

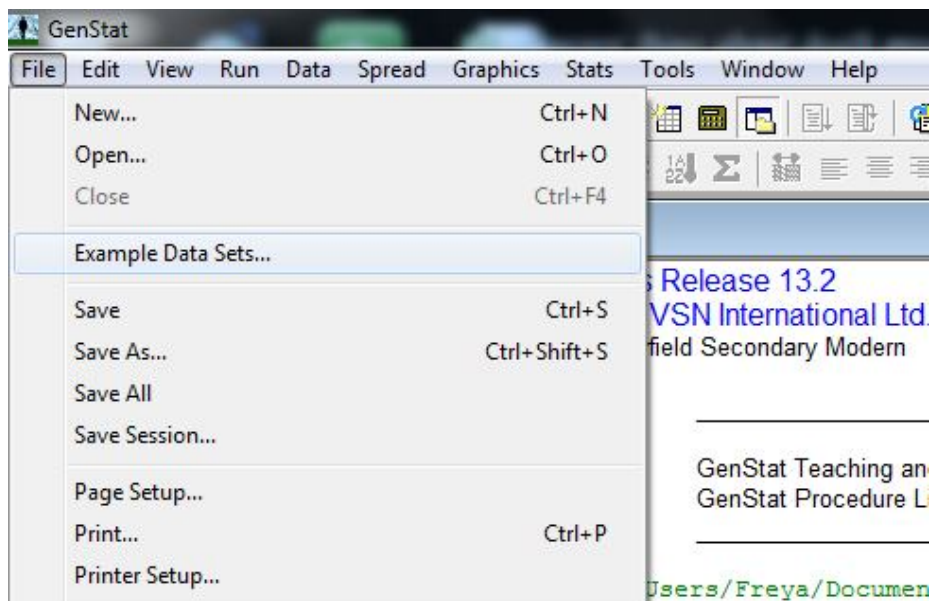


## Lesson: Use of Infrared Thermography for Non-Invasive Assessment of Animal Welfare

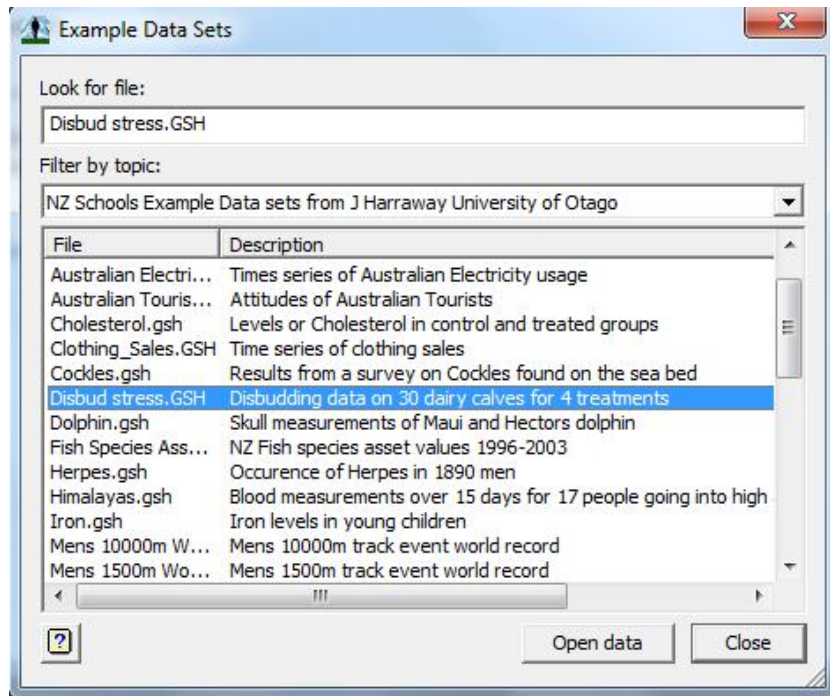
The development of a non-invasive tool to measure stress and pain in animals has significant applications in assuring the welfare standards of the New Zealand agricultural industry and boosting the value of its products in overseas markets. Here we are interested in stress involved in the disbudding (removal of horns) of cattle.

This lesson investigates the emotional and or stress responses of cattle during disbudding using four different treatment options.

1. To open the data we click on **File>Example Data Sets:**



This brings up the Example Data Sets dialog shown below. Click on the **Filter by topic** drop down menu and select the **NZ Schools Example Data sets** option. Choose the file **Disbud stress.GSH** and click on **Open data**.



