

Assumptions of the two-way ANOVA

To use a two-way ANOVA your data should meet certain assumptions. Two-way ANOVA makes all of the normal assumptions of a parametric test of difference:

1. Homogeneity of variance (a.k.a. homoscedasticity)

The variation around the mean for each group being compared should be similar among all groups. If your data don't meet this assumption, you may be able to use a [non-parametric alternative](#), like the Kruskal-Wallis test.

2. Independence of observations

Your independent variables should not be dependent on one another (i.e. one should not cause the other). This is impossible to test with categorical variables – it can only be ensured by good [experimental design](#).

In addition, your dependent variable should represent unique observations – that is, your observations should not be grouped within locations or individuals.

If your data don't meet this assumption (i.e. if you set up experimental treatments within blocks), you can include a blocking variable and/or use a repeated-measures ANOVA.

3. Normally-distributed dependent variable

The values of the dependent variable should follow a bell curve. If your data don't meet this assumption, you can try a data transformation.

Two Way ANOVA in Excel with replication: Steps

Step 1: Click the “Data” tab and then click “Data Analysis.” If you don’t see the Data analysis option, [install the Data Analysis Toolpak](#).

Step 2: Click “ANOVA two factor with replication” and then click “OK.” The two way ANOVA window will open.

Step 3: Type an Input Range into the Input Range box. For example, if your data is in cells A1 to A25, type “A1:A25” into the Input Range box. Make sure you include *all* of your data, including headers and group names.

Step 4: Type a number in the “Rows per sample” box. Rows per sample is actually a bit misleading. What this is asking you is how many individuals are in each group. For example, if you have 12

individuals in a group taking two tests (as in the picture below) you would type “12” into the Rows per Sample box.

| A | B | C |
|----------|------|---------|
| group | math | english |
| School A | 80 | 87 |
| | 87 | 89 |
| | 78 | 84 |
| | 77 | 86 |
| | 89 | 99 |
| | 98 | 91 |
| | 88 | 92 |
| | 81 | 99 |
| | 84 | 88 |
| | 92 | 77 |
| | 96 | 71 |
| | 93 | 86 |
| School B | 81 | 99 |
| | 78 | 77 |
| | 54 | 71 |
| | 55 | 77 |
| | 45 | 81 |
| | 56 | 90 |
| | 79 | 68 |
| | 68 | 77 |
| | 67 | 90 |
| | 89 | 89 |
| | 12 | 91 |
| | 51 | 68 |

Step 5: Select an Output Range. For example, click the “new worksheet” radio button to display the data in a new worksheet.

Step 6: Select an alpha level. In most cases, an alpha level of 0.05 (5 percent) works for most tests.

Step 7: Click “OK” to run the two way ANOVA. The data will be returned where you specified in Step 5.

Step 8: Read the results. To figure out if you are going to reject the null hypothesis or not, you'll basically be looking at two factors:

1. 1. If the F-value (f) is larger than the f critical value (f_{crit})
2. If the p-value is smaller than your chosen alpha level.

Question: Two way Anova with replication:

Suppose a botanist wants to know if plant growth is influenced by sunlight exposure and watering frequency. She plants 40 seeds and lets them grow for one month under different conditions for sunlight exposure and watering frequency.

After one month, she records the height of each plant. The results are shown below:

| | | Sunlight Exposure | | | |
|--------------------|-----|-------------------|-----|--------|------|
| Watering Frequency | | None | Low | Medium | High |
| Daily | 4.8 | 5 | 6.4 | 6.3 | |
| | 4.4 | 5.2 | 6.2 | 6.4 | |
| | 3.2 | 5.6 | 4.7 | 5.6 | |
| | 3.9 | 4.3 | 5.5 | 4.8 | |
| | 4.4 | 4.8 | 5.8 | 5.8 | |
| Weekly | 4.4 | 4.9 | 5.8 | 6 | |
| | 4.2 | 5.3 | 6.2 | 4.9 | |
| | 3.8 | 5.7 | 6.3 | 4.6 | |
| | 3.7 | 5.4 | 6.5 | 5.6 | |
| | 3.9 | 4.8 | 5.5 | 5.5 | |

In the table above, we see that there were five plants grown under each combination of conditions.

