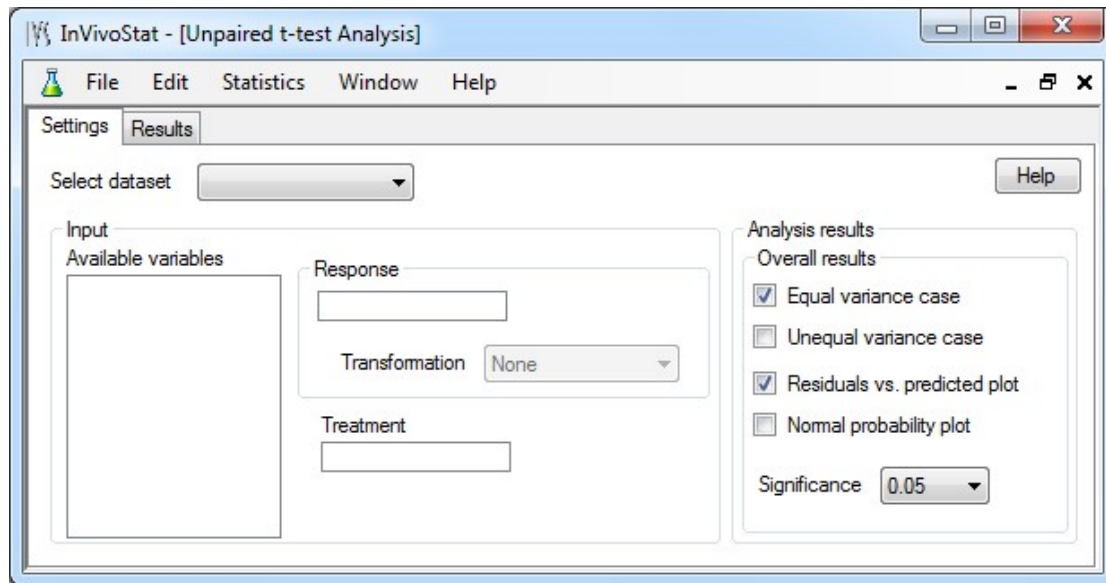


# InVivoStat

## Unpaired $t$ -test Analysis Module

### Tipsheet

The Unpaired t-test Analysis module in InVivoStat is available within the Additional Analyses sub-menu in the Statistics drop-down menu and is entitled “Unpaired t-test Analysis”. The user interface is:

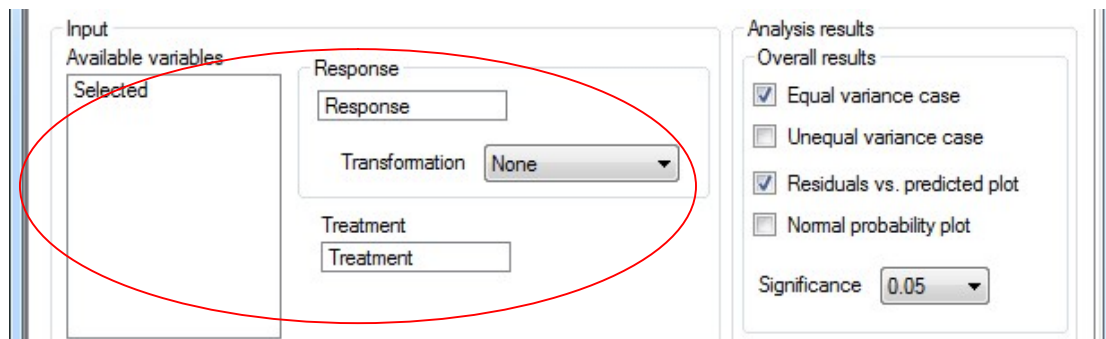


The Unpaired t-test Analysis module performs a t-test assuming equal and/or unequal variances between the two groups. The latter is an implementation of Welch’s t-test for unequal variances.

For more complex designs, with more factors and/or more than two factor levels, we recommend using the Single Measures Parametric Analysis module.