This opens a spreadsheet (displayed below) containing data from a study investigating the effects of 4 different disbudding treatments on dairy calves, which was carried out by Mairi Stewart (AgResearch, Ruakura):

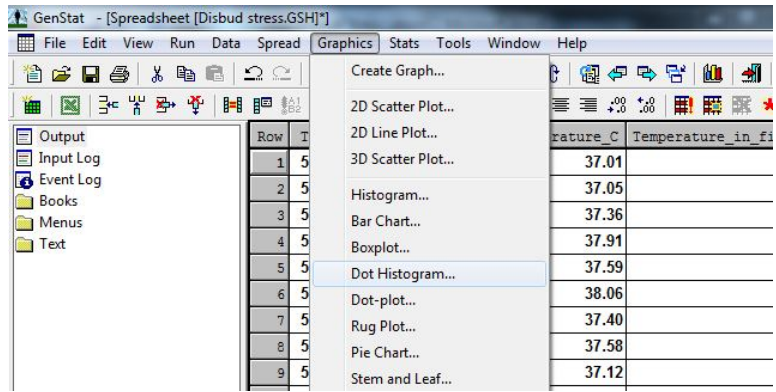
Row	Tag	Treatment	Base_eye_temperature_C	Temperature_in_first_3_mins_post_treat	Change_in_eye_temperature_C
1	5124	Control	37.01	37.23	0.21
2	5659	Control	37.05	36.75	-0.30
3	5656	Control	37.36	37.60	0.24
4	5677	Control	37.91	37.82	-0.09
5	5132	Control	37.59	37.85	0.26
6	5950	Control	38.06	37.90	-0.16
7	5685	Control	37.40	37.33	-0.07
8	5129	Control	37.58	37.65	0.07
9	5678	LA	37.12	37.36	0.24
10	5538	LA	37.55	37.42	-0.13
11	5657	LA	37.37	37.30	-0.07
12	5682	LA	37.44	37.45	0.01
13	5673	LA	37.34	37.30	-0.04
14	5697	LA	37.59	37.69	0.10

In this study 30 calves were allocated to one of 4 treatments for disbudding and stress levels were monitored using infrared thermography (a non-invasive method which measured eye-temperature).

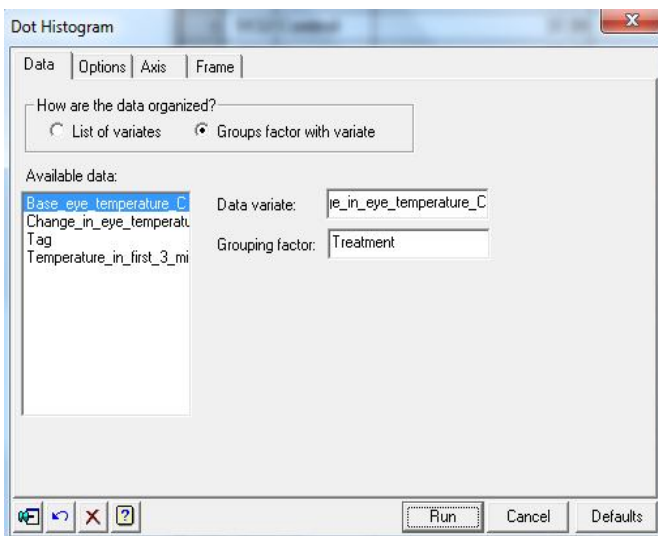
In the spreadsheet the data recorded is **Tag**, **Treatment**, **Base\_eye\_temperature\_C** (eye temperature at baseline), **Temperature-in\_first\_3\_mins\_post\_treat**, and **Change\_in\_eye\_temperature\_C**. The four treatment types recorded are **Control**, **LA** (local anaesthetic injection, mock disbudding), **DB** (disbudded with saline injection) and **LADB** (disbudded with local anaesthetic injection).

It is important to know what our data looks like, as it may help us to understand the results of the calculations that we will be doing later on.

