

## **LATIN SQUARE DESIGN (LS)**

### **Facts about the LS Design**

- With the Latin Square design you are able to control variation in two directions.
- Treatments are arranged in rows and columns
- Each row contains every treatment.
- Each column contains every treatment.
- The most common sizes of LS are 5x5 to 8x8

### **Advantages of the LS Design**

1. You can control variation in two directions.
2. Hopefully you increase efficiency as compared to the RCBD.

### **Disadvantages of the LS Design**

1. The number of treatments must equal the number of replicates.
2. The experimental error is likely to increase with the size of the square.
3. Small squares have very few degrees of freedom for experimental error.
4. You can't evaluate interactions between:
  - a. Rows and columns
  - b. Rows and treatments
  - c. Columns and treatments.

### **Effect of the Size of the Square on Error Degrees of Freedom**

SOV	Df	2x2	3x3	4x4	5x5	8x8
Rows	$r-1$	1	2	3	4	7
Columns	$r-1$	1	2	3	4	7
Treatments	$r-1$	1	2	3	4	7
Error	$(r-1)(r-2)$	0	2	6	12	42
Total	$r^2 - 1$	3	8	15	24	63

Where  $r$  = number of rows, columns, and treatments.

- One way to increase the Error df for small squares is to use more than one square in the experiment (i.e. repeated squares).

### Example

Two 4x4 Latin squares.

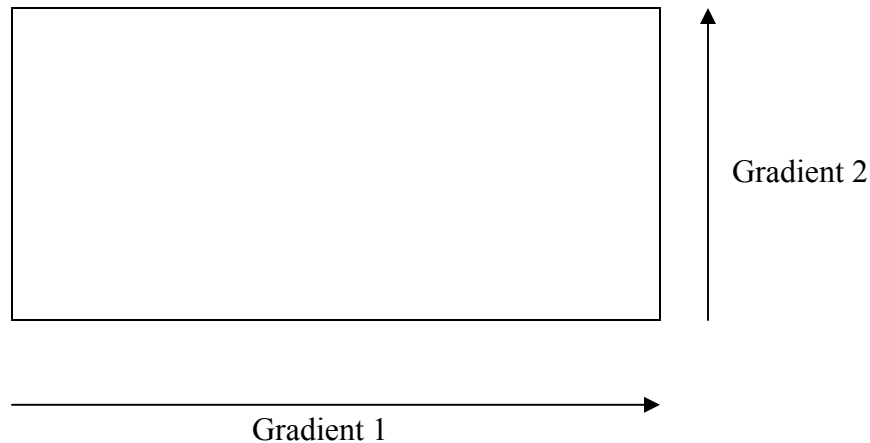
SOV	Df
Squares	$sq - 1 = 1$
* <b>Row(square)</b>	$sq(r-1) = 6$
* <b>Column(square)</b>	$sq(r-1) = 6$
Treatment	$r-1 = 3$
Square x Treatment	$(sq-1)(r-1) = 3$
* <b>Error</b>	$sq(r-1)(r-2) = 12$
Total	$sqr^2 - 1 = 31$

\*Additive across squares.

Where sq = number of squares.

### Examples of Uses of the Latin Square Design

1. Field trials in which the experimental error has two fertility gradients running perpendicular each other or has a unidirectional fertility gradient but also has residual effects from previous trials.



2. Animal science feed trials.
3. Insecticide field trial where the insect migration has a predictable direction that is perpendicular to the dominant fertility gradient of the experimental field.
4. Greenhouse trials in which the experimental pots are arranged in a straight line perpendicular to the glass walls, such that the difference among rows of pots and distance from the glass wall are expected to be the major sources of variability.

