M.Sc. (Semester-II) Forensic Science, FS-201 Forensic Analytical Chemistry

Tests of significance:

1. Tests of significance are used to validate a result.
2. They are also used to find out whether a new method is significant or not by comparing with a standard (or reference) method.

t-test:

t-test is classified into three types.

1. Student's t-test
2. Paired t-test
3. t-test or multiple samples

Student's t-test

It is applied when the true mean (or accepted value) of a measurement is known. This is a test used for small samples. Its purpose is to compare the mean from a sample with standard value and to express some level of confidence in the significance of comparison.

In the first step, t value is calculated based on the equation 1 (t_{cal}).

$$\pm t = \frac{(\bar{x} - \mu) \times \sqrt{N}}{S} \quad \text{.... 1}$$

where $\bar{x}$ = mean
μ = true value or accepted value
N = number of experiments and
S = standard deviation

Then in the second step, the calculated t value (t_{cal}) is compared with the tabulated t values (t_{tab}). **t_{tab} values are usually available in all standard books and websites. t_{tab} values varies with N (number of experiments) and confidence level. Usually we have to compare the t_{cal} values with the t_{tab} values at 95% confidence level and at N-1 degrees of freedom.**

After comparison, the third step is to derive inference, like the following.

If $t_{tab} > t_{cal}$, then there is no significant difference, the result can be accepted.

If $t_{tab} \leq t_{cal}$, then there is a significant difference, the result may be biased.

Example: If $\bar{x}$ the mean of a 12 determinations is 8.37, and μ the true value is 7.91, say whether or not the result is significant if the S is 0.17.

$$\pm t = \frac{(\bar{x} - \mu) \times \sqrt{N}}{S} = \frac{(8.37 - 7.91)\sqrt{12}}{0.17} = 9.4$$

t_{cal} = 9.4

From the t table, for eleven degrees of freedom at 95% confidence level, the t_{tab} value is 2.2 (given).

Since $t_{tab} < t_{cal}$, the result has significant difference. It implies that there is a particular bias exists in the laboratory procedure.

Paired t test

When the t test is applied to two sets of data, µ is replaced by the mean of the second test, usually the mean of results obtained from a standard (or reference) method for the same sample.

This method is mainly useful to test a new method whether it is significant or not (acceptable or not acceptable).

In this paired t test, in the first step, the t value is calculated from equation 2

$$\pm t = \frac{(\bar{x}1-\bar{x}2)}{Sp} \times \sqrt{\frac{N1 \times N2}{N1+N2}} \qquad \ldots.. 2$$

Where Sp is the pooled standard deviation which can be calculated from equation 3 (below).

$$Sp = \sqrt{\frac{\sum(xi1-\bar{x}1)+\sum(xi2-\bar{x}2)}{N1+N2-2}} \qquad \ldots. 3$$

$\bar{x}_1$ and $\bar{x}_2$ are the means of method 1 and method 2, respectively.
x_{i1} and $x i_2$ represent the individual measurements of method 1 and method 2, respectively.
N1 and N2 represent the number of experiments in method 1 and method 2, respectively.

The second and third steps are comparison with the t_{tab} value and deriving inference respectively, similar to the student's t-test.

If $t_{tab} > t_{cal}$, then there is no significant difference between the two methods, the new method can be accepted.
If $t_{tab} \leq t_{cal}$, then there is a significant difference between the two methods and the new method cannot be accepted.

t test for multiple samples:

When we use multiple samples for the analysis, the t value is calculated in a different way.

The difference between each of the paired measurements on each sample is computed. An average difference $\bar{D}$ is calculated and the individual deviation of each are used to compute a standard deviation (S_D).

In the first step, t is calculated from the equation 4.

$$\pm t = \frac{\bar{D}}{SD}\sqrt{N} \qquad \ldots 4$$

where $S_D = \sqrt{\frac{\Sigma(Di-\bar{D})}{N-1}}$

Di = Individual difference between the two methods for each sample.

$\bar{D}$ = Mean of the all individual difference
N = Number of samples

The second and third steps are comparison with the t_{tab} value and deriving inference respectively, similar to the student's t-test.

If $t_{tab} > t_{cal}$, then there is no significant difference between the two methods, the new method can be accepted.
If $t_{tab} \leq t_{cal}$, then there is a significant difference between the two methods and the new method cannot be accepted.

F test

This test is used to compare the precisions of two sets of data for example, the results of two different analytical methods or the results from two different laboratories.
In the first step, F value is calculated from the equation 5.

$$F = S_A^2/S_B^2 \quad \ldots\ldots 5$$

S_A and S_B represent the standard deviation of the method A and method B, respectively.

The large value of S is always used as the numerator so that the value of F is always greater than unity.

Then in the second step, the F value is checked for its significance against values in the F table obtained from an F distribution corresponding to the N-1 number of degrees of freedom for the two sets of data. F tables are also available in all standard books.

In the third step, an inference can be derived like the following.

If $F_{tab} > F_{cal}$, then there is no significant difference in the precision of the two methods/laboratories.
If $F_{tab} \leq F_{cal}$, then there is a significant difference in the precision of the two methods/laboratories.

Example: If S_A is 0.210 and S_B is 0.641 for two methods, then is there any significant difference between the precision of the two sets of results?