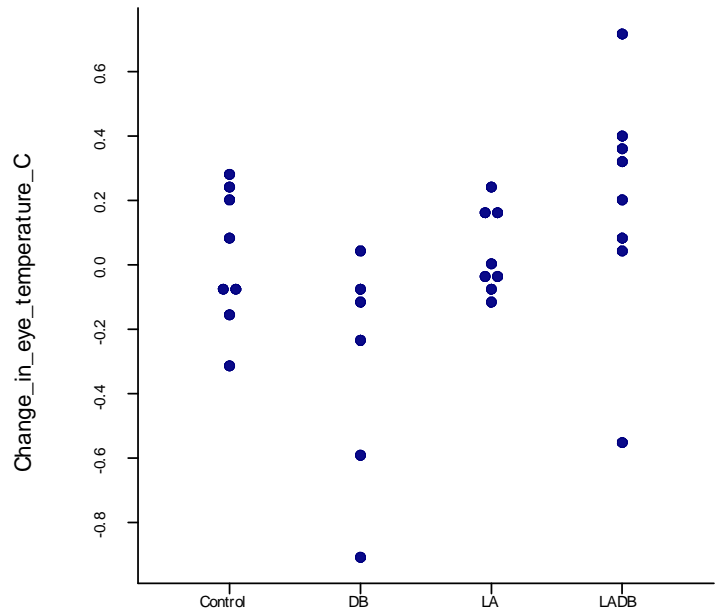
- The first step of the analysis is to plot the change in eye temperature against the four treatment groups. First click on **Graphics>Dot Histogram**:



In the **How are the data organized** box click on **Groups factor with variate**, then enter **Change\_in\_eye\_temperature** into the **Data variate** box and **Treatment** into the **Grouping factor** box. Either click **Run** to plot the graph with default settings, or click on the **Options** tab to modify the graph (e.g. add a title):

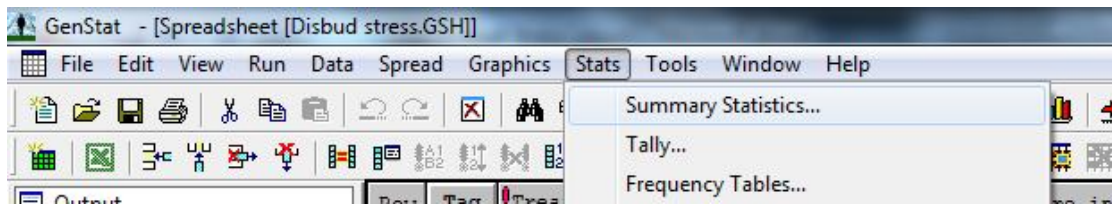


Change in eye temperature for treatment groups

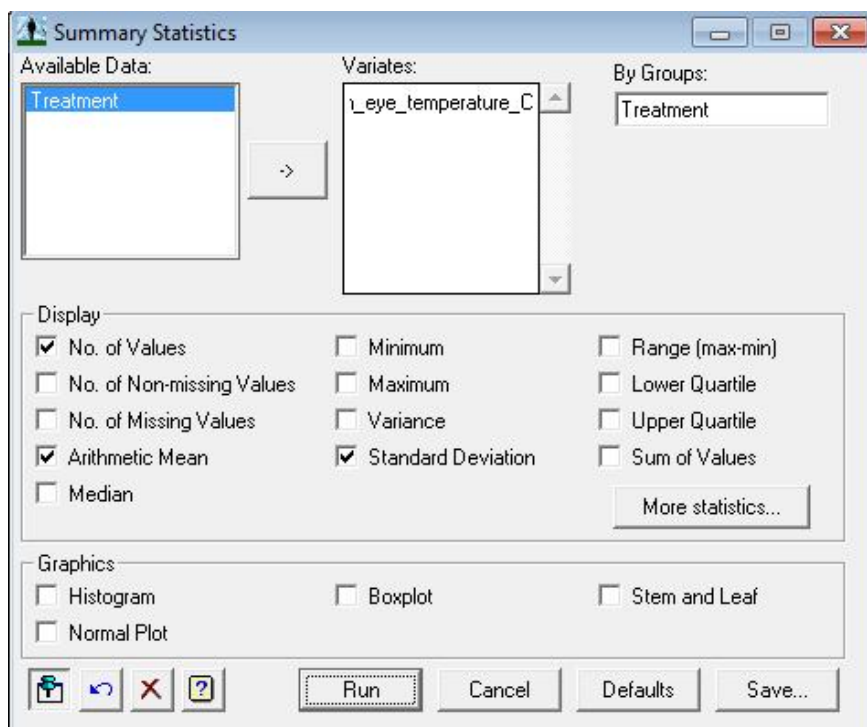


What can you see from the graph?

3. Now we want to calculate the mean change in eye temperature and the standard error for each treatment mean (SEM). To do this we use **Stats>Summary Statistics**:



Enter the change in eye temperature variable into the **Variates** box, and the Treatment variable into the **By Groups** box. Tick the **No. Of Values**, **Arithmetic Mean**, and **Standard Deviation** boxes, then click on the **More statistics** button and tick the **Standard Error of Mean** box. Click on **Run** then click on **Save**.



