

## Chapter 234

# Analysis of 2x2 Cross-Over Designs using T-Tests

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### Introduction

This procedure analyzes data from a two-treatment, two-period (2x2) cross-over design. The response is assumed to be a continuous random variable that follows the normal distribution. When the normality assumption is suspect, the nonparametric Mann-Whitney U (or Wilcoxon Rank-Sum) Test may be employed.

In the two-period cross-over design, subjects are randomly assigned to one of two groups. One group receives treatment *R* followed by treatment *T*. The other group receives treatment *T* followed by treatment *R*. Thus, the response is measured at least twice on each subject.

Cross-over designs are used when the treatments alleviate a condition, rather than effect a cure. After the response to one treatment is measured, the treatment is removed and the subject is allowed to return to a baseline response level. Next, the response to a second treatment is measured. Hence, each subject is measured twice, once with each treatment.

Examples of the situations that might use a cross-over design are the comparison of anti-inflammatory drugs in arthritis and the comparison of hypotensive agents in essential hypertension. In both of these cases, symptoms are expected to return to their usual baseline level shortly after the treatment is stopped.

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### Advantages of Cross-Over Designs

A comparison of treatments on the same subject is expected to be more precise. The increased precision often translates into a smaller sample size. Also, patient enrollment into the study may be easier because each patient will receive both treatments.

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### Disadvantages of Cross-Over Designs

The statistical analysis of a cross-over experiment is more complex than a parallel-group experiment and requires additional assumptions. It may be difficult to separate the treatment effect from the time effect and the carry-over effect of the previous treatment.

The design cannot be used when the treatment (or the measurement of the response) alters the subject permanently. Hence, it cannot be used to compare treatments that are intended to effect a cure.

Because subjects must be measured at least twice, it may be more difficult to keep patients enrolled in the study. It is arguably simpler to measure a subject once than to obtain their measurement twice. This is particularly true when the measurement process is painful, uncomfortable, embarrassing, or time consuming.

## Technical Details

### Cross-Over Analysis

In the discussion that follows, we summarize the presentation of Chow and Liu (1999). We suggest that you review their book for a more detailed presentation.

The general linear model for the standard 2x2 cross-over design is

$$Y_{ijk} = \mu + S_{ik} + P_j + F_{(j,k)} + C_{(j-1,k)} + e_{ijk}$$

where  $i$  represents a subject (1 to  $n_k$ ),  $j$  represents the period (1 or 2), and  $k$  represents the sequence (1 or 2). The  $S_{ik}$  represent the random effects of the subjects. The  $P_j$  represent the effects of the two periods. The  $F_{(j,k)}$  represent the effects of the two formulations (treatments). In the case of the 2x2 cross-over design

$$F_{(j,k)} = \begin{cases} F_R & \text{if } k = j \\ F_T & \text{if } k \neq j \end{cases}$$

where the subscripts  $R$  and  $T$  represent the *reference* and *treatment* formulations, respectively.

The  $C_{(j-1,k)}$  represent the carry-over effects. In the case of the 2x2 cross-over design

$$C_{(j-1,k)} = \begin{cases} C_R & \text{if } j = 2, k = 1 \\ C_T & \text{if } j = 2, k = 2 \\ 0 & \text{otherwise} \end{cases}$$

where the subscripts  $R$  and  $T$  represent the *reference* and *treatment* formulations, respectively.

Assuming that the average effect of the subjects is zero, the four means from the 2x2 cross-over design can be summarized using the following table.

Sequence	Period 1	Period 2
1 (RT)	$\mu_{11} = \mu + P_1 + F_R$	$\mu_{21} = \mu + P_2 + F_T + C_R$
2 (TR)	$\mu_{12} = \mu + P_1 + F_T$	$\mu_{22} = \mu + P_2 + F_R + C_T$

where  $P_1 + P_2 = 0$ ,  $F_T + F_R = 0$ , and  $C_T + C_R = 0$ .

### Treatment Effect

#### Two-Sample T-Test for Treatment Effect

The presence of a treatment (drug) effect can be studied by testing whether  $F_T - F_R = H_0$  using a  $t$  test. This test is calculated as follows

$$T_d = \frac{\hat{F} - H_0}{\hat{\sigma}_d \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

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where

$$\hat{F} = \bar{d}_{.1} - \bar{d}_{.2}$$

$$\bar{d}_{.k} = \frac{1}{n_k} \sum_{i=1}^{n_k} d_{ik}$$

$$\hat{\sigma}_d^2 = \frac{1}{(n_1 - n_2 - 2)} \sum_{k=1}^2 \sum_{i=1}^{n_k} (d_{ik} - \bar{d}_{.k})^2$$

$$d_{ik} = \frac{Y_{i2k} - Y_{i1k}}{2}$$

The null hypothesis of no drug effect is rejected at the  $\alpha$  significance level if

$$|T_d| > t_{\alpha/2, n_1 + n_2 - 2}.$$

A  $100(1 - \alpha)\%$  confidence interval for  $F = F_T - F_R$  is given by

$$\hat{F} \pm (t_{\alpha/2, n_1 + n_2 - 2}) \hat{\sigma}_d \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}.$$

### Mann-Whitney U (or Wilcoxon Rank-Sum) Test for Treatment Effect

Senn (2002) pages 113-114 describes Koch's adaptation of the Wilcoxon-Mann-Whitney rank sum test that tests treatment effects in the presence of period effects. The test is based on the period differences and assumes that there are no carryover effects. Koch's method calculates the ranks of the period differences for all subjects in the trial and then uses the Mann-Whitney U (or Wilcoxon Rank-Sum) Test to analyze these differences between the two sequence groups. The Mann-Whitney U (or Wilcoxon Rank-Sum) Test is described in detail in the Two-Sample T-Test chapter of the documentation.

### Carryover Effect

The 2x2 cross-over design should only be used when there is no carryover effect from one period to the next. The presence of a carryover effect can be studied by testing whether  $C_T = C_R = 0$  using a  $t$  test. This test is calculated as follows

$$T_c = \frac{\hat{C}}{\hat{\sigma}_u \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

where

$$\hat{C} = \bar{U}_{.2} - \bar{U}_{.1}$$

$$\bar{U}_{.k} = \frac{1}{n_k} \sum_{i=1}^{n_k} U_{ik}$$

$$\hat{\sigma}_u^2 = \frac{1}{(n_1 - n_2 - 2)} \sum_{k=1}^2 \sum_{i=1}^{n_k} (U_{ik} - \bar{U}_{.k})^2$$

$$U_{ik} = Y_{i1k} + Y_{i2k}$$

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The null hypothesis of no carryover effect is rejected at the  $\alpha$  significance level if

$$|T_c| > t_{\alpha/2, n_1+n_2-2}.$$

A  $100(1-\alpha)\%$  confidence interval for  $C = C_T - C_R$  is given by

$$\hat{C} \pm (t_{\alpha/2, n_1+n_2-2}) \hat{\sigma}_u \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}.$$