## 1 Setting up the model

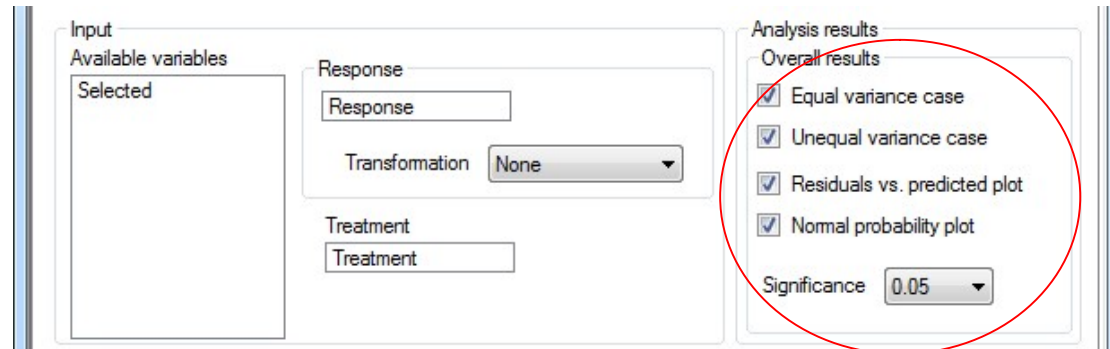
Once the dataset has been opened, the user can select the variables for the analysis by dragging and dropping them from the “Available variables” list into the “Response” and “Treatment” boxes.



Once selected, the user has the option of applying a transformation to the response variable, either  $\log_{10}$ ,  $\log_e$ , square root, arcsine or rank.

## 2 Selecting the analysis options

There are several results that are available to the user. These are selected before running the analysis.



The screenshot shows the InVivoStat software interface. On the left, under 'Input', there is a list of 'Available variables' with 'Selected' listed below it. In the center, there are fields for 'Response' (containing 'Response'), 'Transformation' (set to 'None'), and 'Treatment' (containing 'Treatment'). On the right, under 'Analysis results', the 'Overall results' section is circled in red. It contains four checked checkboxes: 'Equal variance case', 'Unequal variance case', 'Residuals vs. predicted plot', and 'Normal probability plot'. Below these is a 'Significance' dropdown menu set to '0.05'.

The output options include:

1) Equal variance case

Produces the result of the t-test under the assumption that the variances are the same in both groups. Analysis is performed using a pooled variance estimate.

2) Unequal variance case

Produces the result of Welch's t-test under the assumption that the variances are not the same across groups.

3) Residuals vs. predicted plot

Allows the user to check the homogeneity of variance assumption of the parametric analysis. Plot produced under the equal variance assumption.

4) Normal probability plot

Allows the user to check the normality assumption of the parametric analysis. Plot produced under the equal variances assumption.

5) Significance level

The default is 5%, although this can be changed.

## 3 Output Results

### Response

InVivoStat identifies the response being analysed. This section also describes any transformation that has been applied.

### Scatterplot of the raw data

InVivoStat produces a scatterplot of the raw data. This should be used to identify possible outliers. On the plot the X-axis corresponds to the two levels of the treatment factor and the Y-axis corresponds to the response.

t-test assuming equal variance table

This table gives results of the t-test under the assumption of equal variances between the groups.

Table of the least square (predicted) means

InVivoStat produces a table of the least square (predicted) means from the model assuming equal variances, with confidence intervals.

Comparison of the least square (predicted) means

InVivoStat produces a table of the difference between the least square (predicted) means from the model assuming equal variances, with confidence interval.

t-test assuming unequal variance table

This table gives results of the t-test under the assumption of unequal variances between the groups.

Table of the least square (predicted) means

InVivoStat produces a table of the least square (predicted) means from the model assuming unequal variances, with confidence intervals.

Comparison of the least square (predicted) means

InVivoStat produces a table of the difference between the least square (predicted) means from the model assuming unequal variances, with confidence interval.

Diagnostic plots

If requested InVivoStat produces the residuals vs. predicted plot and the normal probability plot. The residuals plotted on the residuals vs. predicted plot are the standardized residuals as these can provide a test for outliers. Any observation with a residual greater (or less than) 3 could be considered an outlier. These plots are produced using the equal variance model assumption.

References

Finally a list of references for the methods applied in the analysis is given.

