

## Chapter 263

# Latin Square Designs

## Introduction

This module generates Latin Square and Graeco-Latin Square designs. Designs for from three to ten treatments are available.

Latin Square designs are similar to randomized block designs, except that instead of the removal of one blocking variable, these designs are carefully constructed to allow the removal of two blocking factors. They accomplish this while reducing the number of experimental units needed to conduct the experiment.

Following is an example of a four treatment Latin Square. The experimental layout is as follows:

<u>Rows</u>	<u>Columns</u>			
	<u>Col1</u>	<u>Col2</u>	<u>Col3</u>	<u>Col4</u>
Row 1	A	B	C	D
Row 2	B	C	D	A
Row 3	C	D	A	B
Row 4	D	A	B	C

In the above table, the four treatments are represented by the four letters: A, B, C, and D. The letters are arranged so that each letter occurs only once within each row and each column. Notice that a simple random design would require  $4 \times 4 \times 4 = 64$  experimental units. This Latin Square needs only 16 experimental units—a reduction of 75%!

The influence of a fourth factor may also be removed from the design by introducing a second set of letters, this time lower case. This design is known as the *Graeco-Latin Square*.

<u>Rows</u>	<u>Columns</u>			
	<u>Col1</u>	<u>Col2</u>	<u>Col3</u>	<u>Col4</u>
Row 1	Aa	Bb	Cc	Dd
Row 2	Bd	Ca	Db	Ac
Row 3	Cb	Dc	Ad	Ba
Row 4	Dc	Ad	Ba	Cb

Four factors at four levels each would normally require 256 experimental units, but this design only requires 16—a reduction in experimental units of almost 94%!

The Graeco-Latin Square is formed by combining two orthogonal Latin Squares. Graeco-Latin Squares are available for all numbers of treatments except six.

## Latin Square Assumptions

It is important to understand the assumptions that are made when using the Latin Square design. The large reduction in the number of experimental units needed by this design occurs because it assumes the magnitudes of the interaction terms are small enough that they may be ignored. That is, the Latin Square design is

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a main effects only design. Another way of saying this is that the treatments, the row factor, and the column factor affect the response independently of one another.

Assuming that there are no interactions is quite restrictive, so before you use this design you should be able to defend this assumption. In practice, the influence of the interactions is averaged into the experimental error of the analysis of variance table. We say that the experimental error is inflated. This results in a reduced F-ratio for testing the treatment factor, and a reduced F-ratio lessens the possibility of achieving statistical significance.

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## Randomization

Probability statements made during the analysis of the experimental data require strict attention to the randomization process. The randomization process is as follows:

1. Randomly select a design from the set of orthogonal designs available.
2. Randomly assign levels of the row factor to the rows.
3. Randomly assign levels of the column factor to the columns.
4. Randomly assign treatments to the treatment letters (or numbers as the case may be).

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## Orthogonal Sets

These designs were taken from Rao, Mitra, and Matthai (1966). We have included designs with up to ten treatments. The number of available squares depends on the number of treatments. The following table shows the number of orthogonal squares stored within this procedure.

<u>Number of Treatments</u>	<u>Number of Orthogonal Designs</u>
3	2
4	3
5	4
6	1
7	6
8	7
9	8
10	2

Graeco-Latin Squares are generated by combining two of the available orthogonal squares. Note that there are no six-level Graeco-Latin Squares.

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## Procedure Options

This section describes the options available in this procedure.

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### Design Tab

This panel specifies the parameters that will be used to create the design values.

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#### Experimental Setup

##### Row Values

The values used to represent the rows are specified here. These values may be letters, digits, words, or numbers. The list is delimited by blanks or commas. The number of rows is implied by the number of items in this list. The number of row, column, and treatment values must be equal. From three to ten values are allowed.

##### Column Values

The values used to represent the columns are specified here. These values may be letters, digits, words, or numbers. The list is delimited by blanks or commas. The number of rows is implied by the number of items in this list. The number of row, column, and treatment values must be equal. From three to ten values are allowed.

##### Treatment 1 Values

The values used to represent the treatments are specified here. These values may be letters, digits, words, or numbers. The list is delimited by blanks or commas. The number of rows is implied by the number of items in this list. The number of row, column, and treatment values must be equal. From three to ten values are allowed.

##### Treatment 2 Values

The values used to represent the second set of treatments are specified here. These values may be letters, digits, words, or numbers. The list is delimited by commas. The number of rows is implied by the number of items in this list. The number of row, column, and treatment values must be equal. From three to ten values are allowed.

Note that this value is left blank unless you want to generate a Graeco-Latin Square.

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#### Experimental Setup – Orthogonal Designs

##### Orthogonal Design Number I

Select one of the available orthogonal designs. The number of available orthogonal designs is given in the table in Orthogonal Sets section above. Good scientific protocol requires that you randomly choose which of these designs is used.