### Period Effect

The presence of a period effect can be studied by testing whether  $P_1 = P_2 = 0$  using a  $t$  test. This test is calculated as follows

$$T_P = \frac{\hat{P}}{\hat{\sigma}_d \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

where

$$\hat{P} = \bar{O}_{.1} - \bar{O}_{.2}$$

$$\bar{O}_{.1} = \bar{d}_1$$

$$\bar{O}_{.2} = -\bar{d}_2$$

$$\hat{\sigma}_d^2 = \frac{1}{(n_1 - n_2 - 2)} \sum_{k=1}^2 \sum_{i=1}^{n_k} (d_{ik} - \bar{d}_{.k})^2$$

$$d_{ik} = \frac{Y_{i2k} - Y_{i1k}}{2}$$

The null hypothesis of no drug effect is rejected at the  $\alpha$  significance level if

$$|T_P| > t_{\alpha/2, n_1+n_2-2}.$$

A  $100(1-\alpha)\%$  confidence interval for  $P = P_2 - P_1$  is given by

$$\hat{P} \pm (t_{\alpha/2, n_1+n_2-2}) \hat{\sigma}_d \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}.$$

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## Data Structure

The data for a cross-over design is entered into three variables. The first variable contains the sequence number, the second variable contains the response in the first period, and the third variable contains the response in the second period. Note that each row of data represents the complete response for a single subject.

Chow and Liu (1999) give the following data on page 73. We will use these data in our examples to verify the accuracy of our calculations. These data are contained in the dataset called ChowLiu73.

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## ChowLiu73 dataset

Sequence	Period 1	Period 2
1	74.675	73.675
1	96.400	93.250
1	101.950	102.125
1	79.050	69.450
1	79.050	69.025
1	85.950	68.700
1	69.725	59.425
1	86.275	76.125
1	112.675	114.875
1	99.525	116.250
1	89.425	64.175
1	55.175	74.575
2	74.825	37.350
2	86.875	51.925
2	81.675	72.175
2	92.700	77.500
2	50.450	71.875
2	66.125	94.025
2	122.450	124.975
2	99.075	85.225
2	86.350	95.925
2	49.925	67.100
2	42.700	59.425

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## Validation

Chow and Liu (1999) use the above dataset throughout their book. Except for some obvious typographical errors that exist in their book, our results match their results exactly. We have also tested the algorithm against examples in other texts. In all cases, NCSS matches the published results.

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## Procedure Options

This section describes the options available in this procedure. To find out more about using a procedure, turn to the Procedures chapter.

Following is a list of the procedure's options.

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## Variables Tab

The options on this panel specify which variables to use.

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### Sequence Variable

#### Sequence Group Variable

Specify the variable containing the sequence number. The values in this column should be either 1 (for the first sequence) or 2 (for the second sequence).

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### Period Variables

#### Period 1 Variable

Specify the variable containing the responses for the first period of the cross-over trial, one subject per row.

#### Period 2 Variable

Specify the variable containing the responses for the second period of the cross-over trial, one subject per row.

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### Treatment Labels

#### Label 1

This is the one-letter label given to the first treatment. This identifies the treatment that occurs **first** in sequence 1. Common choices are *R* or *A*.

#### Label 2

This is the one-letter label given to the second treatment. This identifies the treatment that occurs **second** in sequence 1. Common choices are *T* or *B*.

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### Reports Tab

The options on this panel control the reports and plots.

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### Descriptive Statistics and Confidence Intervals

#### Confidence Level

This is the confidence level that is used for the confidence intervals in the Cross-Over Effects and Means Summary Report. Typical confidence levels are 90%, 95%, and 99%, with 95% being the most common.

#### Cross-Over Effects and Means Summary Report

Check this option to show the report containing parameters estimates and confidence intervals for Treatment, Period, and Carryover effects.

#### Cross-Over Analysis Detail Report

Check this option to show a report containing the estimated effects, means, standard deviations, and standard errors of various subgroups of the data.

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### Tests

#### Alpha

Alpha is the significance level used in the hypothesis tests. A value of 0.05 is most commonly used, but 0.1, 0.025, 0.01, and other values are sometimes used. Typical values range from 0.001 to 0.20.

#### H0 Value (H0: $\mu_2 - \mu_1 =$ )

This is the hypothesized difference between the two treatment groups (i.e. the treatment effect). To test only whether there is a difference between the two groups, this value is set to zero. If, for example, a researcher wished to test whether the Group 2 mean is greater than the Group 1 mean by at least 5, this value would be set to 5.

The T-Tests for Period and Carryover Effects, which only test against a null value of 0, are not affected by this choice.

## Analysis of 2x2 Cross-Over Designs using T-Tests

### Alternative Hypothesis Direction

Specify the direction of the alternative hypothesis. This choice applies to the T-Test and Mann-Whitney U Test for Treatment Effect. The T-Tests for Period and Carryover Effects, which only give the two-sided test results, are not affected by this choice.

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### Tests – Parametric

#### T-Test for Treatment Effect

Check this option to display the results of the T-Test for the Treatment Effect.

#### T-Tests for Period and Carryover Effects (Two-Sided)

Check this option to display the results of the two-sided T-Tests for Period and Carryover Effects.

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### Tests – Nonparametric

#### Mann-Whitney U Test (Wilcoxon Rank-Sum Test) for Treatment Effect

This test is a nonparametric alternative to the equal-variance t-test for use when the assumption of normality is not valid. This test uses the ranks of the values rather than the values themselves.

There are 3 different tests that can be conducted:

- **Exact Test**  
The exact test can be calculated if there are no ties and the sample size is  $\leq 20$  in both groups. This test is recommended when these conditions are met.
- **Normal Approximation Test**  
The normal approximation method may be used to approximate the distribution of the sum of ranks when the sample size is reasonably large.
- **Normal Approximation Test with Continuity Correction**  
The normal approximation with continuity correction may be used to approximate the distribution of the sum of ranks when the sample size is reasonably large.

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### Assumptions

#### Tests of Assumptions

This report gives a series of four tests of the normality assumptions.

These tests are:

- **Shapiro-Wilk Normality**
- **Skewness Normality**
- **Kurtosis Normality**
- **Omnibus Normality**

#### Assumptions Alpha

Assumptions Alpha is the significance level used in all the assumptions tests. A value of 0.05 is typically used for hypothesis tests in general, but values other than 0.05 are often used for the case of testing assumptions. Typical values range from 0.001 to 0.20.

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## Additional Information

### Show Written Explanations

Check this option to display written details about each report and plot.

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## Report Options Tab

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### Report Options

#### Variable Names

This option lets you select whether to display only variable names, variable labels, or both.

#### Value Labels

This option applies to the *Group Variable(s)*. It lets you select whether to display data values, value labels, or both. Use this option if you want the output to automatically attach labels to the values (like 1=Yes, 2=No, etc.). See the section on specifying *Value Labels* elsewhere in this manual.

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### Report Options – Decimal Places

#### Precision

Specify the precision of numbers in the report. A single-precision number will show seven-place accuracy, while a double-precision number will show thirteen-place accuracy. Note that the reports were formatted for single precision. If you select double precision, some numbers may run into others. Also note that all calculations are performed in double precision regardless of which option you select here. This is for reporting purposes only.

#### Means ... Test Statistics Decimals

Specify the number of digits after the decimal point to display on the output of values of this type. Note that this option in no way influences the accuracy with which the calculations are done.

