

## Analysis of Variance Overview

The Analysis of Variance (ANOVA) technique is a method for comparing the means of continuous data that are classified into groups. There are many different versions depending on how the data are grouped. Some of the more common ones are outlined here.

### Some of the common types of ANOVA

#### One way ANOVA

This can be thought of as an extension to the unpaired t-test for more than two groups. There is a single continuous outcome variable and a single grouping variable. Each person/experimental unit occurs in only one group.

For example, a researcher is interested in how the nutrient level of soil impacts on yield (measured in grams) and randomly assigns plants to one of three nutrient groups (low level of nutrients, medium and high level nutrients). The one way ANOVA compares the mean growth between the three groups. When group sizes are equal ANOVA is relatively robust to non-normality. The null hypothesis is that all means are the same. If this hypothesis is rejected, the ANOVA doesn't tell you which means are different; this can be examined using post hoc tests (see MASH handout on post hoc tests). A one way ANOVA allows you to examine the following research question:

1. Is there a difference between the means for the different groups

#### (One-way) Repeated measures ANOVA

There is a single outcome variable and one grouping variable but in this case the grouping variable represents different experimental conditions (including different times such as at baseline, after 1 month and after 2 months) and each person/experimental unit is exposed to all the experimental conditions (or measured at all time points, if time is the grouping variable), hence the name 'repeated measures'.

A repeated measures ANOVA allows you to examine the following research question:

1. Is there a difference between the means at each of the different conditions/times

At first glance this looks similar to the one way ANOVA, but it differs from this as each individual or experimental unit is exposed to all the experimental conditions being investigated (in each category of the grouping variable), whereas for a one way ANOVA each experimental unit only occurs once and is thus only in a single group (or measured at a single level of the grouping variable). Differences are based upon comparisons within individuals, rather than between individuals.

If an individual has a missing value for one of the conditions, they are not included in the analysis at all, as the method requires complete data for each individual being studied.

Example: A researcher is interested in reaction times. The researcher measures the reaction times of a group of people at 8am, 1pm, 8pm and 1am. A repeated measures ANOVA could be used to see if the average reaction times were all the same at each measurement point or not.

## Two way ANOVA

There is a single continuous outcome variable and **two** grouping variables. Each person/experimental unit occurs only once (i.e. is in only one variable 1 group and one variable 2 group). Hypothesis tests are based on between groups/individuals comparisons.

Extending the example above, the researcher is also interested in how both the nutrient level of soil and the light intensity impacts on yield (measured in grams). There are three nutrient levels (nutrient poor, medium and nutrient rich) and two light levels (low and bright light) and each plant is randomly assigned to one of the light x nutrient combinations:

Low light x nutrient poor

Low light x medium

Low light x nutrient rich

Bright light x nutrient poor

Bright light x medium

Bright light x nutrient rich

A two way ANOVA allows you to examine the following research questions:

1. Is there a difference between the means for grouping variable 1
2. Is there a difference between the means for grouping variable 2
3. Is there an interaction between grouping variable 1 and grouping variable 2, such as that the impact of being in a particular group for grouping variable 1 differs between different levels of grouping variable 2.

In terms of our example:

1. Is there a difference between the means for different amounts of nutrients?
2. Is there a difference between the means for different light levels?
3. Imagine the plants with more nutrients grew more. Does the amount they grew change depending on the light level?

## Mixed (repeated measures) ANOVA/split plot ANOVA

This can be thought of as a special case of a two-way ANOVA where:

- One of the grouping variables is fixed and each person/experimental unit only occurs in one group. This can be thought of as a 'between subjects' factor.
- The other factor is a repeated measure so that each person/experimental unit is measured at each level. This can be thought of as a 'within subjects' factor.

It is recommended to have more than 5 values in each group. It is used to examine if 2 or more repeated measures from two or more groups are significantly different from each other in terms of the variable of interest.

