

## LATIN SQUARE DESIGN

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## Latin square designs

The Latin square design allows blocking in two directions.

The Latin letters A, B, C, . . . are arranged into a square array, such that each letter appears once in each row and once in each column of the square. Rows and columns represent levels of the two blocking factors; the letters correspond to different treatments. So, we need:  $(\# \text{ of treatments}) = (\# \text{ rows}) = (\# \text{ columns})$ .

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### Example

Nitrogen content of wheat as a function of fertilization method, along soil and irrigation gradients.

Here the treatment of interest is the fertilization method, however the response will also be impacted by two other factors: soil, irrigation

If the soil gradient run perpendicular to the irrigation gradient, A Latin square design will allow us to block on both factors.

To understand the design, it's easier to start with an example. Suppose someone wants to study nitrogen content of wheat as a function of fertilization method, along soil and irrigation gradients.

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If the soil gradient run perpendicular to the irrigation gradient, A Latin square design will allow us to block on both factors.

## Illustration of a Latin square design

**Display 8.3 Arrangement of Experimental Plots for the Wheat Experiment in a Latin Square Design**

<i>Field Row</i>	<i>Column 1</i>	<i>Column 2</i>	<i>Column 3</i>	<i>Column 4</i>	<i>Column 5</i>	<i>Irrigation Gradient</i>
1	59.45(E)	47.28(A)	54.44(C)	50.14(B)	59.45(D)	↓
2	55.16(C)	60.89(D)	56.59(B)	60.17(E)	48.71(A)	
3	44.41(B)	53.72(C)	55.87(D)	47.99(A)	59.45(E)	
4	42.26(A)	50.14(B)	55.87(E)	58.74(D)	55.87(C)	
5	60.89(D)	59.45(E)	49.43(A)	59.45(C)	57.31(B)	
	<i>Soil Gradient</i> →					

The Latin square design allows blocking in two directions.

This Figure shows the layout of a Latin square design.

The letters A, B, C, D and E correspond to levels of the treatment factor (in this example, the fertilization methods).

Field column and row are two blocking factors. We see that the irrigation gradient runs across the rows and the soil gradient runs across the columns.

Note that each treatment appears once in each row and once in each column. This is the key feature in a Latin square design.

With a Latin square design, the numbers of rows and columns have to be the same as the number of treatments, but one can use more than one squares.

## Randomize a Latin square design

To randomize the design:

1. Start with a standard square (e.g., ABCD / BCDA / CDAB / DABC ).
2. Randomly order the rows.
3. Randomly order the columns.
4. Randomly assign treatments to the letters.

To randomize the Latin square design:

We start with a standard square (e.g., ABCD / BCDA / CDAB / DABC ), randomly permute the rows, randomly permute the columns, and randomly assign treatments to the letters.

## Statistical analysis for the Latin square design

The model for a  $t \times t$  Latin square is

$$y_{ijk} = \mu + \rho_i + \gamma_j + \tau_k + e_{ijk}$$

where

- $\mu$  = overall mean
- $\rho_i$  =  $i$ th row effect ( $i = 1, \dots, t$ )
- $\gamma_j$  =  $j$ th column effect ( $j = 1, \dots, t$ )
- $\tau_k$  =  $k$ th treatment effect ( $k = 1, \dots, t$ )
- $e_{ijk}$  = random error.

The model is completely additive, i.e., it assumes no interaction between rows, columns, and treatments.

Here is the model equation for a Latin square design. Similar to the model of a RCBD experiment, there is no interaction terms, since the treatments are not replicated within each row or each column.

Note that here we treated rows and columns as fixed effects. Generally, fixed effects models are easier to handle in computational software. Also, for the purpose of treatment comparisons, in this case, the fixed effect model and the random effect model will give the same results.

### Partitioning of the sum of squares, F-test for treatment effect

The total sum of squares for the  $t^2$  observations is partitioned as follows (Kuehl, p. 281):

$$SS \text{ Total} = SS \text{ Rows} + SS \text{ Columns} + SS \text{ Treatment} + SSE.$$

The corresponding degrees of freedom are

$$t^2 - 1 = (t - 1) + (t - 1) + (t - 1) + (t - 2)(t - 1).$$

Fit the model as a three-way ANOVA without interactions.

In a Latin square design, the total sum of square can be partitioned into SS Rows, SS Columns, SS Treatment, and SSE.

One can find the detailed definitions of these sum of squares one textbook page 281. We will skip the details here.

Intuitively, we should see that a Latin square design is very balanced or symmetric: each treatment level appears in each row exactly once, each treatment level appears in each column exactly once, and furthermore, each column level appear in each row once.

The consequence of this balance or symmetry is that the column effects, row effects and treatment effects are all orthogonal to each other in a statistical sense.

## ANOVA table for a Latin square design

**Table 8.6** Analysis of variance for experiments in a Latin square design

<i>Source of Variation</i>	<i>Degrees of Freedom</i>	<i>Sum of Squares</i>	<i>Mean Square</i>	<i>Expected Mean Square</i>
Total	$t^2 - 1$	$\sum_i \sum_j (y_{ij} - \bar{y}_{..})^2$		
Rows	$t - 1$	$t \sum_i (\bar{y}_{i.} - \bar{y}_{..})^2$	$MSR$	
Columns	$t - 1$	$t \sum_j (\bar{y}_{.j} - \bar{y}_{..})^2$	$MSC$	
Treatments	$t - 1$	$t \sum_k (\bar{y}_{.k} - \bar{y}_{..})^2$	$MST$	$\sigma^2 + t\theta_t^2$
Error	$(t - 1)(t - 2)$	$SS \text{ Error}$	$MSE$	$\sigma^2$

Here is the ANOVA table for a Latin square design.