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## Plots Tab

The options on this panel control the appearance of various plots.

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### Select Plots

#### Means Plot ... Probability Plots

Each of these options indicates whether to display the indicated plots. Click the plot format button to change the plot settings.



## Example 1 – 2x2 Cross-Over Analysis (Validation using Chow and Liu (1999))

This section presents an example of how to run an analysis of data from a 2x2 cross-over design. Chow and Liu (1999) page 73 provide an example of data from a 2x2 cross-over design. These data were shown in the Data Structure section earlier in this chapter. On page 77, they provide the following summary of the results of their analysis.

Effect	MVUE	Variance	95% CI	T	P-Value
Carryover	-9.59	245.63	(-42.10, 22.91)	-0.612	0.5468
Treatment	-2.29	13.97	(-10.03, 5.46)	-0.613	0.5463
Period	-1.73	13.97	(-9.47, 6.01)	-0.464	0.6474

We will use the data found the CHOWLIU73 database. You may follow along here by making the appropriate entries or load the completed template **Example 1** from the Template tab of the Analysis of 2x2 Cross-Over Designs using T-Tests window.

### 1 Open the ChowLiu73 dataset.

- From the **File** menu of the NCSS Data window, select **Open Example Data**.
- Click on the file **ChowLiu73.NCSS**.
- Click **Open**.

### 2 Open the Analysis of 2x2 Cross-Over Designs using T-Tests window.

- Using the Analysis menu or the Procedure Navigator, find and select the **Analysis of 2x2 Cross-Over Designs using T-Tests** procedure.
- On the menus, select **File**, then **New Template**. This will fill the procedure with the default template.

### 3 Specify the variables.

- On the Analysis of 2x2 Cross-Over Designs using T-Tests window, select the **Variables tab**.
- For **Sequence Group Variable** enter **Sequence**.
- For **Period 1 Variable** enter **Period1**.
- For **Period 2 Variable** enter **Period2**.

### 4 Specify the reports.

- On the Analysis of 2x2 Cross-Over Designs using T-Tests window, select the **Reports tab**.
- Check **Show Written Explanations**.

### 5 Run the procedure.

- From the Run menu, select **Run Procedure**. Alternatively, just click the green Run button.

The following reports and charts will be displayed in the Output window.

## Cross-Over Effects and Means Summary

Parameter	Count	Parameter Estimate	Standard Deviation	Standard Error	T*	Lower 95.0% CL	Upper 95.0% CL
Treatment Effect	24	-2.288	9.145	3.733	2.0739	-10.030	5.455
$\mu_1$ (or $\mu_R$ )	24	82.559		4.285			
$\mu_2$ (or $\mu_T$ )	24	80.272		4.395			
Period Effect	24	-1.731	9.145	3.733	2.0739	-9.474	6.011
$\mu (\text{Period}=1)$	24	82.281		4.043			
$\mu (\text{Period}=2)$	24	80.550		4.618			
Carryover Effect	24	-9.592	38.390	15.673	2.0739	-42.095	22.911
$(R+T) (Seq=1)$	12	167.627	33.234	9.594			
$(R+T) (Seq=2)$	12	158.035	42.930	12.393			

### Interpretation of the Above Report

This report shows the estimated effects, means, standard deviations, standard errors, and confidence limits of various parameters and subgroups of the data. The least squares mean of treatment R is 82.559 and of treatment T is 80.272. The treatment effect  $[(\mu_2 - \mu_1)$  or  $(\mu_T - \mu_R)]$  is estimated to be -2.288. The period effect  $[(\mu|(\text{Period}=2)) - (\mu|(\text{Period}=1))]$  is estimated to be -1.731. The carryover effect  $[(R+T)|(Seq=2) - (R+T)|(Seq=1)]$  is estimated to be -9.592. Note that least squares means are created by taking the simple average of their component means, not by taking the average of the raw data. For example, if the mean of the 20 subjects in period 1 sequence 1 is 50.0 and the mean of the 10 subjects in period 2 sequence 2 is 40.0, the least squares mean is  $(50.0 + 40.0)/2 = 45.0$ . That is, no adjustment is made for the unequal sample sizes. Also note that the standard deviation of some of the subgroups is not calculated.

This report summarizes the estimated means and the treatment, period, and carryover effects.

### Parameter

These are the items displayed on the corresponding lines. Note that the *Treatment* line is the main focus of the analysis. The *Period* and *Carryover* information is used for preliminary tests of assumptions.

### Count

The count gives the number of non-missing values. This value is often referred to as the group sample size or  $n$ .

### Parameter Estimate

These are the estimated values of the corresponding parameters. Formulas for the three effects were given in the Technical Details section earlier in this chapter.

### Standard Deviation

The sample standard deviation is the square root of the sample variance. It is a measure of spread.

### Standard Error

These are the standard errors of each of the effects. They provide an estimate of the precision of the effect estimate. The formulas were given earlier in the Technical Details section of this chapter.

### T\*

This is the t-value used to construct the confidence interval. If you were constructing the interval manually, you would obtain this value from a table of the Student's t distribution with  $n - 1$  degrees of freedom.

### Lower and Upper Confidence Limits

These values provide a confidence interval for the estimated effect.

### Interpretation of the Above Report

This section provides a written interpretation of the above report.

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## Cross-Over Analysis Detail

Seq.	Period	Treatment	Count	Least Squares Mean	Standard Deviation	Standard Error
1	1	R	12	85.823	15.691	4.530
2	2	R	12	79.296	25.198	7.274
1	2	T	12	81.804	19.712	5.690
2	1	T	12	78.740	23.207	6.699
1	Difference	(T-R)/2	12	-2.009	6.423	1.854
2	Difference	(T-R)/2	12	0.278	11.225	3.240
1	Total	R+T	12	167.627	33.234	9.594
2	Total	R+T	12	158.035	42.930	12.393
.	.	R	24	82.559		4.285
.	.	T	24	80.272		4.395
1	.	.	24	83.814		
2	.	.	24	79.018		
.	1	.	24	82.281		4.043
.	2	.	24	80.550		4.618

**Interpretation of the Above Report**

This report shows the estimated effects, means, standard deviations, and standard errors of various subgroups of the data. The least squares mean of treatment R is 82.559 and of treatment T is 80.272. Note that least squares means are created by taking the simple average of their component means, not by taking the average of the raw data. For example, if the mean of the 20 subjects in period 1 sequence 1 is 50.0 and the mean of the 10 subjects in period 2 sequence 2 is 40.0, the least squares mean is  $(50.0 + 40.0)/2 = 45.0$ . That is, no adjustment is made for the unequal sample sizes. Also note that the standard deviation and standard error of some of the subgroups are not calculated.

This report provides the least squares means of various subgroups of the data.

**Seq.**

This is the sequence number of the mean shown on the line. When the dot (period) appears in this line, the results displayed are created by taking the simple average of the appropriate means of the two sequences.

**Period**

This is the period number of the mean shown on the line. When the dot (period) appears in this line, the results displayed are created by taking the simple average of the appropriate means of the two periods.

**Treatment**

This is the treatment (or formulation) of the mean shown on the line. When the dot (period) appears in this line, the results displayed are created by taking the simple average of the appropriate means of the two treatments.

