



Chapter 4

Latin Square Design (LSD)

Latin Square Design (LSD)

- **This design is used when the experimental units are different in two direction .**
- **In order to control the error or limiting the error to minimum limit, construct two type of block ,the first is horizontally named Row, while the second block is vertically named Column.**

In this design the number of treatments are equal to the number of rows ,to the number of columns and to the number of replications .


- **N.O.treatment = N.O.Row= N.O.Column**
- **t=r=c**
- **The number of experimental unit =(number of treatment)²**

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- A Latin square is a square array of objects (letters A, B, C, ...) such that each object appears once and only once in each row and each column.
 - Example : 4 x 4 Latin Square.

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

Assignment of Treatments to Experimental Units Using a Latin Square Design

- Each treatment appears exactly once in each row.
- Each treatment appears exactly once in each column.
- Among all possible assignments of treatments to experimental units that satisfy the above properties, one is selected at random.
- All possibilities are equally likely.
- In the 4 x 4 case, there are 576 to choose from*.
- 5 x 5: 161,280 possibilities, 6 x 6: 812,851,200 possibilities*

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- **The treatments are assigned to row-column combinations using a Latin-square arrangement.**
 - **It is assumed that there is *no interaction* between rows, columns. The degrees of freedom for the interactions is used to estimate error.**
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The Model for LSD

$$y_{ij(k)} = \mu + R_i + C_j + \tau_k + \varepsilon_{ij(k)}$$

$$i = 1, 2, \dots, r \quad j = 1, 2, \dots, c \quad k = 1, 2, \dots, t$$

$y_{ij(k)}$: the observation in i^{th} row and the j^{th} column receiving the k^{th} treatment

μ : overall mean.

R_i : The effect of the i^{th} row

C_j : The effect of the j^{th} column

τ_k : The effect of the k^{th} treatment

$\varepsilon_{ij(k)}$: Random error

No interaction between rows, columns and treatments

ANOVA TABEL

| S.O.V | d.f. | S.S. | M.S. | F |
|--------------|--------------------------------|------------------------------|------------------------------|-----------------------------------|
| Treat | r-1 | SS_{Tr} | MS_{Tr} | MS_{Tr}/MS_E |
| Rows | r-1 | SS_{Row} | MS_{Row} | MS_{Row}/MS_E |
| Cols | r-1 | SS_{Col} | MS_{Col} | MS_{Col}/MS_E |
| Error | $(r-1)(r-2)$ | SS_E | MS_E | |
| Total | $r^2 - 1$ | SS_T | | |

$$SS_{Row} = \frac{\sum y_{i.}^2}{r} - \frac{y_{..}^2}{r^2}$$

$$SS_{Col} = \frac{\sum y_{.j}^2}{r} - \frac{y_{..}^2}{r^2}$$

$$SS_{Treat.} = \frac{\sum y_{..k}^2}{r} - \frac{y_{..}^2}{r^2}$$

$$SS_{Total} = \sum y_{ij}^2 - \frac{y_{..}^2}{t^2}$$

$$SSE = SS_{Total} - SS_{treat} - SS_{row} - SS_{col}$$

Missing Data

For each missing value in the experiment, you lose one degree of freedom from error and total.

Reasons for missing data include:

- 1. Animal dies**
- 2. Break a test tube (كسر انبوب الاختبار).**
- 3. Animals eat grain (الحبوب) in the plot.**
- 4. Spill grain (الحبوب) sample**

The value for a missing plot can be estimated by using the formula:

Missing Data

where:

$$y_{ij} = \frac{t(R + C + T) - 2G}{(r - 1)(r - 2)}$$

t : number of treatments

R: Row total of row with missing value

C: Column total of column with missing value

T : Treatment total of treatment with missing value

G : Experiment total (Y..)

$$SS_t' = SS_t - \frac{[G - R - C - (r - 1)T]^2}{(r - 1)^2 (r - 2)^2}$$

Relative Efficiency (RE)

Comparison between LS and CRD

- Relative Efficiency of LS to CRD (how many times as many replicates would be needed for CRD to have as precise of estimates of treatment means as LS does)

$$RE(LSD, CRD) = \frac{MSE_{CRD}}{MSE_{LS}D}$$

$$RE = \frac{MSr + MS_C + (r - 1)MSE}{(r + 1)MSE} \times 100$$

Comparison between LS and CRBD

$$RE(LSD, CRBD) = \frac{MSE_{CRBD}}{MSE_{LS} D}$$

$$\% RE = \frac{MSC + (r - 1)MSE}{rMSE} \times 100 \quad \text{Row is Blocks}$$

$$\% RE = \frac{MSR + (r - 1)MSE}{rMSE} \times 100 \quad \text{Column is Blocks}$$

Graeco-Latin Square Designs

- A Graeco-Latin square consists of two Latin squares (one using the letters A, B, C, ... the other using Greek letters (α , β , γ , ...)) such that when the two Latin squares are superimposed on each other the letters of one square appear once and only once with the letters of the other square. The two Latin squares are called mutually orthogonal.