Under the **Save** options, tick the **Display in Spreadsheet** Box then press **Save**. This will produce your summary statistics in a new spreadsheet. The summary statistics can also be found in the GenStat Output Window.

New Spreadsheet:

Row	_UnitLabels_1	Change_in_eye_temperat ['Control']	Change_in_eye_temperat ['DB']	Change_in_eye_temperat ['LA']	Change_in_eye_temperat ['LADB']
1	NVAL	8	6	8	8
2	MEAN	0.0213255	-0.310566	0.0357405	0.20804
3	SD	0.206937	0.370167	0.132248	0.371898
4	SEM	0.0731634	0.15112	0.0467568	0.131486

GenStat Output Window:

### Summary statistics for Change\_in\_eye\_temperature\_C: Treatment Control

Number of values =	8
Mean =	0.0213
Standard deviation =	0.207
Standard error of mean =	0.0732

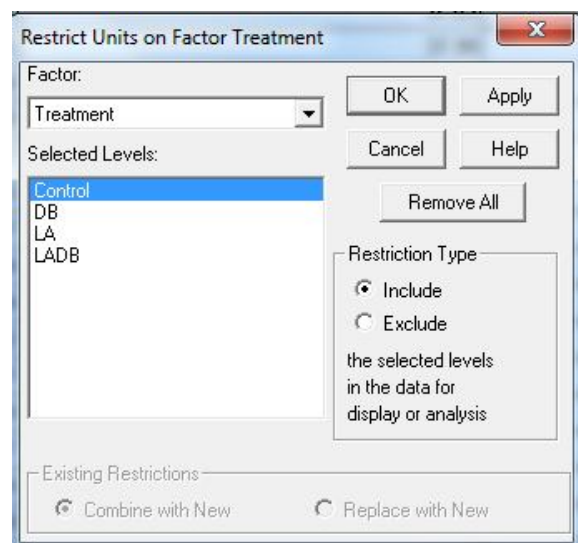
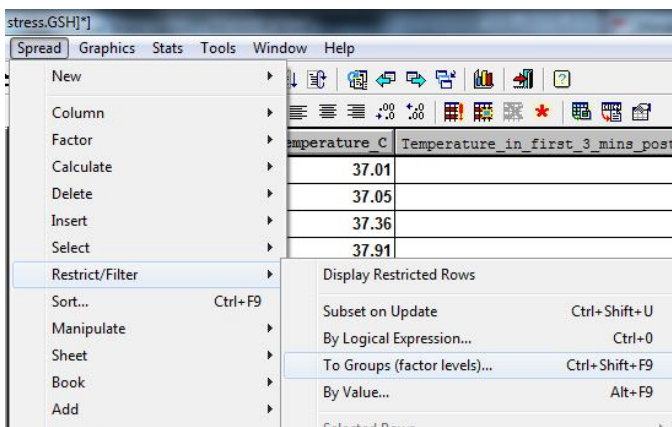
Using these summary statistics, calculate a 95% confidence interval for the mean change in eye temperature for each treatment group (use a large sample value of 1.96 or 2). What do you conclude?

Note: In practice a  $t$ -value should be used as we have small samples for each of the treatments. The  $t$ -value would have  $8-1=7$  degrees of freedom (df) for treatments 1, 2, and 4, but only  $6-1$  df for treatment 3.

- For each of the treatment means, we want to calculate the bootstrap confidence interval. The bootstrap technique involves randomly sampling a new dataset from the original sampled data. This creates a large number of datasets, that may have been seen if the experiment had been repeated many times (i.e. creates new datasets based on the original dataset).

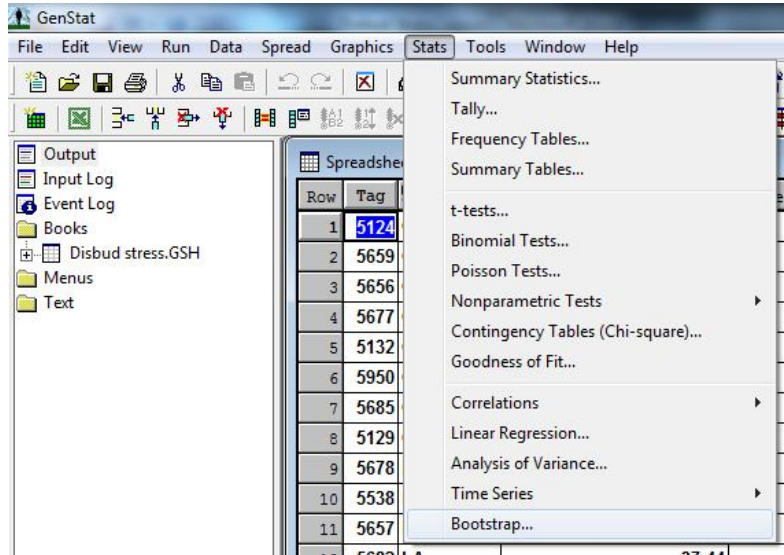
To use the bootstrap technique for each of the treatments we must first separate the data so that only one treatment is sampled per time. To carry out this separation, use **Spread>Restrict/Filter>To Groups (factor levels)**. This allows us to select which group(s) we want the data of.