When the entry is  $(T-R)/2$ , the mean is computed on the quantities created by dividing the difference in each subject's two scores by 2. When the entry is  $R+T$ , the mean is computed on the sums of the subjects two scores.

**Count**

The count is the number of subjects in the mean.

**Least Squares Mean**

Least squares means are created by taking the simple average of their component means, not by taking a weighted average based on the sample size in each component. For example, if the mean of the 20 subjects in period 1 sequence 1 is 50.0 and the mean of the 10 subjects in period 2 sequence 2 is 40.0, the least squares mean is  $(50.0 + 40.0)/2 = 45.0$ . That is, no adjustment is made for the unequal sample sizes. Since least squares means are used in all subsequent calculations, these are the means that are reported.

## Analysis of 2x2 Cross-Over Designs using T-Tests

**Standard Deviation**

This is the estimated standard deviation of the subjects in the mean.

**Standard Error**

This is the estimated standard error of the least squares mean.

**T-Test for Treatment Effect**

Alternative Hypothesis	Mean Difference	Standard Error	T-Value	DF	Prob Level	Reject H0 at $\alpha = 0.050?$
$\mu_T - \mu_R \neq 0$	-2.288	3.733	-0.6127	22	0.54633	No

**Interpretation of the Above Report**

The two treatment means in a 2x2 cross-over study are not significantly different based on a two-sided T-Test at the 0.05 significance level (the actual probability level was 0.54633). The design has 12 subjects in sequence 1 (RT) and 12 subjects in sequence 2 (TR). The average response to treatment R is 82.559, and the average response to treatment T is 80.272. The estimated treatment effect ( $\mu_T - \mu_R$ ) is -2.288.

This report presents the T-test for the treatment effect. In this case, the treatment effect is not significant with a P-value of 0.54633.

**Alternative Hypothesis**

The (unreported) null hypothesis is

$$H_0: \mu_2 - \mu_1 = \mu_T - \mu_R = \text{Hypothesized Difference} = 0$$

and the alternative hypotheses,

$$H_a: \mu_2 - \mu_1 = \mu_T - \mu_R \neq \text{Hypothesized Difference} = 0$$

$$H_a: \mu_2 - \mu_1 = \mu_T - \mu_R < \text{Hypothesized Difference} = 0$$

$$H_a: \mu_2 - \mu_1 = \mu_T - \mu_R > \text{Hypothesized Difference} = 0$$

Since the *Hypothesized Difference* is zero in this example, the null and alternative hypotheses can be simplified to  
Null hypothesis:

$$H_0: \mu_2 = \mu_1 \quad \text{or} \quad H_0: \mu_T = \mu_R$$

Alternative hypotheses:

$$H_a: \mu_2 \neq \mu_1 \quad \text{or} \quad H_a: \mu_T \neq \mu_R,$$

$$H_a: \mu_2 < \mu_1 \quad \text{or} \quad H_a: \mu_T < \mu_R, \text{ or}$$

$$H_a: \mu_2 > \mu_1 \quad \text{or} \quad H_a: \mu_T > \mu_R.$$

In practice, the alternative hypothesis should be chosen in advance.

**Mean Difference**

This is the difference between the treatment means,  $\hat{\mu}_2 - \hat{\mu}_1$ . This is known as the treatment effect.

**Standard Error**

This is the standard error of the treatment effect. It provides an estimate of the precision of the treatment effect estimate. The formula was given earlier in the Technical Details section of this chapter.

## Analysis of 2x2 Cross-Over Designs using T-Tests

### T-Value

This is the test statistic calculated from the data that is used to test whether the effect is different from the null hypothesized value (0 in this case).

### DF

The *DF* is the value of the degrees of freedom. This is two less than the total number of subjects in the study.

### Prob Level

This is the probability level (p-value) of the test. If this value is less than the chosen significance level, then the corresponding effect is said to be significant. For example, if you are testing at a significance level of 0.05, then probabilities that are less than 0.05 are statistically significant. You should choose a value appropriate for your study.

### Reject H0 at $\alpha = 0.050$ ?

This column indicates whether or not the null hypothesis is rejected, in favor of the alternative hypothesis, based on the *p*-value and chosen  $\alpha$ . A test in which the null hypothesis is rejected is sometimes called *significant*.

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## T-Tests for Period and Carryover Effects (Two-Sided)

Parameter	Estimate	Standard Error	T-Value	DF	Prob Level	Reject H0 at $\alpha = 0.050$ ?
Period Effect	-1.731	3.733	-0.4637	22	0.64739	No
Carryover Effect	-9.592	15.673	-0.6120	22	0.54681	No

#### Interpretation of the Above Report

A preliminary test failed to reject the assumption of equal period effects at the 0.05 significance level (the actual probability level was 0.64739). A preliminary test failed to reject the assumption of equal carryover effects at the 0.05 significance level (the actual probability level was 0.54681).

This report presents the T-tests for the period and carryover effects. In this case, both are not significantly different from zero.

### Parameter

These are the items being tested. The *Period* and *Carryover* lines are preliminary tests of assumptions.

### Estimate

These are the estimated values of the corresponding effects. Formulas for these effects were given in the Technical Details section earlier in this chapter.

### Standard Error

These are the standard errors of each of the effects. They provide an estimate of the precision of the effect estimate. The formulas were given earlier in the Technical Details section of this chapter.

### T-Value

These are the test statistics calculated from the data that are used to test whether the effect is different from zero.

### DF

The *DF* is the value of the degrees of freedom. This is two less than the total number of subjects in the study.

### Prob Level

This is the probability level (p-value) of the test. If this value is less than the chosen significance level, then the corresponding effect is said to be significant. Some authors recommend that the tests of assumptions (Period and Carryover) should be done at the 0.10 level of significance.

## Mann-Whitney U or Wilcoxon Rank-Sum Location Difference Test for Treatment Effect

This test is the most common nonparametric substitute for the  $t$ -test when the assumption of normality is not valid. The test is based on the period differences and assumes that there are no carryover effects. This method calculates the ranks of the period differences for all subjects in the trial and then uses the Mann-Whitney U (or Wilcoxon Rank-Sum) Test to analyze these differences between the two sequence groups.

This test has good properties (asymptotic relative efficiency) for symmetric distributions. There are exact procedures for this test given small samples with no ties, and there are large sample approximations. When ties are present in the data, an approximate solution for dealing with ties is available. You can use the approximation provided, but know that the exact results no longer hold.

The Mann-Whitney U (or Wilcoxon Rank-Sum) Test is described in detail in the Two-Sample T-Test chapter of the documentation.

### Mann-Whitney U or Wilcoxon Rank-Sum Location Difference Test for Treatment Effect

Sequence	Mann-Whitney U	Sum of Ranks (W)	Mean of W	Std Dev of W
R/T	87.5	165.5	150	17.31674
T/R	56.5	134.5	150	17.31674

Number of Sets of Ties = 1, Multiplicity Factor = 6

Test Type	Alternative Hypothesis†	Z-Value	Prob Level	Reject H0 at $\alpha = 0.050?$
Exact*	Location Diff. $\neq 0$			
Normal Approximation	Location Diff. $\neq 0$	-0.8951	0.37074	No
Normal Approx. with C.C.	Location Diff. $\neq 0$	-0.8662	0.38637	No

† "Location Diff." refers to the location difference between the period differences of the two treatment groups (T-R).