## 4 Controlling the output

The user has the ability to control various aspects of the output. These are available from the

*Statistics* → *Options* → *Output Options*

menu and should be selected before performing the analysis.

Users can manipulate various global characteristics of the plots produced. Plots can be generated in pdf format and also in black and white.

If the response has been log transformed, by selecting ‘Display geometric means’ the back transformed means are given (and plotted).

Output Options

Graphics: Appearance Graphics: Font Graphics: Colour Advanced Output Options

Points		Lines		Error Bars		Scatterplot Jitter Amount	
Size	4	Lines size	1	Width	0.7	Horizontal	0.1
Shape	21	Solid type	solid			Vertical	0.1
		Dashed type	dashed				

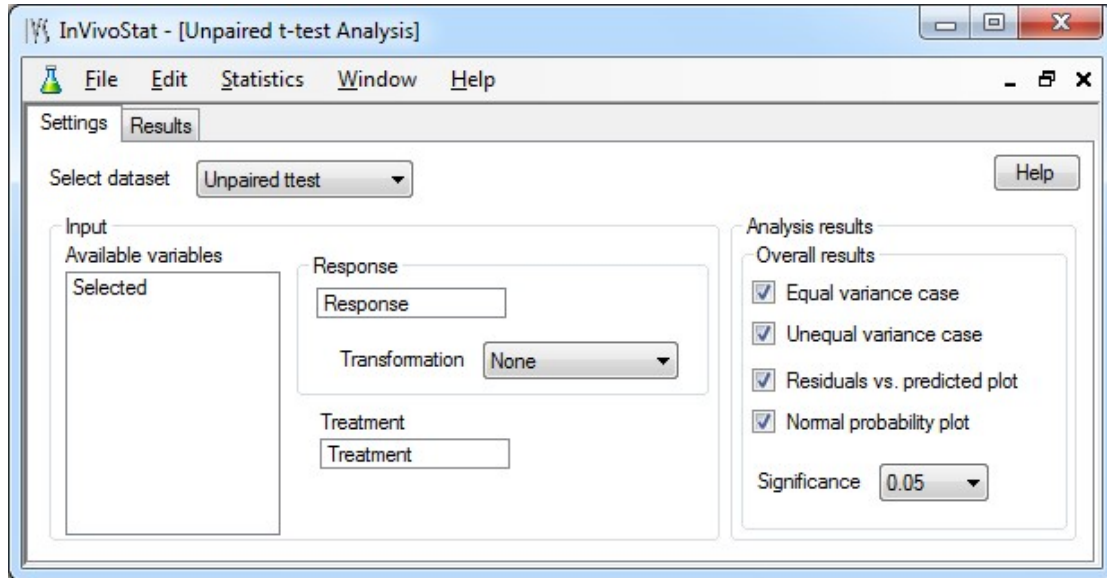
Warning: Be careful when editing the free-text boxes on this interface, output may not be generated as expected if the edited option is not recognised.

Help Reset Save Cancel

More information on the options can be found in the Getting Started Tipsheet.

## 5 Sample output

Options:



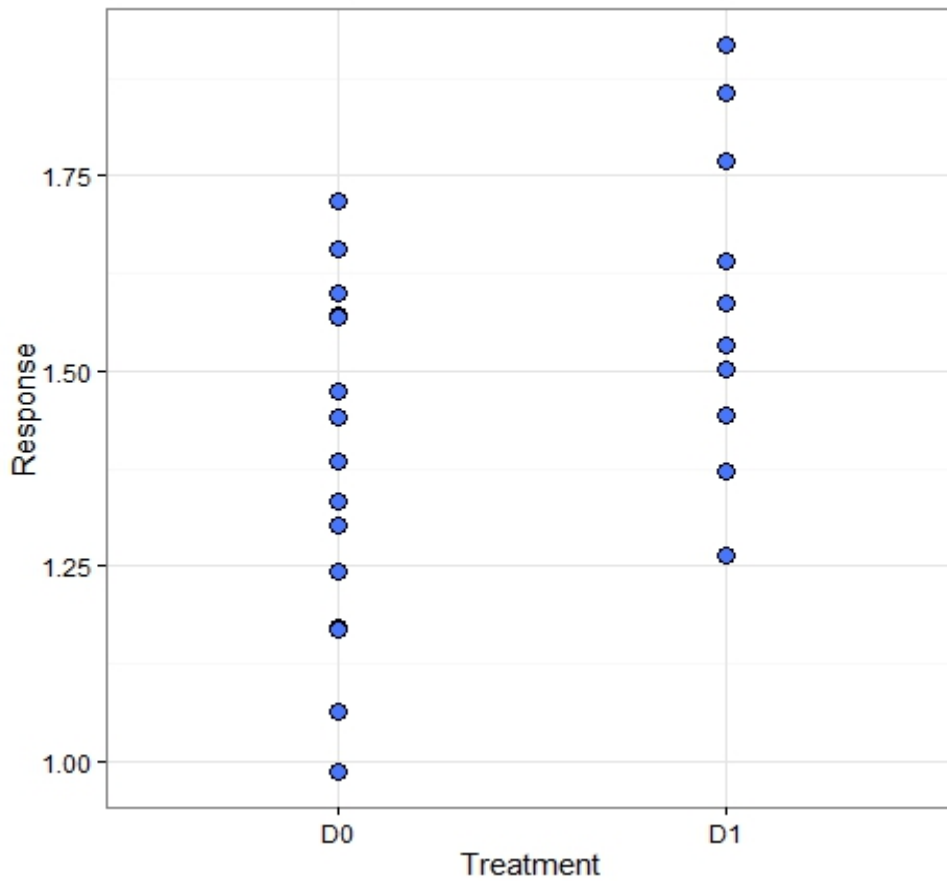
# InVivoStat Unpaired t-test Analysis

## Response

The Response response is currently being analysed by the Unpaired t-test Analysis module.

For more information on the theoretical approaches that are implemented within this module, see Bate and Clark (2014).

## Scatterplot of the raw data



Tip: Use this plot to identify possible outliers.

## Unpaired t-test assuming equal variances

	<b>t-statistic</b>	<b>Degrees of freedom</b>	<b>p-value</b>
Equal variance unpaired t-test	-2.408	36	0.0213

Conclusion: There is a statistically significant difference between the levels of Response at the 5% level.

## Table of the least square (predicted) means with 95% confidence intervals (assuming equal variances)

	<b>Mean</b>	<b>Lower 95% CI</b>	<b>Upper 95% CI</b>
Treatment level			
D0	1.399	1.298	1.500
D1	1.564	1.468	1.660

## Comparison of the least square (predicted) means with 95% confidence interval (assuming equal variances)

	<b>Difference</b>	<b>Lower 95% CI</b>	<b>Upper 95% CI</b>	<b>Std error</b>	<b>p-value</b>
Comparison					
D0 vs. D1	-0.165	-0.305	-0.026	0.069	0.0213

## Unpaired t-test assuming unequal variances

The analysis presented is Welch's t-test for unequal variances.

	<b>t-statistic</b>	<b>Degrees of freedom</b>	<b>p-value</b>
Unequal variance unpaired t-test	-2.387	33.52	0.0228

Conclusion: There is a statistically significant difference between the levels of Response at the 5% level.



Table of the least square (predicted) means with 95% confidence intervals (assuming unequal variances)