The first treatment group is the **Control** group; select this group and press **OK**. This will hide all observations except those who were in the Control treatment group.

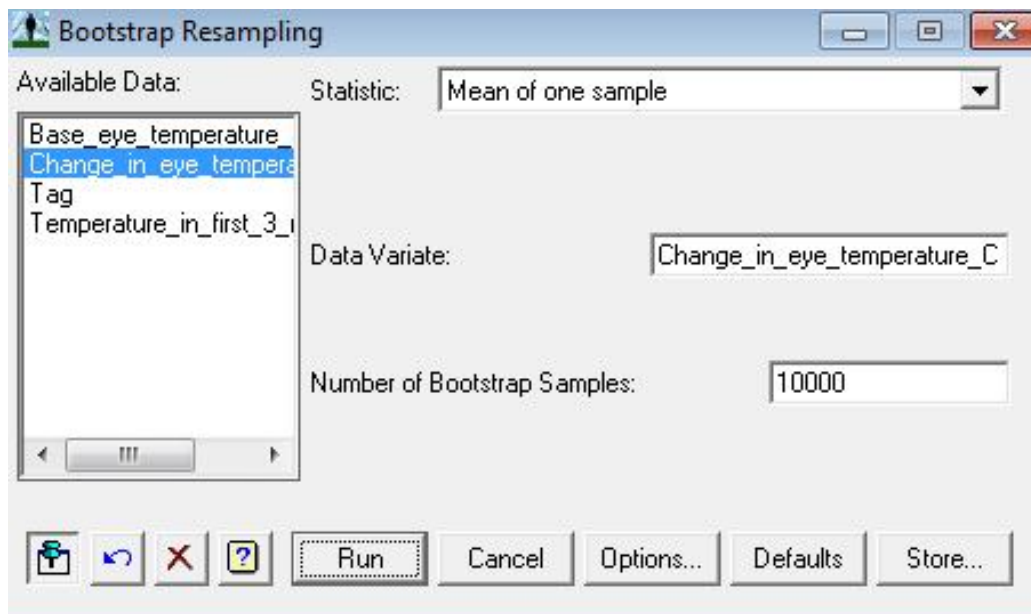


Now we are ready to carry out the bootstrapping and calculate the bootstrap confidence interval (for the control group).

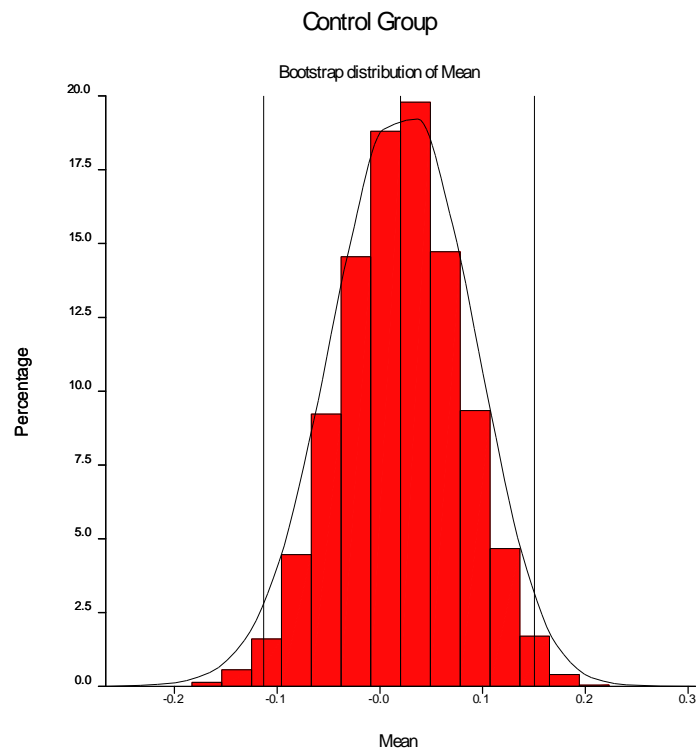
Calculate a bootstrap confidence interval by using **Stats>Bootstrap**:



Choose **Mean of one sample** in the **Statistic** box, and put the variable **Change\_in\_eye\_temperature\_C** into the **Data Variate** box. Set the **Number of Bootstrap Samples** to a large value, for example 10000, then click **Run**:



After running the bootstrap sampling, a distribution graph will be produced in your output. This graph shows the distribution of the mean of the bootstrap samples, and will be similar to the one shown below:



What distribution do the means of the bootstrap samples appear to follow?