\* The Exact Test is provided only when there are no ties and the sample size is  $\leq 20$  in both groups.

#### Interpretation of the Above Report

The two location parameters in a 2x2 cross-over study are not significantly different based on a two-sided Mann-Whitney U or Wilcoxon Rank-Sum Test based on the Normal Approximation at the 0.05 significance level (the actual probability level was 0.37074). The two location parameters in a 2x2 cross-over study are not significantly different based on a two-sided Mann-Whitney U or Wilcoxon Rank-Sum Test based on the Normal Approximation with Continuity Correction at the 0.05 significance level (the actual probability level was 0.38637). The design had 12 subjects in sequence 1 (R/T) and 12 subjects in sequence 2 (T/R). The sum of ranks of the period differences in the first sequence, R/T, was 165.5. The sum of ranks of the period differences in the second sequence, T/R, was 134.5.

This report presents the results of the Wilcoxon-Mann-Whitney test for treatment effect. The null and alternative hypotheses relate to the equality or non-equality of the central tendency of the two distributions of period differences. If a hypothesized difference other than zero is used, the software adds the hypothesized difference to each value of Group 2, and the test is run based on the original Group 1 values and the transformed Group 2 values.

The exact test is only provided when there are no ties and the sample size is less than or equal to 20 in both sequence groups.

## Tests of Assumptions Section

This section presents the results of tests for checking the normality assumption.

Tests of the Normality Assumption for the Period Differences in Sequence 1			
Normality Test	Test Statistic	Prob Level	Reject H0 of Normality at $\alpha = 0.050$ ?
Shapiro-Wilk	0.9418	0.52170	No
Skewness	-0.7849	0.43251	No
Kurtosis	0.3616	0.71767	No
Omnibus (Skewness or Kurtosis)	0.7468	0.68839	No

Tests of the Normality Assumption for the Period Differences in Sequence 2			
Normality Test	Test Statistic	Prob Level	Reject H0 of Normality at $\alpha = 0.050$ ?
Shapiro-Wilk	0.9091	0.20784	No
Skewness	0.9127	0.36138	No
Kurtosis	-0.8364	0.40293	No
Omnibus (Skewness or Kurtosis)	1.5327	0.46472	No

### Shapiro-Wilk Normality

This test for normality has been found to be the most powerful test in most situations. It is the ratio of two estimates of the variance of a normal distribution based on a random sample of  $n$  observations. The numerator is proportional to the square of the best linear estimator of the standard deviation. The denominator is the sum of squares of the observations about the sample mean. The test statistic  $W$  may be written as the square of the Pearson correlation coefficient between the ordered observations and a set of weights which are used to calculate the numerator. Since these weights are asymptotically proportional to the corresponding expected normal order statistics,  $W$  is roughly a measure of the straightness of the normal quantile-quantile plot. Hence, the closer  $W$  is to one, the more normal the sample is.

The probability values for  $W$  are valid for sample sizes greater than 3. The test was developed by Shapiro and Wilk (1965) for sample sizes up to 20. NCSS uses the approximations suggested by Royston (1992) and Royston (1995) which allow unlimited sample sizes. Note that Royston only checked the results for sample sizes up to 5000, but indicated that he saw no reason larger sample sizes should not work.  $W$  may not be as powerful as other tests when ties occur in your data.

### Skewness Normality

This is a skewness test reported by D'Agostino (1990). Skewness implies a lack of symmetry. One characteristic of the normal distribution is that it has no skewness. Hence, one type of non-normality is skewness.

The Value is the test statistic for skewness, while Prob Level is the  $p$ -value for a two-tailed test for a null hypothesis of normality. If this  $p$ -value is less than a chosen level of significance, there is evidence of non-normality. Under Decision ( $\alpha = 0.050$ ), the conclusion about skewness normality is given.

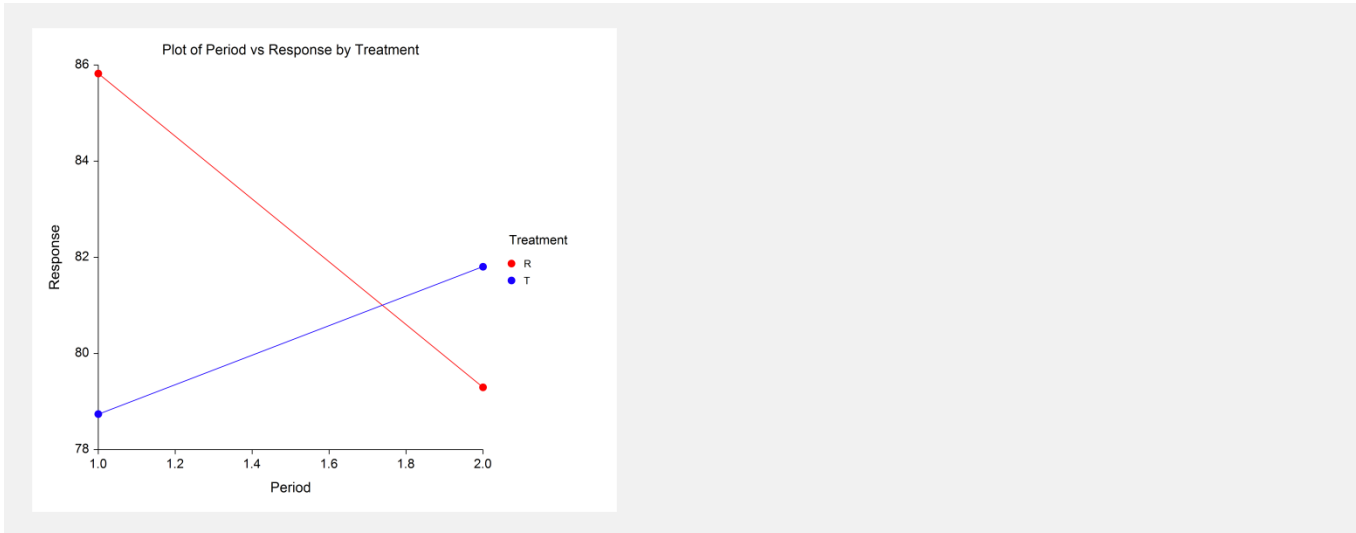
### Kurtosis Normality

Kurtosis measures the heaviness of the tails of the distribution. D'Agostino (1990) reported a second normality test that examines kurtosis. The Value column gives the test statistic for kurtosis, and Prob Level is the  $p$ -value for a two-tail test for a null hypothesis of normality. If this  $p$ -value is less than a chosen level of significance, there is evidence of kurtosis non-normality. Under Decision ( $\alpha = 0.050$ ), the conclusion about normality is given.

### Omnibus Normality

This third normality test, also developed by D'Agostino (1990), combines the skewness and kurtosis tests into a single measure. Here, as well, the null hypothesis is that the underlying distribution is normally distributed. The definitions for Value, Prob Level, and Decision are the same as for the previous two normality tests.