##### Orthogonal Design Number II

This option is only used when the Treatment 2 Values box is non-blank (when you are generating a Graeco-Latin Square). Select a second of the available orthogonal designs to be combined with the first in forming a Graeco-Latin Square. The value here must be different from the value specified in Orthogonal Design I. Good scientific protocol requires that you randomly choose which of these designs is used.

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#### Data Storage

##### Store Data to the Dataset

Check this box to generate the design data on the dataset. The data will be identical to the design data generated on the output window.

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### Store First Factor In

The row values are stored in this column. The column values are stored in the column immediately to the right. The treatment values are stored in the column immediately to the right of that column. If specified, the values of the second treatment are stored in the column immediately to the right of the first treatment column.

Warning: The program fills these variables with data, so any previous data will be replaced.

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## Example 1 – Latin Square Design

This section presents an example of how to generate a Latin Square design using this program. **CAUTION: since the purpose of this routine is to generate (not analyze) data, you should begin with an empty dataset.**

In this example, we will show you how to generate a design with four treatments. You may follow along here by making the appropriate entries or load the completed template **Example 1** by clicking on Open Example Template from the File menu of the Latin Square Designs window.

### 1 Open a new (empty) dataset.

- From the File menu of the NCSS Data window, select **New**.
- Click the **Ok** button.

### 2 Open the Latin Square Designs window.

- Using the Analysis menu or the Procedure Navigator, find and select the **Latin Square Designs** procedure.
- On the menus, select **File**, then **New Template**. This will fill the procedure with the default template.

### 3 Specify the design parameters.

- On the Latin Square Designs window, select the **Design tab**.
- Set **Row Values** to **R1 R2 R3 R4**.
- Set **Column Values** to **C1 C2 C3 C4**.
- Set **Treatment 1 Values** to **A B C D**.
- Set the **Orthogonal Design Number I** to **1**.
- Set the **Orthogonal Design Number II** to **8**.
- Check the box **Store Data with the Dataset**.

### 4 Run the procedure.

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

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## Four-Level Latin Square Design

C1	C2	C3
R1	C1	A
R1	C2	B
R1	C3	C
R1	C4	D
R2	C1	B
R2	C2	A
R2	C3	D
R2	C4	C
R3	C1	C
R3	C2	D
R3	C3	A
R3	C4	B
R4	C1	D
R4	C2	C
R4	C3	B
R4	C4	A

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Three columns are filled with data. The first column, C1, contains the row value. The second column, C2, contains the column value. The third column, C3, contains the treatment letter.

To use this design, you would follow the randomization rules discussed earlier to obtain your experimental layout. After running your experiment, you would replace the random values in, say, C4 with those obtained from your experiment. You would then analyze the data using the GLM procedure. You would specify Factor 1 (C1) as Fixed (or Random as the case may be), Factor 2 (C2) as Fixed (or Random as the case may be), and treatment (C3) as Fixed. The response variable would be C4.

On the Model window of the GLM ANOVA procedure, you would set Which Model Terms to 'Up to 1-Way.' This forces the program to combine all interaction terms into an error term. The results would appear in this format.

## Analysis of Variance Table

### Expected Mean Squares Section

Source	DF	Term	Denominator	Expected
Term		Fixed?	Term	Mean Square
A (C1)	3	Yes	S(ABC)	S+bcsA
B (C2)	3	Yes	S(ABC)	S+acsB
C (C3)	3	Yes	S(ABC)	S+absC
S(ABC)	6	No		S

Note: Expected Mean Squares are for the balanced cell-frequency case.

### Analysis of Variance Table

Source	DF	Sum of	Mean	F-Ratio	Prob	Power
Term		Squares	Square		Level	(Alpha=0.05)
A (C1)	3	80425.69	26808.56	0.33	0.806088	0.071380
B (C2)	3	614617.2	204872.4	2.50	0.156199	0.239479
C (C3)	3	284915.2	94971.73	1.16	0.399286	0.131679
S	6	491094.4	81849.06			
Total (Adjusted)	15	1471053				
Total	16					

\* Term significant at alpha = 0.05

The values of the sum of squares, mean squares, and F-ratios will not match those displayed here. However, the number of degrees of freedom will match.

Note that only six degrees of freedom are available for the error term (S). This is a severe limitation of a Latin Square design with only four-levels. Often, you would replicate the experiment to obtain more error degrees of freedom.

Also note that the Expected Mean Square values are generated from the complete model assumption. Since the Latin Square is not complete (does not include all row-by-column-by-treatment combinations), these values are incorrect. The actual expected mean squares in this case would be  $S+4A$ ,  $S+4B$ , and  $S+4C$ , respectively.