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- A *Greaco-Latin Square* experiment is assumed to be a four-factor experiment.
 - The factors are *rows*, *columns*, *Latin treatments* and *Greek treatments*.
 - It is assumed that there is *no interaction* between rows, columns, Latin treatments and Greek treatments.
 - The degrees of freedom for the interactions is used to estimate error.

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- **Example:** a 4 x 4 Greaco-Latin Square

| | | | |
|------------|------------|------------|------------|
| A^α | B^β | C^γ | D^δ |
| B^γ | A^δ | D^α | C^β |
| C^δ | D^γ | A^β | B^α |
| D^β | C^α | B^δ | A^γ |

Note:

There exists at most $(t - 1)$ $t \times t$ Latin squares L_1, L_2, \dots, L_{t-1} such that any pair are mutually orthogonal.

e.g. It is possible that there exists a set of six 6×6 mutually orthogonal Latin squares $L_1, L_2, L_3, L_4, L_5, L_6$

The Model for a Greaco-Latin Experiment

$$y_{ij(kl)} = \mu + \tau_k + R_i + C_j + G_l + \varepsilon_{ij(kl)}$$

$$i = 1, 2, \dots, r$$

$$j = 1, 2, \dots, r$$

$$k = 1, 2, \dots, r$$

$$l = 1, 2, \dots, r$$

$y_{ij(kl)}$: the observation in i^{th} row and the j^{th} column receiving the k^{th} Latin treatment and the l^{th} Greek treatment

μ : overall mean

τ_k : the effect of the k^{th} Latin treatment



G_l : The effect of the l^{th} Greek treatment

R_i : The effect of the i^{th} row

C_j : The effect of the j^{th} column

$\varepsilon_{ij(k)}$: Random error

**No interaction between rows, columns, Latin treatments and
Greek treatments**

The Anova Table for a Greaco-Latin Square Experiment

| Source | d.f. | S.S. | M.S. | F |
|--------|--------------|----------------------|----------------------|---------------------------|
| Treat | $r-1$ | $SS_{\text{Treat.}}$ | $MS_{\text{Treat.}}$ | $MS_{\text{Treat.}}/MS_E$ |
| Greek | $r-1$ | SS_{Gr} | MS_{Gr} | MS_{Gr}/MS_E |
| Rows | $r-1$ | SS_{Row} | MS_{Row} | MS_{Row}/MS_E |
| Cols | $r-1$ | SS_{Col} | MS_{Col} | MS_{Col}/MS_E |
| Error | $(r-1)(r-3)$ | SS_E | MS_E | |
| Total | $r^2 - 1$ | SS_T | | |

Crossover Designs(C.O.D.)

- An experimental design in which each experimental unit is used with each treatment being studied. The simplest crossover trial uses two groups of experimental units (e.g. hospital patients), 1 and 2, and two treatments (e.g. medicines), A and B. The trial uses two equal-length time periods. In the first period, group 1 is assigned treatment A and group 2 is assigned treatment B. In the second period, the assignments are reversed.

○
A crossover design is balanced with respect to carry-over if each treatment follows every other treatment the same number of times

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- **Two blocking factors: subject and period**
 - **Used in clinical trials**

| | Subject | | | | | |
|-----------------|----------------|----------|----------|----------|----------|----------|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Period 1 | A | A | B | A | B | B |
| Period 2 | B | B | A | B | A | A |

• **Rearrange as a replicated Latin Square**

Subject

| | 1 | 3 | 2 | 5 | 4 | 6 |
|-------------------|----------|----------|----------|----------|----------|----------|
| ○ Period 1 | A | B | A | B | A | B |
| ○ Period 2 | B | A | B | A | B | A |

Effects in Crossover Designs are confounded with the carry-over (residual effects) of previous treatments

We will assume that the carry-over only persists for the treatment in the period immediately before the present period