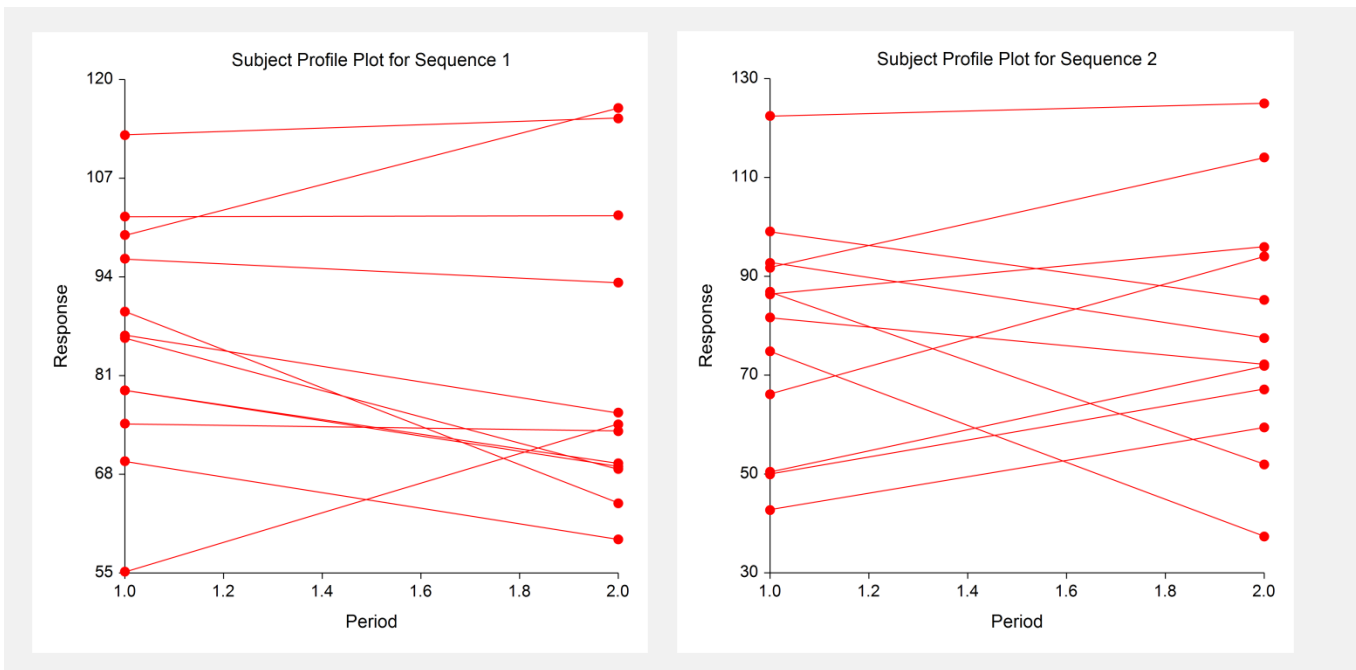
## Plot of Sequence-by-Period Means



The sequence-by-period means plot shows the mean responses on the vertical axis and the periods on the horizontal axis. The lines connect like treatments. The distance between these lines represents the magnitude of the treatment effect.

If there is no period, carryover, or interaction effects, two horizontal lines will be displayed. The tendency for both lines to slope up or down represents period and carryover effects. The tendency for the lines to cross represents period-by-treatment interaction. This is also a type of carryover effect.

## Plot of Subject Profiles



The profile plot displays the raw data for each subject. The response variable is shown along the vertical axis. The two sequences are shown along the horizontal axis. The data for each subject is depicted by two points connected by a line. The subject's response to the reference formulation is shown first followed by their response to the treatment formulation. Hence, for sequence 2, the results for the first period are shown on the right and for the second period on the left.

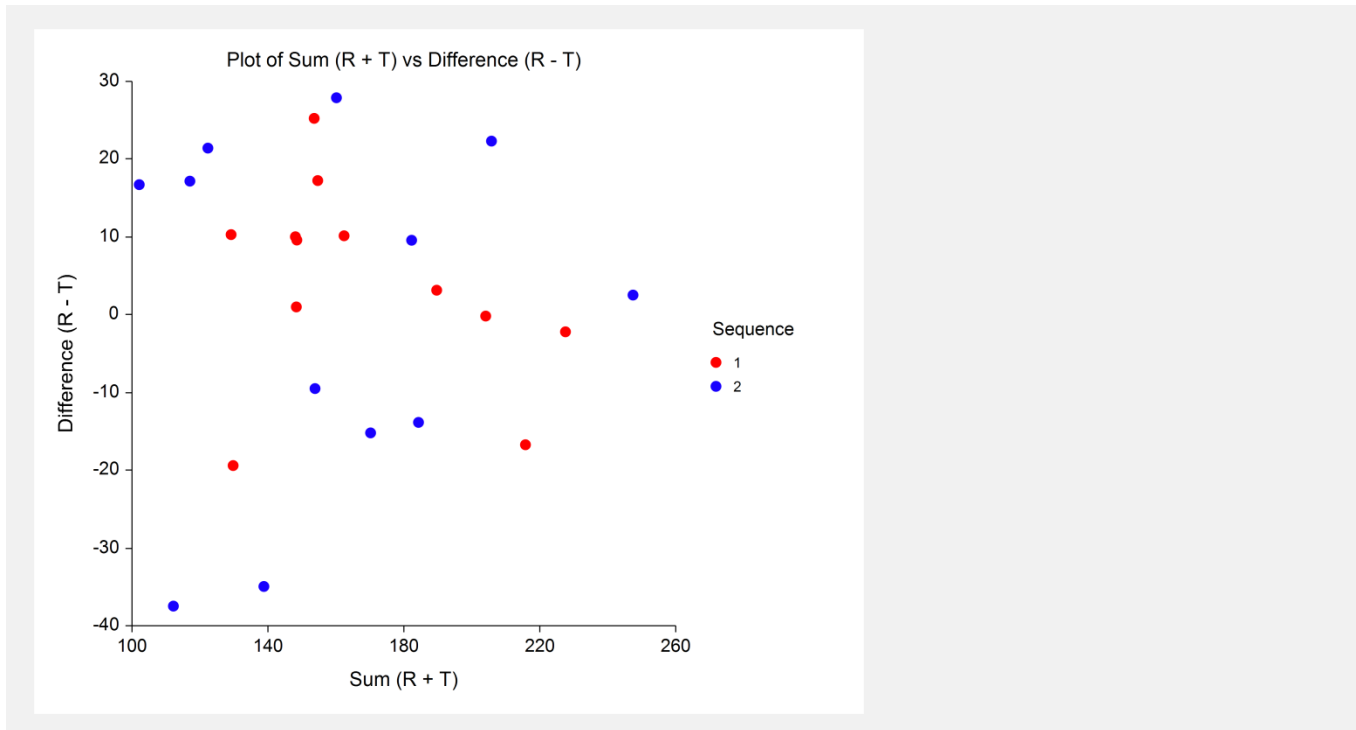


## Analysis of 2x2 Cross-Over Designs using T-Tests

This plot is used to develop a feel for your data. You should view it first as a tool to check for outliers (points and subjects that are very different from the majority). Note that outliers should be removed from the analysis only if a reason can be found for their deletion. Of course, the first step in dealing with outliers is to double-check the data values to determine if a typing error might have caused them. Also, look for subjects whose lines exhibit a very different pattern from the rest of the subjects in that sequence. These might be a signal of some type of data-recording or data-entry error.