By clicking onto the GenStat Output Window, we can see some summary statistics from the bootstrap sampling, including the mean, standard error, and confidence interval:

### Bootstrap estimates, from 10000 bootstrap samples

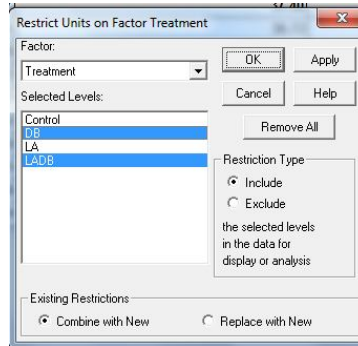
Label	mean	s.e.	95% confidence interval	
			lower	upper
Mean	0.0202	0.0676	-0.1133	0.1504

Repeat the procedure in step 4 for the remaining 3 treatment groups.

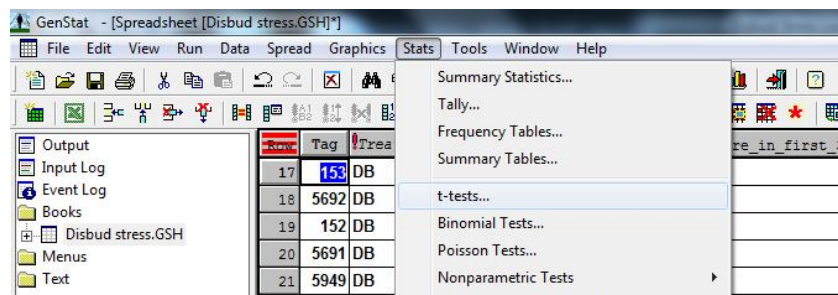
How do the bootstrap estimates of the mean for each treatment group compare to the treatment means we calculated previously? Do the bootstrap confidence intervals lead to the same conclusions as before (for each treatment group)?

- To compare the treatment means for the DB and LABD treatments we want to set up a 95% confidence interval for the difference between the means.

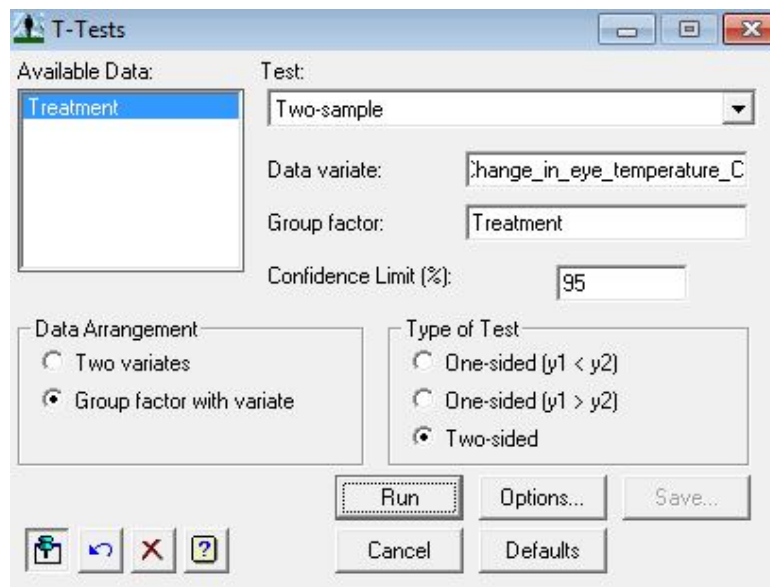
To do this we first use **Spread>Restrict/Filter> To Groups (factor levels)** as was performed at the start of part 4. Click on **DB** so it is highlighted, then hold down **Ctrl** and click on **LABD** so it is also highlighted. Click **OK**:



Calculate the mean and standard error for the difference between means by using **Stats>t-tests**:



In the **Test box** select Two-sample, and in the **Data Arrangement** box select Group factor with variate. Enter **Change\_in\_eye\_temperature\_C** into the **Data variate** box, and **Treatment** into the **Group factor** box. Click **Run**:





In the GenStat Output Window you will find the mean, the standard error, and the confidence interval for the difference between the two means:

### Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
DB	6	-0.3106	0.1370	0.3702	0.1511
LADB	8	0.2080	0.1383	0.3719	0.1315

Difference of means:	-0.519
Standard error of difference:	0.200

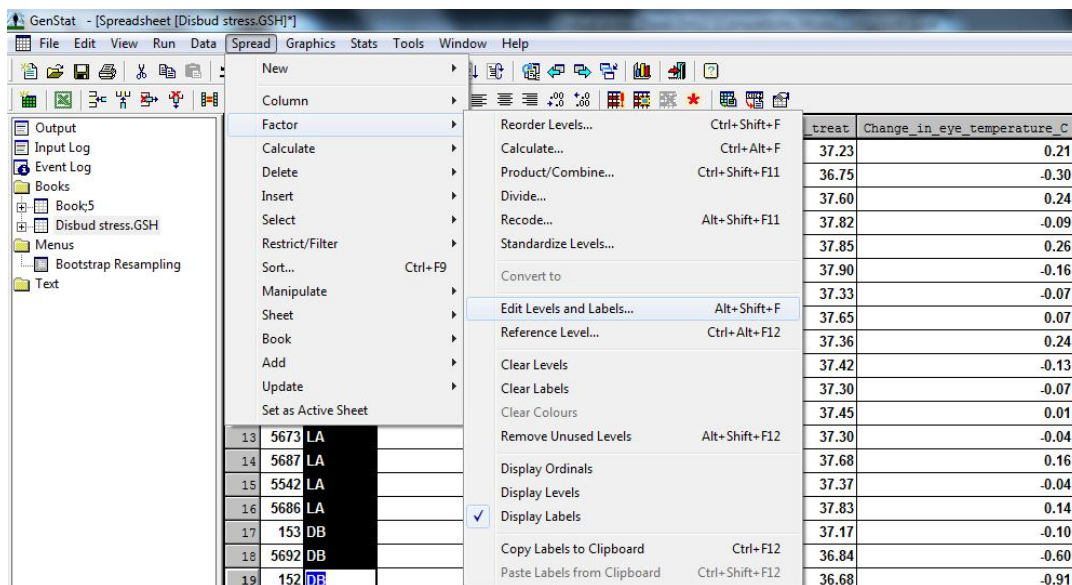
95% confidence interval for difference in means: (-0.9554, -0.08184)

How is the difference of means calculated? Note that this confidence interval is found using a t-value. Calculate the confidence interval using the mean and standard error values above and the large sample value of 1.96 or 2.

What conclusion can be drawn from our confidence interval in regard to the effect of the local anaesthetic?

- We now want to calculate the bootstrap confidence interval for the difference between the two means in 5. To carry this out we must first rearrange the data so that DB and LADB are coded as 1 and 2 respectively (the bootstrap procedure automatically uses codes 1 and 2 in its calculations).

To recode the treatments, we first click on the treatment row, so that all treatments are highlighted. We then use **Spread>Factor>Edit Levels and Labels**:



Here we want to recode the **Ordinals** and **Levels** columns as follows:

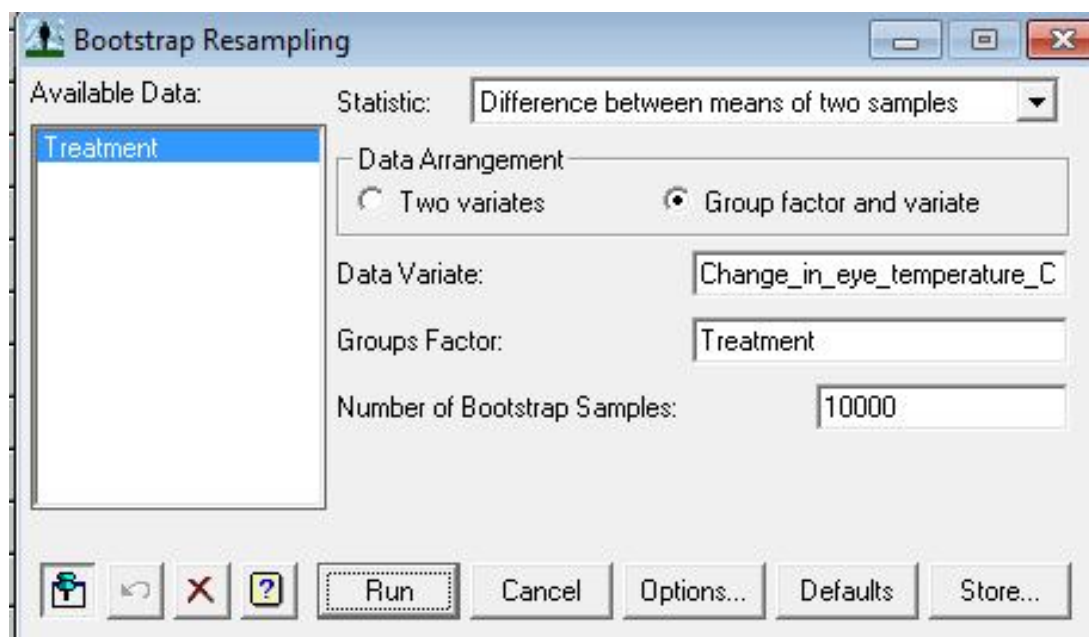
Control = 4      DB = 1      LA = 3      LADB = 2

