80	0.6446
TREAT	1	3.5629762	3.5629762	2.86	0.1165
PER	1	6.2429762	6.2429762	5.01	0.0449

APPENDIX II

SAS CODE:

```
dm 'log;clear;out;clear;';
data exam2;
infile 'exam2.test';
input seq $ per $ treat $ rep $ resp unit $ carry;
p1=0;p2=0;
if treat = 'A' then p1=1;
if treat = 'B' then p2=1;
proc glm;
 class  per treat unit;
 model resp = unit treat per carry;
 contrast 'treat' treat 1 -1;
 contrast 'carryover' carry .5;
 contrast 'peroid' per 1 -1 0,per 1 0 -1;
 estimate 'A vs B' treat 1 -1;
 estimate 'caA vs caB' carry 1 -1;
run;
proc sort;
 by unit;
proc means data=exam2 noprint;
 by unit;
 var resp p1 p2;
 output out=results mean= mresp mp1 mp2 ;
proc print data=results;
proc glm data = results;
 model mresp=mp1 mp2;
 estimate 'A vs B' mp1 1 mp2 -1;
run;
```

SAS OUTPUT:

General Linear Models Procedure

Dependent Variable: RESP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	18	213.611113	11.867284	12.63	0.0001
Error	26	24.426665	0.939487		
Corrected Total	44	238.037778			

R-Square	C.V.	Root MSE	RESP Mean
0.897383	3.873642	0.96927	25.0222

Dependent Variable: RESP

Source	DF	Type I SS	Mean Square	F Value	Pr > F
UNIT	14	178.491111	12.749365	13.57	0.0001
TREAT	1	7.453444	7.453444	7.93	0.0091
PER	2	22.885002	11.442501	12.18	0.0002
CARRY	1	4.781556	4.781556	5.09	0.0327

Source	DF	Type III SS	Mean Square	F Value	Pr > F
UNIT	14	158.510654	11.322190	12.05	0.0001
TREAT	1	12.368552	12.368552	13.17	0.0012
PER	2	22.139042	11.069521	11.78	0.0002
CARRY	1	4.781556	4.781556	5.09	0.0327

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
treat	1	12.3685516	12.3685516	13.17	0.0012
carryover	1	4.7815555	4.7815555	5.09	0.0327
peroid	2	22.1390424	11.0695212	11.78	0.0002

Parameter	Estimate	T for H0: Parameter=0	Pr > |T|	Std Error of Estimate
A vs B	-1.18891408	-3.63	0.0012	0.32766983
caA vs caB	0.42679078	2.26	0.0327	0.18918027

OBS	UNIT	_TYPE_	_FREQ_	MRESP	MP1	MP2
1	1	0	3	25.7333	0.66667	0.33333
2	10	0	3	26.1000	0.33333	0.66667
3	11	0	3	21.0333	0.66667	0.33333
4	12	0	3	25.6667	0.66667	0.33333
5	13	0	3	23.3667	0.66667	0.33333
6	14	0	3	25.6000	0.66667	0.33333
7	15	0	3	22.3333	0.66667	0.33333
8	2	0	3	22.6333	0.66667	0.33333
9	3	0	3	26.2333	0.66667	0.33333
10	4	0	3	27.9000	0.33333	0.66667
11	5	0	3	23.1000	0.33333	0.66667
12	6	0	3	27.5333	0.33333	0.66667
13	7	0	3	26.7667	0.33333	0.66667
14	8	0	3	24.7000	0.33333	0.66667
15	9	0	3	26.6333	0.33333	0.66667

General Linear Models Procedure

Dependent Variable: MRESP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	15.3810847	15.3810847	4.53	0.0529
Error	13	44.1159524	3.3935348		
Corrected Total	14	59.4970370			

R-Square	C.V.	Root MSE	MRESP Mean
0.258518	7.362076	1.84215	25.0222

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MP1	1	15.3810847	15.3810847	4.53	0.0529
MP2	0	0.0000000	.	.	.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
MP1	0	0	.	.	.
MP2	0	0	.	.	.

Parameter	Estimate	T for H0: Parameter=0	Pr > |T|	Std Error of Estimate
A vs B	-6.08928571	-2.13	0.0529	2.86021727

Dependent Variable: MRESP

Parameter	Estimate	T for H0: Parameter=0	Pr > |T|	Std Error of Estimate
INTERCEPT	28.13452381 B	18.30	0.0001	1.53732059
MP1	-6.08928571 B	-2.13	0.0529	2.86021727
MP2	0.00000000 B	.	.	.