F_{cal} = (0.641)2/(0.210)2 = 0.411/0.044 = 7.4
(The large value of S is always used as the numerator)

F_{tab} value at the 95% confidence level is 2.91 (given).

Since $F_{tab} < F_{cal}$, there is a significant difference in the precision of the two methods/laboratories.

Example for paired t test (and F test):

A new gravimetric method for the determination of iron(III) is developed. If we want to compare this result with the reference method, the analysis is as follows.

Results of the test method: 20.10, 20.50, 18.65, 19.25, 19.40, 19.99
(% of iron content)

Results from the reference (standard method): 18.89, 19.20, 19.00, 19.70, 19.40
(% of iron content)

Is there any significant difference between the two methods? Analyze by t test and F test. t_{tab} for 9 degrees of freedom at 95% confidence level is 1.83 (given).
(In the present example, 9 degrees of freedom should be selected since N1+N2-2 = 9. For each method, N-1 degrees of freedom)
F_{tab} value for $F_{5,4}$ at 95% confidence level is 6.26 (given).

Solution:

$xi1$	$(xi1 - \bar{x}1)$	$(xi1 - \bar{x}1)^2$	$xi2$	$(xi2 - \bar{x}2)$	$(xi2 - \bar{x}2)^2$
20.10	0.45	0.202	18.89	0.35	0.122
20.50	0.85	0.722	19.20	0.04	0.002
18.65	1.00	1.000	19.00	0.24	0.058
19.25	0.40	0.160	19.70	0.46	0.212
19.40	0.25	0.062	19.40	0.16	0.026
19.99	0.34	0.116			

$\bar{x}_1 = \frac{\sum(xi1)}{6}$ = 19.65 and $\bar{x}_2 = \frac{\sum(xi2)}{5}$ = 19.24

$\sum(xi1 - \bar{x}1)^2$ = 2.262 and $\sum(xi2 - \bar{x}2)^2$ = 0.420

<u>Paired t test</u>

$$Sp = \sqrt{\frac{\sum(xi1-\bar{x}1)+\sum(xi2-\bar{x}2)}{N1+N2-2}} = \sqrt{\frac{2.262+0.420}{6+5-2}} = \sqrt{\frac{2.682}{9}} = \sqrt{0.298} = 0.546$$

$$\pm\, t = \frac{(\bar{x}1-\bar{x}2)}{Sp} \times \sqrt{\frac{N1 \times N2}{N1+N2}} = \frac{(19.65-19.24)}{0.546} \times \sqrt{\frac{6\times5}{6+5}} = \frac{0.41}{0.546} \times \sqrt{\frac{30}{11}} = 1.23$$

t_{cal} = 1.23 and t_{tab} = 1.83 (given)

$t_{tab} > t_{cal}$, therefore there is no significant difference between the two methods, the new method can be accepted.

<u>F test</u>

$F = S_A^2/S_B^2$ = (2.262/5)/(0.420/4) = 0.4524/0.105 = 4.31

F_{cal} = 4.31 and F_{tab} = 6.26 (given)

$F_{tab} > F_{cal}$, therefore there is no significant difference in the precision of the two methods.

<u>Chi-square test (χ^2 test):</u>

It is used to test whether the predicted hypothesis is correct or not (accepted or not).

This determines whether or not a set of data differs significantly from a theoretical or defined distribution, that is, whether the observed frequencies of an occurrence corresponds to the predicted frequencies. Chi-square (χ^2) is calculated from the equation 6.

$$\chi^2 = \sum \frac{(O-E)2}{E} \qquad \text{…. 6}$$

where O is the observed frequency and E is the expected frequency.

<u>Sequences of steps in the χ^2 test:</u>

1. Determine the expected value from hypothesis.
2. Make a table with observed and expected values.
3. Calculate the value of χ^2.

4. Determine the degree of freedoms
5. See χ^2 value for χ^2 table for that particular degrees of freedom. χ^2 tables are available in all standard books. If $\chi^2_{cal} < \chi^2_{tab}$ for 95% confidence level, then the hypothesis is accepted and if not then it is rejected.

Example:
A coin is tossed 100 times. The head appears 60 and tail 40 times. Will you accept or reject the hypothesis that the tossing was normal? χ^2_{tab} for 1 degree of freedom at 95% confidence level is 3.84 (given).

Solution:
Since it is a question of acceptance or rejection of a hypothesis, we can use the χ^2 test. Expected frequency (or hypothesis) for head and tail is 50. Therefore,

	Head	Tail	Total
Observed frequency (O):	60	40	100
Expected frequency (E):	50	50	100
$\frac{(O-E)2}{E}$:	$(10)^2/50$	$(-10)^2/50$	

$$\chi^2 = \sum \frac{(O-E)2}{E} = (10)^2/50 + (-10)^2/50 = 200/50 = 4.$$

Since there are only two variables, head and tail, the degree of freedom is 2-1 = 1.

Thus, χ^2_{cal} is 4 and χ^2_{tab} is 3.84 (given).

Since $\chi^2_{cal} > \chi^2_{tab}$ for one degree of freedom at 95%confidence level, the hypothesis (occurrence of 50 times head and 50 times tail) is not accepted.