Question: Two-way ANOVA

A Two way ANOVA without replication can compare a group of individuals performing more than one task. For example, you could compare students' scores across different research areas or subjects...

| Students | A.I | Machine Learning | Statistical Analysis |
|----------|-----|------------------|----------------------|
| 1 | 89 | 68 | 89 |
| 2 | 87 | 74 | 90 |
| 3 | 99 | 89 | 99 |
| 4 | 100 | 90 | 85 |
| 5 | 96 | 84 | 96 |
| 6 | 100 | 82 | 100 |

Question 2: (without replication)

A new method to determine the amount of low-calorie sweetener in different food samples has been introduced by a company. The company wants to apply this method on four food samples. The company has four labs. So the tests that involve the application of this new method to each of the food samples will be carried out in each of the four labs. Each of the labs have reported the mean recovery percentages of the amount of low-calorie sweetener they could detect on each of the food samples. The data are given below.

| Lab | Food samples | | | |
|-----|--------------|-------|-------|-------|
| | 1 | 2 | 3 | 4 |
| 1 | 99.5 | 83.0 | 96.5 | 96.8 |
| 2 | 105.0 | 105.5 | 104.0 | 108.0 |
| 3 | 95.4 | 81.9 | 87.4 | 86.3 |
| 4 | 93.7 | 80.8 | 84.5 | 70.3 |

It seems that different labs have different results for each sample. What we have to ascertain is if any of these results have occurred due to chance variation. To establish this, we go for two-way ANOVA without replication. Why ‘without replication’? Because from each lab we have only one value for percentage recovery (which is the mean value). If there had been more than one value for percentage recovery from each lab, for each food sample, we would have to go for two-way ANOVA with replication.

Using two-way ANOVA without replication, we are going to calculate the F statistic for both the groups (i.e. food samples/columns) and the blocks (i.e. labs/rows).

The main equations for two-way ANOVA without replication are given below with the expanded meaning of each term.

$$SST = SSG + SSB + SSE \quad (1)$$

$$F_{groups} = \frac{\left(\frac{SSG}{df_{groups}} \right)}{\left(\frac{SSE}{df_{error}} \right)} \quad (2)$$

$$F_{blocks} = \frac{\left(\frac{SSB}{df_{blocks}} \right)}{\left(\frac{SSE}{df_{error}} \right)} \quad (3)$$

SST = Sum of squares total

SSG = Sum of squares groups (i.e. columns)

SSB = Sum of squares blocks (i.e. rows)

SSE = Sum of squares error

df_{groups} = degrees of freedom groups

df_{blocks} = degrees of freedom blocks

df_{error} = degrees of freedom error

Calculating { F }_{ groups } F_{groups} and { F }_{ blocks } F_{blocks} manually

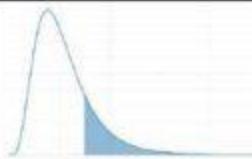
1. Add all the 16 mean recovery values and divide by 16 to get the overall mean,
2. Find the difference between each of the 16 recovery values and the overall mean, square the differences, and add them up to obtain SST
3. Find the difference between each group mean and the overall mean, square the differences, add them up, and multiply by the number of items in each group to obtain SSG

| Lab | Food samples | | | |
|-----|--------------|-------|-------|-------|
| | 1 | 2 | 3 | 4 |
| 1 | 99.5 | 83.0 | 96.5 | 96.8 |
| 2 | 105.0 | 105.5 | 104.0 | 108.0 |
| 3 | 95.4 | 81.9 | 87.4 | 86.3 |
| 4 | 93.7 | 80.8 | 84.5 | 70.3 |

4. Find the difference between each block mean and the overall mean, square the differences, add them up, and multiply by the number of items in each group to obtain SSB
5. Since we have already calculated SST, SSG, and SSB, we can calculate SSE using equation (1)

$$SSE = SST - SSG - SSB$$

6. Calculating degrees of freedom
7. Calculating $\{ F \}_{\text{groups}}$ using equation (2)
8. Calculating $\{ F \}_{\text{blocks}}$ using equation (3)