The profile plot allows you to assess the consistency of the responses to the two treatments across subjects. You may also be able to evaluate the degree to which the variation is equal in the two sequences.

### Plot of Sums and Differences



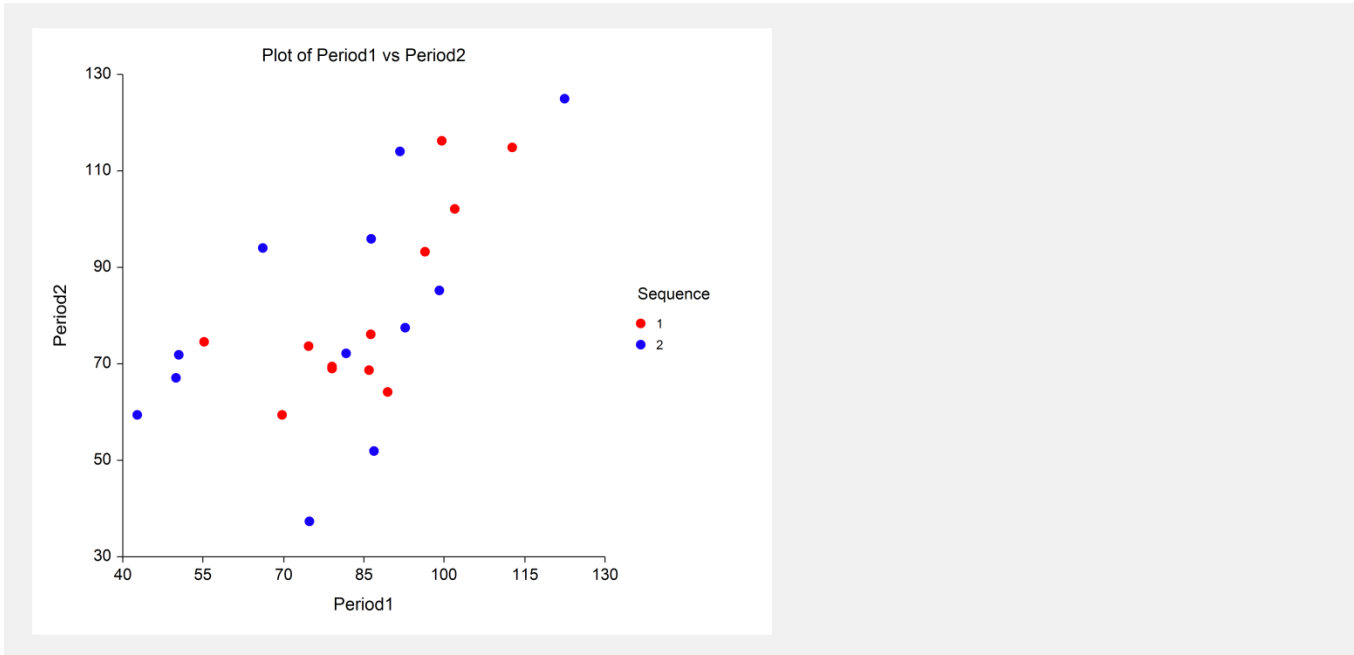
The sums and differences plot shows the sum of each subject's two responses on the horizontal axis and the difference between each subject's two responses on the vertical axis. Dot plots of the sums and differences have been added above and to the right, respectively.

Each point represents the sum and difference of a single subject. Different plotting symbols are used to denote the subject's sequence. A horizontal line has been added at zero to provide an easy reference from which to determine if a difference is positive (favors treatment R) or negative (favors treatment T).

The degree to which the plotting symbols tend to separate along the horizontal axis represents the size of the carryover effect. The degree to which the plotting symbols tend to separate along the vertical axis represents the size of the treatment effect.

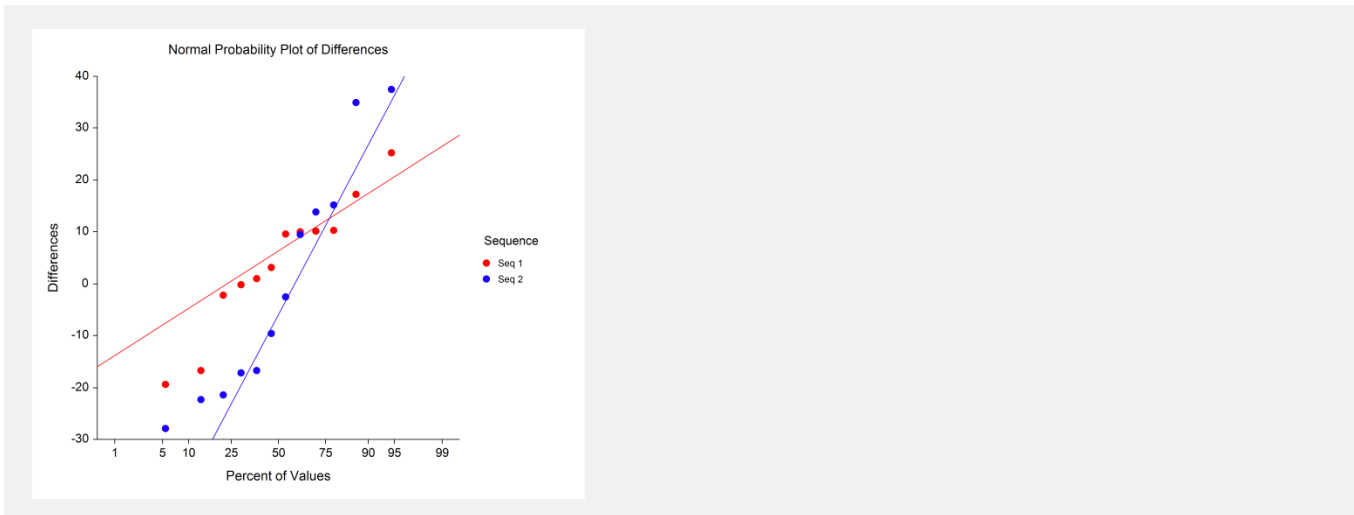
Outliers are easily detected on this plot. Outlying subjects should be reviewed for data-entry errors and for special conditions that might have caused their responses to be unusual. Outliers should not be removed from an analysis just because they are different. A compelling reason should be found for their removal and the removal should be well documented.

## Period Plot



The Period Plot displays a subject's period 1 response on the horizontal axis and their period 2 response on the vertical axis. The plotting symbol is the sequence number. The plot is used to find outliers and other anomalies.

## Probability Plots



These plots show the differences ( $P1 - P2$ ) on the vertical axis and values on the horizontal axis that would be expected if the differences were normally distributed. The first plot shows the differences for sequence 1 and the second plot shows the differences for sequence 2.

If the assumption of normality holds, the points should fall along a straight line. The degree to which the points are off the line represents the degree to which the normality assumption does not hold. Since the normality of these differences is assumed by the  $t$ -test used to test for a difference between the treatments, these plots are useful in assessing whether that assumption is valid.