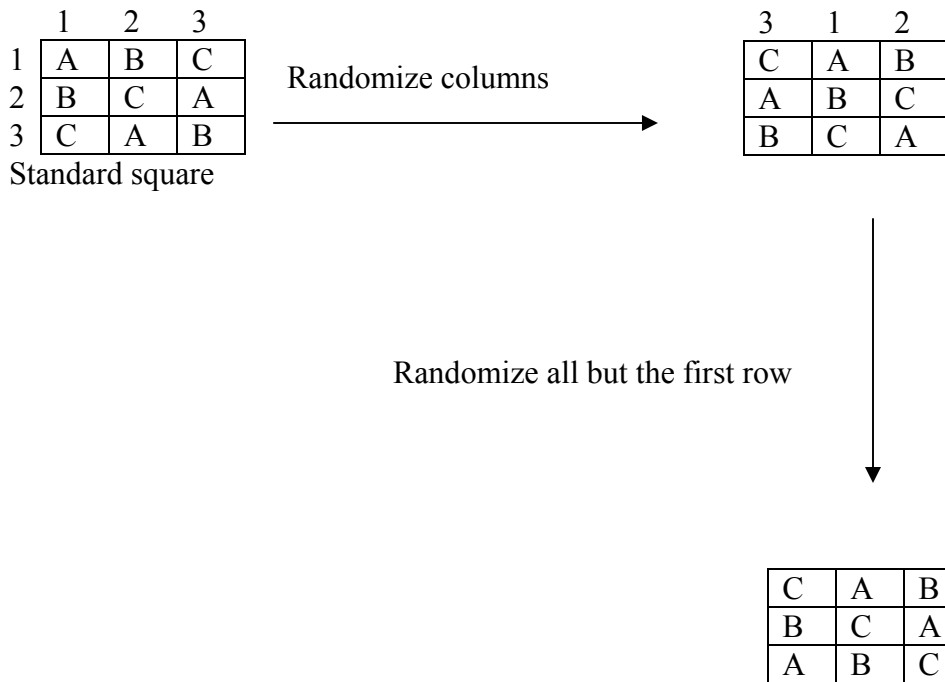
A	D	C	B	B	C	A	D	D	A	B	C	C	B	D	A
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

## Randomization Procedure

-Depends on the type of Latin Square you use.

### 3x3 Latin Square

-Start with the standard square and randomize all columns and all but the first row.



### 4x4 Latin Square

- Randomly choose a standard square.
- Randomize all columns and all but the first row.

### 5x5 Latin Square

- Randomly choose a standard square.
- Randomize all columns and rows.

### Analysis of a Single Latin Square

#### Example

Grain yield of three maize hybrids (A, B, and D) and a check (C).

Row	Column 1	Column 2	Column 3	Column 4	Row ( $\sum R$ )
1	1.640 (B)	1.210 (D)	1.425 (C)	1.345 (A)	5.620
2	1.475 (C)	1.185 (A)	1.400 (D)	1.290 (B)	5.350
3	1.670 (A)	0.710 (C)	1.665 (B)	1.180 (D)	5.225
4	1.565 (D)	1.290 (B)	1.655 (A)	0.660 (C)	5.170
Column total ( $\sum C$ )	6.350	4.395	6.145	4.475	21.365

Step 1. Calculate treatment totals.

Treatment	Total
A	5.855
B	5.885
C	4.270
D	5.355

Step 2. Compute the Correction Factor (CF).

$$CF = \frac{Y^2}{r^2}$$

$$= \frac{21.365^2}{4^2}$$

$$= 28.53$$

Step 3. Calculate the Total SS

$$TotalSS = \sum Y_{ij}^2 - CF$$

$$= (1.64^2 + 1.210^2 + 1.425^2 + \dots + 0.66^2) - CF$$

$$= 1.4139$$

Step 4. Calculate the Row SS

$$\begin{aligned} RowSS &= \frac{\sum Row^2}{r} - CF \\ &= \frac{(5.62^2 + 5.35^2 + 5.225^2 + 5.17^2)}{4} - CF \\ &= 0.0302 \end{aligned}$$

Step 5. Calculate the Column SS.

$$\begin{aligned} Col.SS &= \frac{\sum Col^2}{r} - CF \\ &= \frac{(6.35^2 + 4.395^2 + 6.145^2 + 4.475^2)}{4} - CF \\ &= 0.8273 \end{aligned}$$

Step 6. Calculate the Treatment SS

$$\begin{aligned} TrtSS &= \frac{\sum Y_{i.}^2}{r} - CF \\ &= \frac{(5.855^2 + 5.885^2 + 4.270^2 + 5.355^2)}{4} - CF \\ &= 0.4268 \end{aligned}$$

Step 7. Calculate the Error SS

$$\begin{aligned} \text{Error SS} &= \text{Total SS} - \text{Row SS} - \text{Column SS} - \text{Trt SS} \\ &= 0.1296 \end{aligned}$$