 F-Table Upper Tail Area of 0.05

| Denominator df | Numerator df | | | | | | | | | | | |
|----------------|--------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| | 161.45 | 199.50 | 215.71 | 224.58 | 230.16 | 233.99 | 236.77 | 238.88 | 240.54 | 241.88 | 242.98 | 244.08 |
| 1 | 161.45 | 199.50 | 215.71 | 224.58 | 230.16 | 233.99 | 236.77 | 238.88 | 240.54 | 241.88 | 242.98 | 244.08 |
| 2 | 18.51 | 19.00 | 19.16 | 19.25 | 19.30 | 19.33 | 19.35 | 19.37 | 19.38 | 19.40 | 19.40 | 19.40 |
| 3 | 10.13 | 9.55 | 9.28 | 9.12 | 9.01 | 8.94 | 8.89 | 8.85 | 8.81 | 8.79 | 8.76 | 8.73 |
| 4 | 7.71 | 6.94 | 6.59 | 6.39 | 6.26 | 6.16 | 6.09 | 6.04 | 6.00 | 5.96 | 5.94 | 5.91 |
| 5 | 6.61 | 5.79 | 5.41 | 5.19 | 5.05 | 4.95 | 4.88 | 4.82 | 4.77 | 4.74 | 4.70 | 4.66 |
| 6 | 5.99 | 5.14 | 4.76 | 4.53 | 4.39 | 4.28 | 4.21 | 4.15 | 4.10 | 4.06 | 4.03 | 3.99 |
| 7 | 5.59 | 4.74 | 4.25 | 4.12 | 3.97 | 3.87 | 3.79 | 3.73 | 3.68 | 3.64 | 3.60 | 3.56 |
| 8 | 5.32 | 4.46 | 4.07 | 3.84 | 3.69 | 3.58 | 3.50 | 3.44 | 3.39 | 3.35 | 3.31 | 3.27 |
| 9 | 5.12 | 4.26 | 3.86 | 3.63 | 3.48 | 3.37 | 3.29 | 3.23 | 3.18 | 3.14 | 3.10 | 3.06 |
| 10 | 4.96 | 4.10 | 3.71 | 3.48 | 3.33 | 3.22 | 3.14 | 3.07 | 3.02 | 2.98 | 2.94 | 2.90 |
| 11 | 4.84 | 3.98 | 3.59 | 3.36 | 3.20 | 3.09 | 3.01 | 2.95 | 2.90 | 2.85 | 2.82 | 2.78 |
| 12 | 4.75 | 3.89 | 3.49 | 3.26 | 3.11 | 3.00 | 2.91 | 2.85 | 2.80 | 2.75 | 2.72 | 2.68 |
| 13 | 4.67 | 3.81 | 3.41 | 3.18 | 3.03 | 2.92 | 2.83 | 2.77 | 2.71 | 2.67 | 2.63 | 2.59 |
| 14 | 4.60 | 3.74 | 3.34 | 3.11 | 2.96 | 2.85 | 2.76 | 2.70 | 2.65 | 2.60 | 2.57 | 2.53 |
| 15 | 4.54 | 3.68 | 3.29 | 3.06 | 2.90 | 2.79 | 2.71 | 2.64 | 2.59 | 2.54 | 2.51 | 2.47 |
| 16 | 4.49 | 3.63 | 3.24 | 3.01 | 2.85 | 2.74 | 2.66 | 2.59 | 2.54 | 2.49 | 2.46 | 2.42 |
| 17 | 4.45 | 3.59 | 3.20 | 2.96 | 2.81 | 2.70 | 2.61 | 2.55 | 2.49 | 2.45 | 2.41 | 2.37 |
| 18 | 4.41 | 3.55 | 3.16 | 2.93 | 2.77 | 2.66 | 2.58 | 2.51 | 2.46 | 2.41 | 2.37 | 2.33 |
| 19 | 4.38 | 3.52 | 3.13 | 2.90 | 2.74 | 2.63 | 2.54 | 2.48 | 2.42 | 2.38 | 2.34 | 2.30 |
| 20 | 4.35 | 3.49 | 3.10 | 2.87 | 2.71 | 2.60 | 2.51 | 2.45 | 2.39 | 2.35 | 2.31 | 2.27 |
| 21 | 4.32 | 3.47 | 3.07 | 2.84 | 2.68 | 2.57 | 2.49 | 2.42 | 2.37 | 2.32 | 2.28 | 2.24 |
| 22 | 4.30 | 3.44 | 3.05 | 2.82 | 2.66 | 2.55 | 2.46 | 2.40 | 2.34 | 2.30 | 2.26 | 2.22 |
| 23 | 4.28 | 3.42 | 3.03 | 2.80 | 2.64 | 2.53 | 2.44 | 2.37 | 2.32 | 2.27 | 2.24 | 2.20 |
| 24 | 4.26 | 3.40 | 3.01 | 2.78 | 2.62 | 2.51 | 2.42 | 2.36 | 2.30 | 2.25 | 2.22 | 2.18 |
| 25 | 4.24 | 3.39 | 2.99 | 2.76 | 2.60 | 2.49 | 2.40 | 2.34 | 2.28 | 2.24 | 2.20 | 2.16 |
| 26 | 4.23 | 3.37 | 2.98 | 2.74 | 2.59 | 2.47 | 2.39 | 2.32 | 2.27 | 2.22 | 2.18 | 2.14 |
| 27 | 4.21 | 3.35 | 2.96 | 2.73 | 2.57 | 2.46 | 2.37 | 2.31 | 2.25 | 2.20 | 2.17 | 2.13 |
| 28 | 4.20 | 3.34 | 2.95 | 2.71 | 2.56 | 2.45 | 2.36 | 2.29 | 2.24 | 2.19 | 2.15 | 2.11 |