For example, you could examine whether light levels had an impact on growth over time. If you measured time at several time points, say baseline, one month and two months. A significant interaction would imply that light level had a differential effect on growth over time. The mixed ANOVA is what's known as an

omnibus test – if significant it tells you that differences exist somewhere in your model, but it doesn't tell you where they are.

With a mixed ANOVA there are several effects that are examined, but of primary importance is (3) the interaction between the two factors:

1. Within subjects effect: did the outcome variable in the groups change over time
2. Between subjects effect: was the outcome variable different between the groups
3. Interaction effect: did the outcome variable change differently over time in the groups. Is the effect of time different depending on what group the individual is in?

In terms of our example:

1. Is there a difference between the mean heights at different time points?
2. Is there a difference between the means heights at different light levels?
3. Imagine the plants had different heights over time (ie, they all grew enough for it to be considered significant). Does the amount they grew change depending on the light level?

## Two-way repeated measures ANOVA

This differs from a Mixed ANOVA in that in a two-way repeated measures ANOVA every experimental unit is assessed at every level of both factors. With a Mixed ANOVA one of the factors is fixed such that each experimental unit experiences only one of the levels. For the other factor they are measured at each level. As with a mixed ANOVA, the primary interest is in investigating whether there is an interaction between the two factors. An overall significant result only tells you that there were significant differences between groups, it doesn't tell you where they were. This needs to be investigated using post-hoc tests.

**Example:** *The classic example of a two-way repeated measures ANOVA is a crossover trial. Each person has all treatments and is measured both before and after each treatment. Note that the order in which treatments are given should be randomised to guard against carryover effects.*

## Assumptions for ANOVA

1. Data within groups are continuous
2. Data within groups are normally distributed, or at the very least that there are no significant outliers.
3. Homogeneity of variance. The variability is reasonably constant across groups
4. Independence of observations (except with repeated measures ANOVA and mixed ANOVA!)
5. Sphericity (for repeated measures ANOVA and mixed ANOVA): assumes that the variances of the differences between each of the pairs of repeated measurements is similar– this requires that the variance of the population difference scores for any two conditions are the same as the variances population difference scores for any other two conditions – usually tested by Mauchly's test of sphericity. For example, let's assume there are three time points. Sphericity assumes that the variance of the differences (T1-T2) is similar to the variance of the difference (T2-T3), and the difference (T1-T3).

## Summary table:

| Number of factors                                                                       | Name                                               | Data organisation                                                                                                                                                                                                                                                                                               | Example                                                                                                                                                                                           |
|-----------------------------------------------------------------------------------------|----------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| One factor                                                                              | One-way ANOVA                                      | Several groups. Each experimental unit can only be in one group                                                                                                                                                                                                                                                 | Plant yields measured at different nutrient levels: low, medium and high nutrient level). Each plant can only be in one nutrient level group                                                      |
|                                                                                         | (One-way) repeated measures ANOVA                  | Several related groups. Each experimental unit occurs in all groups. The related groups can be time, or different experimental conditions, the key is that all experimental units experience all conditions                                                                                                     | Plant yield measured at different time points. The same plants are measured over time                                                                                                             |
| Two factors<br>(Key interest lies in assessing the interaction between the two factors) | Two-way ANOVA                                      | Each experimental unit can only occur at one combination of the two factors                                                                                                                                                                                                                                     | Plant yields measured at different nutrient levels (low, medium and high) and different light levels (low and bright light). Each plant can only be in one nutrient x light level combination     |
|                                                                                         | Mixed (repeated measures) ANOVA / split plot ANOVA | One factor is fixed and each experimental unit can only occur at one level/in one group. The main effect of this factor is assessed between groups. Second factor contains related groups and each experimental unit occurs at all levels. The main effect of this factor is assessed within groups/individuals | Plant yields at different nutrient levels measured over time. Each plant can only be in one nutrient level group (fixed) but is measured at every time point eg, one week, one month, two months. |
|                                                                                         | Two-way repeated measures ANOVA                    | Both factors contain related groups                                                                                                                                                                                                                                                                             | In a crossover study, each person receives both treatments and outcome is measured at multiple time points                                                                                        |