The expected mean square column suggests that to test the treatment effects, we should use the MSE as the denominator.

This ANOVA table left out the expected mean square for MS Rows and MS Columns. Because the randomization is restricted, the F-test for row or column effects will only be approximate. Recall that the made the same remark for testing the block effect in an RCBD design.



### Test for treatment effect

To test  $H_0: \tau_1 = \dots = \tau_t = 0$ , compare  $F_0 = \text{MST}/\text{MSE}$  to an  $F_{t-1, (t-2)(t-1)}$  distribution.

The tests for row and column effects may not be appropriate, because of the restrictions on randomization in the Latin square design.

For testing the treatment effect, the null hypothesis is that all treatment means are equal.

Again, the tests for row and column effects may not be appropriate, because of the restrictions on randomization in the Latin square design.

## Example

Example. Grain yield of wheat as a function of seeding rate, blocking by field row and column ( $t = 5$ ). See Kuehl, p. 283 (data set wheat2).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
row.f	4	99.20	24.801	5.2553	0.0111
col.f	4	38.48	9.620	2.0385	0.1527
treat.f	4	522.30	130.574	27.6685	5.619e-06
Residuals	12	56.63	4.719		

There is a strong treatment effect, after adjusting for field row and column. ☐

In the grain yield example, the ANOVA F-test for the treatment effect gives a p-value of (5.9 times  $10^{-6}$ ).

There is strong treatment effect adjusting for field row and column.

### Example.

In a study of the effect of traffic-light sequence (the treatment of interest) on waiting time at traffic lights (the response), investigators blocked by intersection and time of day. There were  $t = 5$  levels of the treatment and the two blocking factors.

		Intersection					
		1	2	3	4	5	
Time of day	1		D	C	B	A	E
	2						
	3		(A-E = traffic light sequences)				
	4						
	5						

Note that the rows and columns in a Latin square represent two factors that can impact the response, they do not have to correspond to physical locations.

In a study of the effect of traffic-light sequence on waiting time at traffic lights. The traffic-light sequence is the treatment of interest, the waiting time is the response.

But the waiting time tend to vary at different intersections and at different time of day. So one can block by intersection and time of day to increase the precision for treatment comparisons.

In this example, there were  $t = 5$  levels of the treatment and the two blocking factors.

## The cost of blocking

Note that the MSE in a Latin square design has only  $(t - 2)(t - 1)$  degrees of freedom.

Design	error d.f.	d.f. when $t = 5$
Completely randomized	$t(t - 1)$	20
Randomized complete block	$(t - 1)(t - 1)$	16
Latin square	$(t - 2)(t - 1)$	12

Considerable power is lost in the tests of treatment effects, unless the reduction in the error sum of squares effected by blocking is substantial.

One disadvantage of the Latin square design is that we lose quite many degrees of freedom for error.

In this table, we compare the numbers of error degrees of freedom for a completely randomized design, a RCBD, and a Latin square design.

With 25 observations and 5 treatment levels, the numbers of d.f. for the three types of designs are 20, 16, and 12.

In a Latin square design, considerable power is lost in the tests of treatment effects, unless the reduction in the error sum of squares effected by blocking is substantial.

### Relative efficiency

If we blocked only on rows, in a RCB design, the estimated mean square error is

$$s_{rcb}^2 = \frac{\text{MS Columns} + (t - 1) \cdot \text{MSE}}{t} = \frac{9.62 + 4(4.719)}{5} = 5.699,$$

where  $\text{MSE} \equiv s_{ls}^2$  is from the Latin square analysis.

The relative efficiency of column blocking is then

$$\text{RE}_{col} = s_{rcb}^2 / s_{ls}^2 = 5.699 / 4.719 = 1.21.$$

If we blocked only on columns,

$$s_{rcb}^2 = \frac{\text{MS Rows} + (t - 1) \cdot \text{MSE}}{t} = \frac{24.80 + 4(4.719)}{5} = 8.735,$$

and the relative efficiency of row blocking is

$$\text{RE}_{row} = s_{rcb}^2 / s_{ls}^2 = 8.735 / 4.719 = 1.85.$$

To quantify the effectiveness of blocking, we can compute the relative efficiency of a Latin square design as compared to a RCBD.

The formulas for computing the error variance estimates and relative efficiency are listed here.

For the wheat yield data, the relative efficiency of column blocking is 1.21. That is, if we only block on rows, we would need 21% more replicates to achieve the same power as achieved by the Latin square design.

The relative efficiency of row blocking is 1.21. That is, if we only blocked on columns, we would need 81% more replicates to achieve the same power as achieved by the Latin square design.

## Analysis in R

See R code and notes in [example8.2.html](#)

For analyzing Latin square designs in R, see the R code and notes in [example8.2.html](#)

## Summary

### Latin square design

- Blocking in two directions
- How to randomize the design
- ANOVA table and F-test
- Relative efficiency

In this lecture, we discussed the Latin square design. A Latin square design allows us to simultaneously block on two factors.

The topics here run parallel with the topics in the lecture on RCBD.

We discussed how to generate a randomization plan for a Latin square experiment.

As usual, the ANOVA table summarizes important information regarding the partition of the sum of squares, the expected values of the mean squares, and the F-test for treatment effects.

To evaluate the effectiveness of column or row blocking, we can compute the relative efficiency.