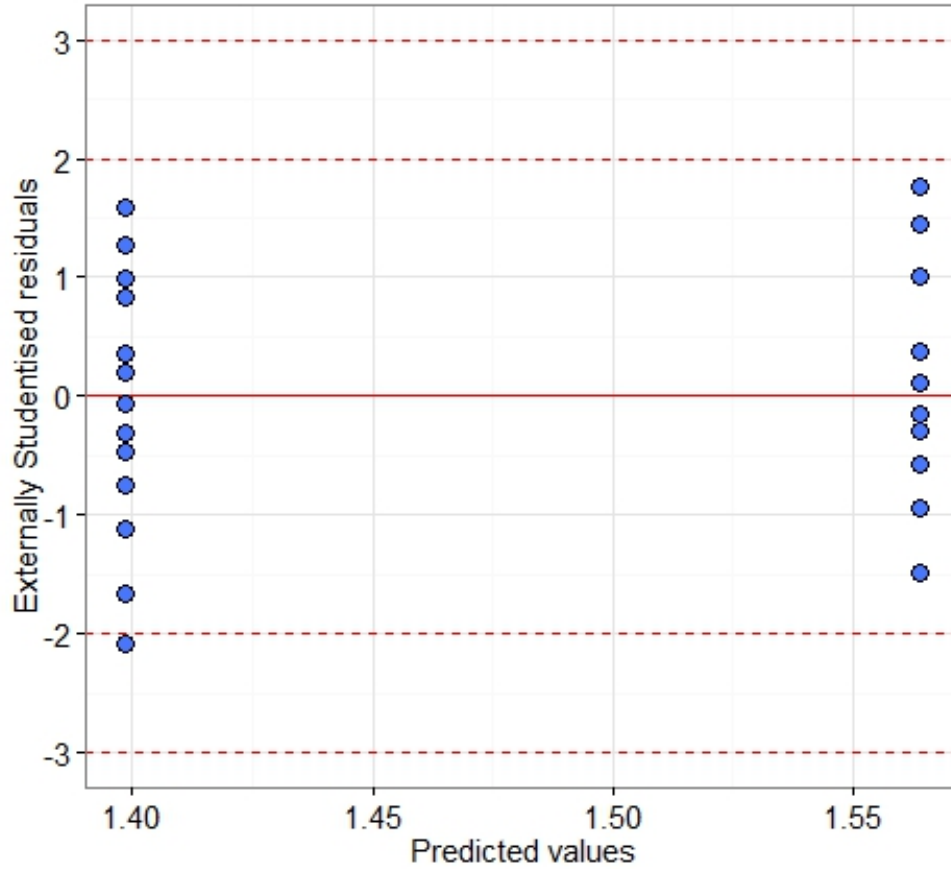
	Mean	Lower 95% CI	Upper 95% CI
Treatment level			
D0	1.399	1.285	1.513
D1	1.564	1.473	1.655

Comparison of the least square (predicted) means with 95% confidence interval (assuming unequal variances)

	Difference	Lower 95% CI	Upper 95% CI	p-value
Comparison				
D0 vs. D1	-0.165	-0.306	-0.024	0.0228

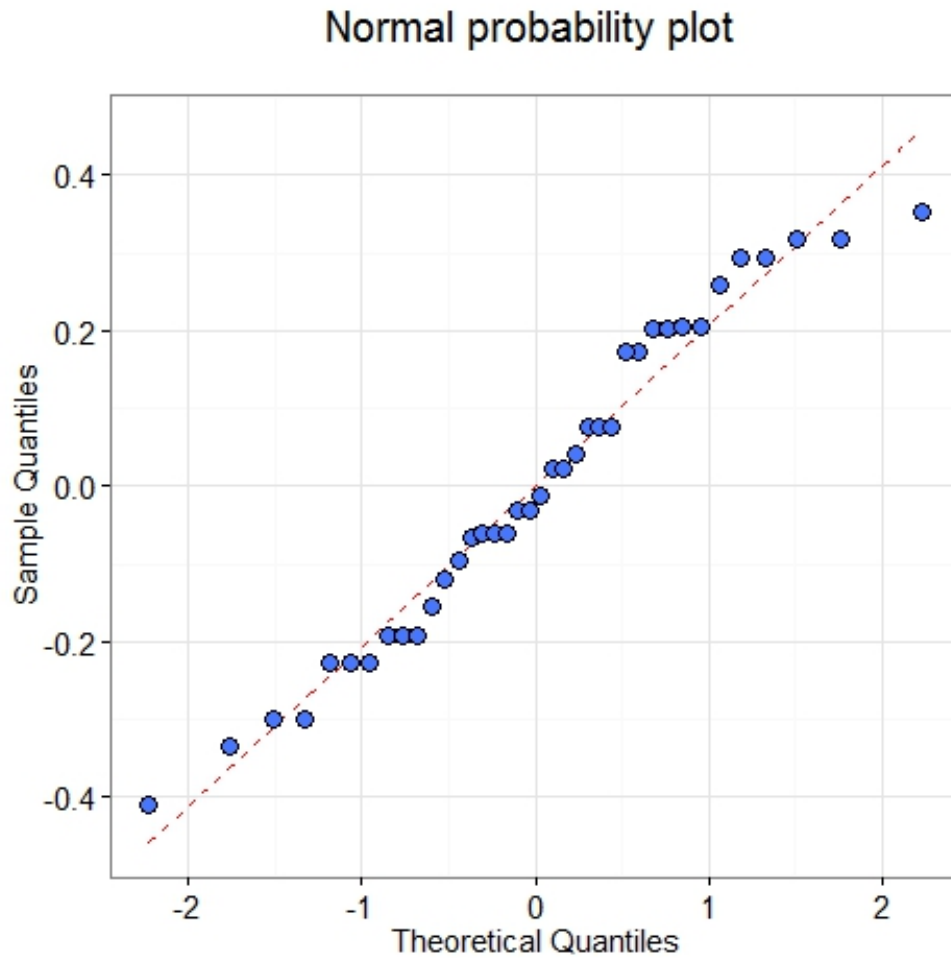
## Diagnostic plots (assuming equal variances)