ANOVA TABEL

| Source o f Variation | d.f. | S.S. | M.S. | F |
|----------------------|------------|--------------------|--------------------|-------------------------|
| Replication(Column) | c-1 | SS_{Repl} | MS_{Repl} | MS_{Repl}/MS_E |
| Periods(Row) | p-1 | SS_p | MS_p | MS_p/MS_E |
| Treat | t-1 | SS_t | MS_t | MS_t/MS_E |
| Error | (t-1)(c-2) | SS_E | MS_E | |
| Total | tc - 1 | SS_T | | |

$$SSR_{Period} = \frac{\sum y_{i.}^2}{r} - \frac{y_{..}^2}{rt}$$

$$SSC_{Repli.} = \frac{\sum y_{.j}^2}{t} - \frac{y_{..}^2}{rt}$$

$$SS_{Tret.} = \frac{\sum y_{..k}^2}{r} - \frac{y_{..}^2}{rt}$$

$$SS_{Total} = \sum y_{ijk}^2 - \frac{y_{..}^2}{rt}$$

$$SSE = SS_{Total} - SS_{treat} - SS_{Period} - SS_{Repli.}$$

The Model for C.O.D

$$y_{ijk} = \mu + R_i + C_j + T_k + \varepsilon_{ijk}$$

$$i = 1, 2, \dots, t \quad j = 1, 2, \dots, r \quad k = 1, 2, \dots, c$$

$y_{ij(kl)}$: The observation in i^{th} row and the j^{th} column receiving the k^{th} Latin treatment and the l^{th} Greek treatment

μ : Overall mean

T_k : The effect of the k^{th} Latin treatment

R_i : The effect of the i^{th} row

C_j : The effect of the j^{th} column

$\varepsilon_{ij(k)}$: Random error

Advantages

- A crossover study has two advantages over a non-crossover longitudinal study.
- First, the influence of confounding covariates is reduced (يتم (تقليل تأثير المتغيرات المتداخلة) because each crossover patient serves as his or her own . In a non-crossover study, even randomized, it is often the case that different treatment-groups are found to be on some covariates. In a controlled, randomized crossover designs, such imbalances are implausible (unless covariates were to change systematically during the study).
 - Second, optimal crossover designs المثلّى بالكفاءة are statistically efficient and so require fewer subjects than do non-crossover designs (even other repeated measures designs).

Disadvantages

Crossover studies often have two problems:

- First is the issue of "order" effects, because it is possible that the order in which treatments are administered may affect the outcome. An example might be a drug with many adverse effects given first, making patients taking a second, less harmful medicine, more sensitive to any adverse effect.
- Second is the issue of "carry-over" between treatments, which confounds the estimates of the treatment effects. In practice, "carry-over" effects can be avoided with a sufficiently long "wash-out" period between treatments. However, the planning for sufficiently long wash-out periods does require expert knowledge of the dynamics of the treatment, which often is unknown, of course.

Youden Square Design

Example : Given Five Treatment
A,B,C,D,E

| 1 | 2 | 3 | 4 |
|---|---|---|---|
| A | B | C | D |
| E | A | B | C |
| D | E | A | B |
| C | D | E | A |
| B | C | D | E |

The Model for Youden Square Design

$$y_{ijk} = \mu + R_i + C_j + T_k + \varepsilon_{ijk}$$

$$i = 1, 2, \dots, t \quad j = 1, 2, \dots, r \quad k = 1, 2, \dots, c$$

$y_{ij(k)}$: The observation in i^{th} row and the j^{th} column receiving the k^{th} Latin treatment and the i^{th} Greek treatment

μ : Overall mean

T_k : The effect of the k^{th} Latin treatment

R_i : The effect of the i^{th} row

C_j : The effect of the j^{th} column

$\varepsilon_{ij(k)}$: Random error

ANOVA TABEL

| Source | d.f. | S.S. | M.S. | F |
|----------------|-------------|---------------------|---------------------|----------------------------|
| Treat | t-1 | SS_{Treat} | MS_{Treat} | MS_{Treat} / MS_E |
| Blocks(row) | r-1 | SS_{Repli} | MS_{Repli} | MS_{Repli} / MS_E |
| Location (col) | c-1 | SS_{Col} | MS_{Col} | MS_{Col} / MS_E |
| Error | Subtraction | SS_E | MS_E | |
| Total | N - 1 | SS_T | | |

$$\lambda = \frac{r(k-1)}{a-1}$$

r: عدد تكرارات كل معالجة في التجربة
k: عدد القطع التجريبية في القطاع
a: عدد المعالجات

$$SS_{\text{Repl.}} = \frac{\sum y_{i..}^2}{r} - \frac{y_{..}^2}{N}$$

$$SS_{\text{adj}(treat)} = \frac{k \sum Q_i^2}{\lambda \times a}$$

$$SS_{\text{Location}(Col.)} = \frac{\sum y_{.j}^2}{t} - \frac{y_{..}^2}{N}$$

$$SS_{\text{Total}} = \sum y_{ijk}^2 - \frac{y_{..}^2}{N}$$

$$SSE = SS_{\text{Total}} - SS_{\text{treat}} - SS_{\text{Repli.}} - SS_{\text{Col.}}$$