Ordinals	Levels	Labels	Counts	Colour
4	4	Control	8	
1	1	DB	6	
3	3	LA	8	
2	2	LADB	8	

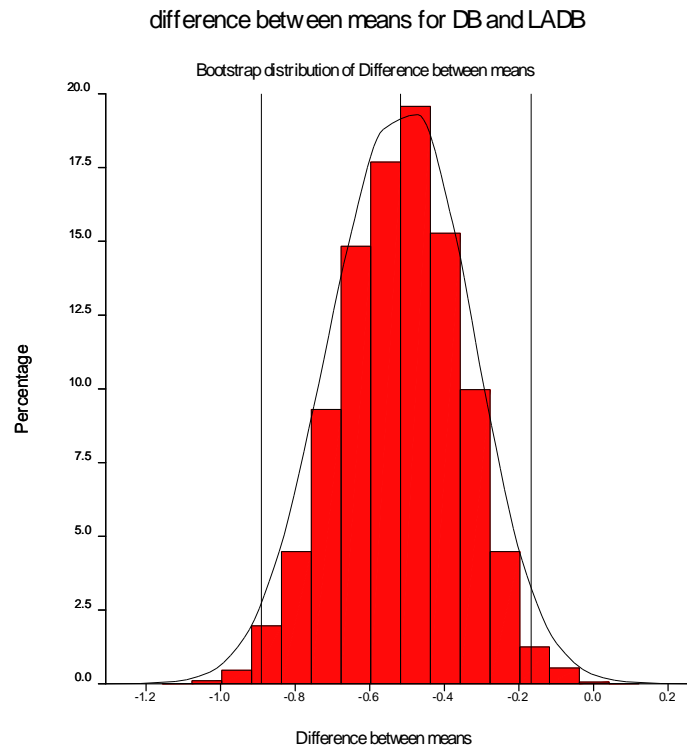
Press **OK**. This means that DB and LADB will be the two treatments chosen when bootstrap sampling below (as 1 and 2 are the default bootstrap values)

To carry out the bootstrapping for the difference between the two means (DB and LADB) we use **Stats>Bootstrap**.

In the **Statistic** box select **Difference between means of two samples**, and in the **Data Arrangement** box select **Group factor and variate**. Enter the variable **Change\_in\_eye\_temperature\_C** into the **Data Variate** box and **Treatment** into the **Groups Factor** box. Choose a large value for the **Number of Bootstrap Samples**, for instance 10000, then press **Run**:



The graph that appears after the bootstrapping has run shows the distribution of the difference between means (DB and LADB), and will be similar to the one below:



In the GenStat Output Window we find some summary statistics from the bootstrap sampling, including an estimate for the difference between means for the DB and LADB treatments, and the 95% confidence interval for the difference:

**Bootstrap estimates, from 10000 bootstrap samples**

Label	mean	s.e.	95% confidence interval	
			lower	upper
Difference between means	-0.517	0.186	-0.890	-0.167

How does the bootstrap estimate of the mean difference between DB and LADB compare with the estimate of the mean difference found in 5? Does the bootstrap confidence interval lead to the same conclusion as before? Was this outcome expected?

Notice that the bootstrap mean and confidence interval are both negative. What causes the negative difference between the means and could we make it a positive difference?

- Repeat steps 5 and 6 for the other four comparisons of pairs of means (eventually you will see a better way of making all these comparisons using the Analysis of Variance procedure: ANOVA). What conclusions can you draw from the results you have obtained so far? Report your findings.