Residuals vs. predicted plot



Tip: On this plot look to see if the spread of the points increases as the predicted values increase. If so the response may need transforming or the unequal variance assumption selected.

Tip: Any observation with a residual less than -3 or greater than 3 (SD) should be investigated as a possible outlier.



Tip: Check that the points lie along the dotted line. If not then the data may be non-normally distributed.

## Statistical references

Bate ST and Clark RA. (2014). *The Design and Statistical Analysis of Animal Experiments*. Cambridge University Press.

Welch BL. (1947). The generalization of Student's problem when several different population variances are involved. *Biometrika*, 34(1-2), 28-35.

## R references

R Development Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org>.

Barret Schloerke, Jason Crowley, Di Cook, Heike Hofmann, Hadley Wickham, Francois Briatte, Moritz Marbach and Edwin Thoen (2014). GGally: Extension to ggplot2. R package version 0.4.5. <http://CRAN.R-project.org/package=GGally>

Erich Neuwirth (2011). RColorBrewer: ColorBrewer palettes. R package version 1.0-5. <http://CRAN.R-project.org/package=RColorBrewer>

H. Wickham. ggplot2: elegant graphics for data analysis. Springer New York, 2009.

H. Wickham. Reshaping data with the reshape package. Journal of Statistical Software, 21(12), 2007.

Hadley Wickham (2011). The Split-Apply-Combine Strategy for Data Analysis. Journal of Statistical Software, 40(1), 1-29. URL <http://www.jstatsoft.org/v40/i01/>.

Hadley Wickham (2012). scales: Scale functions for graphics. R package version 0.2.3. <http://CRAN.R-project.org/package=scales>

Lecoutre, Eric (2003). The R2HTML Package. R News, Vol 3. N. 3, Vienna, Austria.

Louis Kates and Thomas Petzoldt (2012). proto: Prototype object-based programming. R package version 0.3-10. <http://CRAN.R-project.org/package=proto>

Torsten Hothorn, Frank Bretz and Peter Westfall (2008). Simultaneous Inference in General Parametric Models. Biometrical Journal 50(3), 346--363.

## Analysis dataset

	Response	Treatment
1	1.17	D0
2	1.30	D0
3	1.72	D0
4	1.17	D0
5	1.39	D0
6	1.44	D0
7	1.60	D0
8	1.57	D0
9	1.33	D0

10	1.57	D0
11	1.66	D0
12	1.47	D0
13	1.72	D0
14	1.17	D0
15	1.06	D0
16	1.24	D0
17	1.60	D0
18	0.99	D0
19	1.37	D1
20	1.50	D1
21	1.59	D1
22	1.37	D1
23	1.50	D1
24	1.37	D1
25	1.64	D1
26	1.53	D1
27	1.50	D1
28	1.59	D1
29	1.77	D1
30	1.86	D1
31	1.26	D1
32	1.64	D1
33	1.53	D1
34	1.77	D1
35	1.44	D1
36	1.92	D1
37	1.86	D1
38	1.26	D1