STATISTICAL SIGNIFICANCE

Whenever biologists collect quantitative (numerical) data on organisms being studied, the individual measurements always vary to at least some extent, even amongst those individuals kept in exactly the same environment. This individual variation is natural and expected, but it tends to make comparisons difficult when comparing two groups of organisms exposed to slightly different conditions. The average values for each group might be different, but is that difference great enough to be considered significant? That is to say, does the difference in averages suggest that the variable was somehow responsible, or was it merely due to the random chance variation normally expected in small populations?

This is the question that exists with any biological research, so that results can seldom (if ever) be interpreted with absolute certainty as to a cause-effect relationship. However, there are statistical ways that can give us some degree of *confidence* for the *probability* of that relationship. The simple difference in averages is not reliable enough to determine this degree of confidence. Studies have shown that several other factors are involved, but mainly they narrow down to 1) the sample sizes (numbers of individuals in each group), and 2) the range (extent) of variation in each group.

Fortunately, certain calculations can be performed on the data that take into account those factors and present us with a measure of the separation of the data in the two sets, and a measure of confidence that those data reflect real (significant) differences, or not. The following formula (the "t-Test") is based on the well-known "Student's t-Test" used for comparing two sets of data. It is reasonably simple and suitable for most uses likely to be encountered in this course.

$$t = \frac{\sqrt{(A1 - A2)^2}}{\sqrt{[(T1 + T2) / (N1 + N2 - 2)] \times [(1/N1) + (1/N2)]}}$$

t = t value of confidence (calculate this and see where it falls in the t-Table of Significance
A1 = Arithmetic average ("mean") of measurements in group 1
A2 = Arithmetic average ("mean") of measurements in group 2
T1 = Total of squared deviations from A1 (mean of group 1
T2 = Total of squared deviations from A2 (mean of group 2)
N1 = Number of items measured in group 1
N2 = Number of items measured in group 2
N1+N2-2 = "Degrees of Freedom" or "Sample Size" value

PROCEDURE (long method):
1. Record the data and make preliminary calculations indicated on the data sheet provided.
2. Copy the t-test formula, replacing the letters with the appropriate values.
3. Calculate the value for "t", using calculator (see sample solution as a guide).
4. Find where the "t" value would fit in the "**t-Table of Significance**" on the "2P" line (Sample Size line) that equals N1+N2-2 (a number from 1-10).

A. If the t-value (on its Sample Size line) is less than the number in the 0.05 column (95%), then the difference in averages is considered to be "**not significant**." This difference could be due to chance *more* than 1 out of 20 times (5%), or due to the variable factor less than 95% of time. Such a value is *not* high enough to say that the variable in the study caused the differences noted; those differences could have been the result of random variations in both populations. There is "no significant difference."

B. If the t-value on its Sample Size line) is equal to or more than the value in the 0.05 column AND less than the value in the 0.01 column (99%), then the difference in averages is considered to be "**significant**." This difference (minimum standard) could be due to chance *less* than 1 out of 20 times (5%), or due to the variable factor 95+% of the time. The difference is probably a real one, caused by the experimental variable.

C. If the t-value (on the Sample Size line) is in the 0.01 column (99%), or higher, then the difference in averages is considered to be "***very* significant**." This difference could be due to chance less than 1 out of 100 times (1%), or due to the variable factor 99+% of the time. Such a value gives us a *very* high level of confidence that the variable in the study *did* cause the differences measured. Almost certainly, the experimental factor being studied caused the differences observed (with 99+% confidence).

Very often, scientists will report the specific level of confidence (e.g., "0.05" or "0.01") that their data show. That's because they have calculated the level using a formula appropriate for the nature of their study and the data collected. Of course, computer programs can process the data entered and provide the level of confidence directly. Such a program for the t-Test can be found at: http://www.physics.csbsju.edu/stats/t-test.html or http://graphpad.com/quickcalcs/ttest1.cfm

AUTO-CALC: We also have an Excel sheet (STATISTICAL SIGNIFICANCE: T-TEST) with tables into which your measurements can be entered, then the t-value is automatically calculated, looked up in the t-Table, and a statement of significance is produced. If you want to use this layout (second pair of tables) to enter and process your own data, just clear the values from the "Values" columns ONLY (select data in column for Group 1, then select... Edit>Clear>Contents; repeat for Group 2); then enter your data. Type "omitted" for any data not to be used (little or no growth). Then print the page for your report.

ONLINE RESOURCES
explanation and a data-input online solution
http://www.physics.csbsju.edu/stats/t-test.html

Includes procedure and a t-test table:
http://helios.bto.ed.ac.uk/bto/statistics/tress4a.html

Explains, shows diagrams and formula:
http://www.socialresearchmethods.net/kb/stat_t.htm

Experimental Biosciences (explanation, formula, table):
http://www.ruf.rice.edu/~bioslabs/tools/stats/ttest.html