If the plots show a pronounced pattern of non-normality, you might try taking the square roots or the logs of the responses before beginning the analysis.

## Example 2 – Wilcoxon-Mann-Whitney Rank-Sum Test (Validation using Senn (2002))

This section presents an example of how to run a non-parametric analysis of data from a 2x2 cross-over design. Senn (2002) page 93 presents an example of data from a 2x2 cross-over design. The data consist of 12 patients' judgements of efficacy for two type of bronchodilators used for the treatment of asthma: Salbutamol (S) and Formoterol (F).

On page 94, Senn (2002) reports the results of a standard cross-over analysis. He reports the estimated treatment effect (Formoterol – Salbutamol) to be -28.67mm with a standard error of 5.415mm, a “highly significant” t-value of -5.29, and a confidence interval for the effect of -41mm to -16mm.

On pages 113 and 114, Senn (2002) reports the results of the non-parametric Wilcoxon-Mann-Whitney Rank-Sum Test for treatment effect in the presence of a period effect. He reports the sum of ranks for the F/S sequence group to be 21 and the sum of ranks for the S/F sequence group to be 57. The Mann-Whitney U statistic is equal to 0. The two-sided p-value is equal to 0.0022.

We will use the data found the SENN93 database. You may follow along here by making the appropriate entries or load the completed template **Example 2a** from the Template tab of the Analysis of 2x2 Cross-Over Designs using T-Tests window.

### 1 Open the Senn93 dataset.

- From the **File** menu of the NCSS Data window, select **Open Example Data**.
- Click on the file **Senn93.NCSS**.
- Click **Open**.

### 2 Open the Analysis of 2x2 Cross-Over Designs using T-Tests window.

- Using the Analysis menu or the Procedure Navigator, find and select the **Analysis of 2x2 Cross-Over Designs using T-Tests** procedure.
- On the menus, select **File**, then **New Template**. This will fill the procedure with the default template.

### 3 Specify the variables.

- On the Analysis of 2x2 Cross-Over Designs using T-Tests window, select the **Variables tab**.
- For **Sequence Group Variable** enter **Sequence**.
- For **Period 1 Variable** enter **Period1**.
- For **Period 2 Variable** enter **Period2**.
- For **Label 1** enter **S**.
- For **Label 2** enter **F**.

### 4 Specify the report.

- On the Analysis of 2x2 Cross-Over Designs using T-Tests window, select the **Reports tab**.
- Check only **Cross-Over Effects and Means Summary Report**, **T-Test for Treatment Effect**, and **Mann-Whitney U Test (Wilcoxon Rank-Sum Test) for Treatment Effect** (and sub-tests).

### 5 Run the procedure.

- From the Run menu, select **Run Procedure**. Alternatively, just click the green Run button.

The following reports and charts will be displayed in the Output window.

## Analysis of 2x2 Cross-Over Designs using T-Tests

## Output

## Cross-Over Effects and Means Summary

Parameter	Count	Parameter Estimate	Standard Deviation	Standard Error	T*	Lower 95.0% CL	Upper 95.0% CL
Treatment Effect	12	-28.667	9.379	5.415	2.2281	-40.732	-16.601
$\mu_1$ (or $\mu_S$ )	12	54.750		6.245			
$\mu_2$ (or $\mu_F$ )	12	26.083		4.363			
Period Effect	12	3.000	9.379	5.415	2.2281	-9.065	15.065
$\mu (Period=1)$	12	38.917		5.824			
$\mu (Period=2)$	12	41.917		4.910			
Carryover Effect	12	39.000	32.262	18.627	2.2281	-2.503	80.503
(S+F) (Seq=1)	6	61.333	37.511	15.314			
(S+F) (Seq=2)	6	100.333	25.974	10.604			

## T-Test for Treatment Effect

Alternative Hypothesis	Mean Difference	Standard Error	T-Value	DF	Prob Level	Reject H0 at $\alpha = 0.050?$
$\mu_F - \mu_S \neq 0$	-28.667	5.415	-5.2939	10	0.00035	Yes

## Mann-Whitney U or Wilcoxon Rank-Sum Location Difference Test for Treatment Effect

Sequence	Mann-Whitney U	Sum of Ranks (W)	Mean of W	Std Dev of W
S/F	36	57	39	6.244998
F/S	0	21	39	6.244998

Number of Sets of Ties = 0, Multiplicity Factor = 0

Test Type	Alternative Hypothesis†	Z-Value	Prob Level	Reject H0 at $\alpha = 0.050?$
Exact*	Location Diff. $\neq 0$		0.00216	Yes
Normal Approximation	Location Diff. $\neq 0$	-2.8823	0.00395	Yes
Normal Approx. with C.C.	Location Diff. $\neq 0$	-2.8022	0.00507	Yes

† "Location Diff." refers to the location difference between the period differences of the two treatment groups (F-S).

\* The Exact Test is provided only when there are no ties and the sample size is  $\leq 20$  in both groups.

The results match those from Senn (2002). There is a highly significant difference between the two treatments.

The previous results assume a null hypothesized treatment difference of 0. On page 114, Senn (2002) presents results for testing the null hypothesis that treatment effect is equal to -15mm. He reports the sum of ranks for the F/S sequence group to be 25 and the sum of ranks for the S/F sequence group to be 53. The Mann-Whitney U statistic is equal to 4. He indicates that the null hypothesis is rejected but does not specify a P-value in this case.

To run this example in NCSS, you may follow along here by making the appropriate changes to the previous settings or load the completed template **Example 2b** from the Template tab of the Analysis of 2x2 Cross-Over Designs using T-Tests window.

## 6 Specify the report.

- On the Analysis of 2x2 Cross-Over Designs using T-Tests window, select the **Reports** tab.
- Set **H0:  $\mu_2 - \mu_1 = -15$** .
- Check only **Mann-Whitney U Test (Wilcoxon Rank-Sum Test) for Treatment Effect** (and sub-tests).

## 7 Run the procedure.

- From the Run menu, select **Run Procedure**. Alternatively, just click the green Run button.

## Analysis of 2x2 Cross-Over Designs using T-Tests

## Mann-Whitney U or Wilcoxon Rank-Sum Location Difference Test for Treatment Effect

Sequence	Mann-Whitney U	Sum of Ranks (W)	Mean of W	Std Dev of W
S/F	32	53	39	6.244998
F/S	4	25	39	6.244998

Number of Sets of Ties = 0, Multiplicity Factor = 0

Test Type	Alternative Hypothesis†	Z-Value	Prob Level	Reject H0 at $\alpha = 0.050$ ?
Exact*	Location Diff. $\neq -15$		0.02597	Yes
Normal Approximation	Location Diff. $\neq -15$	-2.2418	0.02497	Yes
Normal Approx. with C.C.	Location Diff. $\neq -15$	-2.1617	0.03064	Yes

† "Location Diff." refers to the location difference between the period differences of the two treatment groups (F-S).

\* The Exact Test is provided only when there are no ties and the sample size is  $\leq 20$  in both groups.

Again, the results from **NCSS** match those from Senn (2002). The treatment effect is significantly different from -15mm.