**NO REPEATED MEASURES**

This refers to the situation where the response of each independent subject in the study (mouse, well, plate) is measured/assayed only once.

**Test Assumptions**

If your group size is >10, consider removing data points that are above or below the median by 2 x the interquartile (25-75%) range (i.e., median 2IQR). There may be other reasons to exclude a data point, even if it falls within these brackets, e.g., a known technical problem with the measurement or a wrong genotype.

Determine whether the data in all groups are distributed normally (Gaussian distribution)

Recommended test: D’Agostino-Pearson test

If not, determine whether transformation of the dependent variable results in a normal data distribution in all groups.

Recommended approach: Log transformation (any base)

The untransformed or transformed data is normally distributed:

Yes 🡪 Use parametric tests

No 🡪 Use non-parametric tests

***Use parametric tests if untransformed or transformed data are normally distributed.***

***Use non-parametric tests if they are not.***

Also check for evidence that variances among groups are not comparable.

Recommended test: F-test or Bartlett’s test (for normally distributed data); Levene’s test or Brown-Forsythe test (for non-normally distributed data)

**Parametric Tests**

2 Groups

Use tests in blue if group sizes are equal (even if variances are unequal).

Use tests in green if group sizes and variances are unequal.

Direction of change is unpredictable: Unpaired, two-tailed Student’s *t*-test or Welch’s *t*-test

Specific direction of change expected: Unpaired, one-tailed Student’s *t*-test or Welch’s *t*-test

3 Groups

Use tests in blue (or black) if variances among groups are equal.

Use tests in green (or black) if variances among groups are unequal.

One-way or two-way ANOVA (for 1 vs. 2 independent categorical variables, respectively) with Holm-Sidak, Tukey or Dunnett’s posthoc test for pairwise comparisons:

|  |  |  |  |
| --- | --- | --- | --- |
|  | Holm-Sidak | Tukey | Dunnett’s |
| Power, given assumptions | +++ | ++ | ++ |
| Restrictive assumptions |  | Normality of dependent variables, equal group sizes and variances, independence of individual comparisons | Normality of dependent variables, equal variances, independence of individual comparisons |
| Computes confidence intervals | No | Yes | Yes |
| Compares | Selected pairs of means (based on *a priori* hypotheses) | Every mean with every other mean | Every mean with a control mean |

Statistical linear models (for >2 independent categorical or continuous variables) with Holm-Sidak posthoc test for pairwise comparisons

Multiple Welch’s *t*-tests with Holm-Sidak correction for multiple comparisons

(See also permutation test under Non-parametric Tests)

**Non-parametric Tests**

2 Groups

Use tests in blue if group sizes are equal (even if variances are unequal).

Use tests in green if group sizes and variances are unequal.

Direction of change is unpredictable: Unpaired, two-sided Mann-Whitney (Wilcoxon rank-sum) test

Specific direction of change expected: Unpaired, one-sided Mann-Whitney (Wilcoxon rank-sum) test

3 Groups

Use tests in blue if variances among groups are equal.

Use tests in green if variances among groups are unequal.

Kruskall-Wallis test (if there is only one independent variable) with Holm-Sidak test for pairwise comparisons

Permutation test (if all groups are n 10) with Holm-Sidak correction for multiple comparisons (use COIN in R)

**REPEATED MEASURES IN SAME SUBJECTS/SAMPLES**

This refers to the situation where the response of each independent subject in the study (mouse, well, plate) is measured/assayed more than once.

**Test Assumptions**

If your group size is >10, consider removing data points that are above or below the median by 2 x the interquartile (25-75%) range. (There may be other reasons to exclude a data point, even if it falls within these brackets, e.g., a known technical problem with the measurement or a wrong genotype.)

2 Measures in 1 group

Determine whether the differences between the two measures across samples are distributed normally (Gaussian distribution)

Recommended test: D’Agostino-Pearson test

If not, determine whether transformation of the dependent variable results in a normal distribution of differences between the two measures.

Recommended approach: Log transformation (any base)

2 Measures in 2 groups

Carry out linear mixed effects model analysis to determine whether all random effects and residuals are normally distributed.

Recommended test: D’Agostino-Pearson test

If not, determine whether transformation of the dependent variable results in a normal distribution of random effects and residuals in ALL groups.

Recommended approach: Log transformation (any base)

***Use parametric tests if normality assumptions are met.***

***Use non-parametric tests if they are violated.***

**Parametric Tests**

2 Measures in 1 group

Direction of change is unpredictable: Paired, two-tailed Student’s *t*-test

Specific direction of change expected: Paired, one-tailed Student’s *t*-test

2 Measures in 2 groups

Linear mixed model analysis with lmerTest (in R) for pairwise comparisons.

If assumptions of the linear mixed effects models are not met but you have at least 20-25 biological replicates, try using the Generalized Estimating Equation (GEE) modeling framework, which is less sensitive to deviations from normality. This type of modeling can be carried out in R using geepack or geese.

We do not recommend using repeated-measures ANOVA because it makes too many restrictive assumptions for most of our experimental designs.

**Non-parametric Tests**

2 Measures in 1 group

Direction of change is unpredictable: Paired, two-tailed Wilcoxon Signed Rank test.

Specific direction of change expected: Paired, one-tailed Wilcoxon Signed Rank test.

2 Measures in 2 groups

For data obtained in the Morris water maze or comparable designs, use the RankSummary package in R or compute the area under the curve for each mouse and then use the approaches described in the “NO REPEATED MEASURES” section above. If your experimental design is different and you have at least 10 biological replicates per group, consider using permutation tests in the permuco package in R.