Step 8. Complete the ANOVA table

SOV	Df	SS	MS	F
Row	$r-1 = 3$	0.030		
Column	$r-1 = 3$	0.827		
Trt	$r-1 = 3$	0.427	0.142	Trt MS/Error MS = 6.60*
Error	$(r-1)(r-2) = 6$	0.129	0.0215	
Total	$r^2-1 = 15$	1.414		

Step 9. Calculate the LSD.

$$LSD = t_{\alpha/2} \sqrt{\frac{2ErrorMS}{r}}$$

$$= 2.447 \sqrt{\frac{2(.0215)}{4}}$$

$$= 0.254$$

### **Linear Model**

$$Y_{ij(t)} = \mu + \beta_i + \kappa_j + \tau_t + \varepsilon_{ij(t)}$$

where:  $\mu$  = the experiment mean.

$\beta_i$  = the row effect,

$\kappa_j$  = the column effect,

$\tau_t$  = the treatment effect, and

$\varepsilon_{ij(t)}$  = the random error.

## Latin Square - Combined Analysis Across Squares

-The squares can be at the same location, or three different locations, or three different years, etc.

### Example

Three 3x3 Latin squares

Square 1

				$\sum R$	
	41 (B)	25 (C)	15 (A)	81	SS Row <sub>1</sub> = 126.89
	20 (A)	32 (B)	24 (C)	76	SS Column <sub>1</sub> = 89.55
	22 (C)	12 (A)	21 (B)	55	SS Treatment <sub>1</sub> = 368.22
$\sum C$	83	69	60	212	SS Error <sub>1</sub> = 21.56

Square 2

				$\sum R$	
	27 (C)	28 (B)	3 (A)	58	SS Row <sub>2</sub> = 130.89
	4 (A)	17 (C)	9 (B)	30	SS Column <sub>2</sub> = 110.22
	22 (B)	4 (A)	17 (C)	43	SS Treatment <sub>2</sub> = 534.22
$\sum C$	53	49	29	131	SS Error <sub>2</sub> = 14.89

Square 3

				$\sum R$	
	43 (B)	27 (C)	17 (A)	87	SS Row <sub>3</sub> = 126.89
	22 (A)	34 (B)	26 (C)	82	SS Column <sub>3</sub> = 89.55
	24 (C)	14 (A)	23 (B)	61	SS Treatment <sub>3</sub> = 368.22
$\sum C$	89	75	66	230	SS Error <sub>3</sub> = 21.56

Step 1. Test the homogeneity of the Error MS from each square using Bartlett's Chi-square test.

Step 1.1 Calculate the Error SS for each square.

Step 1.2 Calculate the Error MS for each square.

Step 1.3 Calculate the Log of each Error MS

Square	Error SS	Error df	Error MS	Log Error MS
1	21.56	2	10.78	1.0326
2	14.89	2	7.45	0.8722
3	21.56	2	10.78	1.0326
			$\sum s_i^2 = 29.01$	$\sum \log s_i^2 = 2.9374$

Step 1.4 Calculate the Pooled Error MS ( $s_p^2$ )

$$s_p^2 = \frac{\sum s_i^2}{\#sq} = \frac{29.01}{3} = 9.67$$

Step 1.5 Calculate Bartlett's  $\chi^2$

$$\chi^2 = \frac{2.3026(Error df) \left[ (sq \log s_p^2) - \sum \log s_i^2 \right]}{1 + \left[ \frac{(sq + 1)}{3 * sq * Error df} \right]}$$

Where Error df = df for one square.

$$\begin{aligned} \chi^2 &= \frac{2.3026(2) \left[ (3 \log 9.67) - 2.9374 \right]}{1 + \left[ \frac{(3 + 1)}{3 * 3 * 2} \right]} \\ &= \frac{0.0869}{1.2222} \\ &= 0.0711 \end{aligned}$$

Step 1.6 Look up the Table  $\chi^2$ -value at the 99.5% level of confidence and df = #sq-1.

$$\chi_{0.005; 2df}^2 = 10.6$$

Step 1.7 Make conclusions

Since  $\chi_{calc}^2 < \chi_{table}^2$  we fail to reject  $H_o : \sigma_1^2 = \sigma_2^2 = \sigma_3^2$  at the 99.5% level of confidence; thus, we can do the combined analysis across squares



Step 2. Calculate Treatment Totals for each square.