APPENDIX III

SAS CODE:

```
dm 'log;clear;out;clear;';
data exam3;
infile 'exam3.dat';
input seq $ per $ treat $ rep $ resp unit $ ca cb cc;
proc glm;
 class  per treat unit;
 model resp = unit treat per ca cb cc;
 contrast 'treat' treat 1 -1 0,treat 1 0 -1;
 contrast 'carryover' ca 1 cb -1,ca 1 cc -1;
 contrast 'period' per 0 1 -1,per -1 .5 .5 ca .3333333 cb .3333333 cc .3333333;
 estimate 'A-B' treat 1 -1 0;
 estimate 'A-C' treat 1 0 -1;
 estimate 'B-C' treat 0 1 -1;
 estimate 'ca-cb' ca 1 cb -1;
 estimate 'ca-cc' ca 1 cc -1;
 estimate 'cb-cc' cb 1 cc -1;
run;
proc sort;
 by unit;
proc means data=exam3 noprint;
 by unit;
 var resp ca cb cc;
 output out=results mean= mresp mca mcb mcc;
proc glm data = results;
 model mresp=mca mcb mcc;
 contrast 'carry' mca 1 mcb -1 mcc 0,mca 1 mcb 0 mcc -1;
 estimate 'ca-cb' mca 1 mcb -1;
 estimate 'ca-cc' mca 1 mcc -1;
 estimate 'cb-cc' mcb 1 mcc -1;
run;
```

SAS OUTPUT:

General Linear Models Procedure

Dependent Variable: RESP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	41	822.034213	20.049615	19.99	0.0001
Error	66	66.186528	1.002826		
Corrected Total	107	888.220741			

R-Square	C.V.	Root MSE	RESP Mean
0.925484	4.097307	1.00141	24.4407

Source	DF	Type I SS	Mean Square	F Value	Pr > F
UNIT	35	360.967407	10.313354	10.28	0.0001
TREAT	2	349.972407	174.986204	174.49	0.0001
PER	2	106.645185	53.322593	53.17	0.0001
CA	1	0.097963	0.097963	0.10	0.7556
CB	1	4.351250	4.351250	4.34	0.0411
CC	0	0.000000	.	.	.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
UNIT	35	361.451472	10.327185	10.30	0.0001
TREAT	2	249.726361	124.863181	124.51	0.0001
PER	1	15.125000	15.125000	15.08	0.0002
CA	0	0.000000	.	.	.
CB	0	0.000000	.	.	.
CC	0	0.000000	.	.	.

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
treat	2	249.726361	124.863181	124.51	0.0001
carryover	2	4.449213	2.224606	2.22	0.1168
period	2	106.645181	53.322591	53.17	0.0001

Parameter	Estimate	T for H0: Parameter=0	Pr > |T|	Std Error of Estimate
A-B	0.82847222	3.14	0.0025	0.26389526
A-C	-3.12013889	-11.82	0.0001	0.26389526
B-C	-3.94861111	-14.96	0.0001	0.26389526
ca-cb	-0.27291667	-0.77	0.4436	0.35405264
ca-cc	0.46458333	1.31	0.1940	0.35405264
cb-cc	0.73750000	2.08	0.0411	0.35405264

Dependent Variable: MRESP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.06154321	0.03077160	0.01	0.9916
Error	33	120.26092593	3.64427048		
Corrected Total	35	120.32246914			

R-Square	C.V.	Root MSE	MRESP Mean
0.000511	7.810718	1.90900	24.4407

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MCA	1	0.03705247	0.03705247	0.01	0.9203
MCB	1	0.02449074	0.02449074	0.01	0.9352
MCC	0	0.00000000	.	.	.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
MCA	0	0	.	.	.
MCB	0	0	.	.	.
MCC	0	0	.	.	.

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
carry	2	0.06154321	0.03077160	0.01	0.9916

Parameter	Estimate	Parameter=0		Estimate
ca-cb	-0.30000000	-0.13	0.8987	2.33803459
ca-cc	-0.10833333	-0.05	0.9633	2.33803459
cb-cc	0.19166667	0.08	0.9352	2.33803459

Parameter	Estimate	T for H0: Parameter=0	Pr > |T|	Std Error of Estimate
INTERCEPT	24.42222222 B	25.59	0.0001	0.95449862
MCA	-0.10833333 B	-0.05	0.9633	2.33803459
MCB	0.19166667 B	0.08	0.9352	2.33803459
MCC	0.00000000 B	.	.	.