Treatment	Square 1	Square 2	Square 3	$\sum TRT$
A	47	11	53	111
B	94	59	100	253
C	71	61	77	209
$\sum Square$	212	131	230	573

Step 3. Calculate the Correction Factor (CF).

$$CF = \frac{Y^2}{sq * r^2}$$

$$= \frac{573^2}{3 * 3^2}$$

$$= 12,160.333$$

Step 4. Calculate the Total SS

$$TotalSS = (41^2 + 25^2 + 15^2 + ... + 23^2) - CF$$

$$= 2,620.67$$

Step 5. Calculate the Square SS

$$SquareSS = \frac{\sum Sq^2}{r^2} - CF$$

$$= \frac{(212^2 + 131^2 + 230^2)}{3^2} - CF$$

$$= 618.0$$

Step 6. Calculate the Row(Square) SS (Additive across squares)

$$Row(Square) SS = Row_1 SS + Row_2 SS + Row_3 SS$$

$$= 384.67$$

Step 7. Calculate the Column(Square) SS (Additive across squares)

$$\begin{aligned}\text{Column(Square) SS} &= \text{Column}_1 \text{ SS} + \text{Column}_2 \text{ SS} + \text{Column}_3 \text{ SS} \\ &= 289.32\end{aligned}$$

Step 8. Calculate the Treatment SS

$$\begin{aligned}\text{TrtSS} &= \frac{\sum TRT_i^2}{sq * r} - CF \\ &= \frac{(111^2 + 253^2 + 209^2)}{3 * 3} - CF \\ &= 1,174.22\end{aligned}$$

Step 9. Calculate the Square X Treatment SS.

$$\begin{aligned}\text{Sq} * \text{TrtSS} &= \frac{\sum (SqXTrt)^2}{r} - CF - \text{SquareSS} - \text{TrtSS} \\ &= \frac{(47^2 + 94^2 + 71^2 + \dots + 77^2)}{3} - CF - \text{SquareSS} - \text{TrtSS} \\ &= 96.45\end{aligned}$$

Step 10. Calculate Error SS (Additive across squares)

$$\begin{aligned}\text{Error SS} &= \text{Error}_1 \text{ SS} + \text{Error}_2 \text{ SS} + \text{Error}_3 \text{ SS} \\ \text{Error SS} &= 58.01\end{aligned}$$

Step 11. Complete the ANOVA Table.

SOV	Df	SS	MS	F (Squares and Trt are Fixed effects)
Square	Sq-1 = 2	618.0		Non-valid F-test
Row(Sq)	Sq(r-1) = 6	384.67		Non-valid F-test
Column(Sq)	Sq(r-1) = 6	289.32		Non-valid F-test
Trt	r-1 = 2	1174.22	587.11	Trt MS/Error MS = 60.73 <sup>**</sup>
Sq X Trt	(sq-1)(r-1) = 4	96.45	24.11	Sq X Trt MS/Error MS = 2.49 <sup>ns</sup>
Error	Sq(r-1)(r-2) = 6	58.01	9.67	
Total	Sqr <sup>2</sup> -1 = 26	2620.67		

Conclusions:

1. The non-significant Square X Treatment interaction indicates that treatments responded similarly in all squares.

Table 1. Mean for the square x treatment interaction.

Square	Treatment		
	A	B	C
1	15.7	31.3	23.7
2	3.7	19.7	20.3
3	17.7	33.3	25.7
LSD(0.05)	-----ns-----		

2. The significant F-test for Treatment indicates that averaged across all squares, there were differences between treatments.
- 3.

Table 2. Mean for the treatment main effect averaged Across squares.

Treatment	Mean
A	12.3
B	28.1
C	23.2
LSD(0.05)	3.6

Step 12. Calculate LSD's

Square X Trt: Normally, you would not calculate this LSD because the F-test for the interaction was non-significant. However, if it would have been significant, you would have calculated the LSD using the following method:

$$LSD_{SqXTrt} = t_{a/2; error df} \sqrt{\frac{2ErrorMS}{r}}$$

$$= 2.447 \sqrt{\frac{2(9.67)}{3}}$$

$$= 6.2$$

**This LSD would be used for comparisons only in Table 1.**

Treatment:

$$\begin{aligned}LSD_{Trit} &= t_{\alpha/2; error df} \sqrt{\frac{2ErrorMS}{sq * r}} \\&= 2.447 \sqrt{\frac{2(9.67)}{3 * 3}} \\&= 3.6\end{aligned}$$

**This LSD would only be used for comparisons in Table 2.**