***Student´s t*-test**

t-test is used for testing for difference between **2 population means (μ)** . Calculation of a **test statistic *t*** results from the estimation of parameters μ and σ in samples: $\bar{x}$ and ***s***. Calculated test statistic is compared with a table critical value, that we find out in t-distribution tables according to the chosen α error and given ν (degree of freedom).
According to data sets, that are available for comparison, we can employ different types of t-test:

I. Population vs. Sample Comparison (*One sample t-test*)

This test is used for evaluation of experiments, where we know population μ (e.g. physiological value of some biochemical indicator) = const. In the experiment we verify a hypothesis, that a test sample comes from a population with this known μ **(H_0: μ = const.)**

$$t = \frac{|\bar{x} - \mu|}{\sqrt{\frac{s^2}{n}}}$$

($\bar{x}$ -sample mean, μ- population mean, s–sample SD, n–number of items in sample)

Calculated *t* is compared with a critical value $t_{(\alpha,\nu)}$, where ν = n-1 and α = 0.05 or 0.01 :

* If $t \le t_{(\alpha,\nu)}$ ⇒ statistically **insignificant** difference between tested parameters at specific α (it means that experiment has been *ineffective*)

  If $t > t_{(\alpha,\nu)}$ ⇒ statistically **significant** difference between tested parameters (at α = 0.05) or **highly significant** difference (at α = 0.01)
  (it means that the experiment has been *effective* – it caused a change of the mean in comparison with the population: the sample comes from another population with μ ≠const).

II. Samples comparison (population μ is not known)

1) paired experiment – there are 2 sets of measurements in 1 group of animals: 1 before an experimental treatment and 1 after the treatment ⇒ obtained values are paired. We calculate **differences between paired values**, then $\bar{x}$ and SD (s) of differences. We test a hypothesis, that μ of the measurements before and after the treatment are equal (or mean value of the differences between this measurements = 0). **(H_0: μ_{differ}=0)**

$$t = \frac{|\bar{x}|}{\sqrt{\frac{s^2}{n}}} \qquad \nu = n-1$$

* If $t \le t_{(\alpha,\nu)}$ ⇒ stat. **insignificant** difference between means of values before and after the treatment at the specific α (the experiment has not been effective, **H_0: μ_{differ}=0**)

  If $t > t_{(\alpha,\nu)}$ ⇒ statistically **significant** difference between means (at α = 0.05) or **highly significant** difference (at α = 0.01)
  (experiment has been effective - μ after the treatment is different from μ before the treatment: H_0 is not true.)

2) unpaired experiment – 2 different sets of data: **E**xperimental and **C**ontrol group of animals. We test a hypothesis, whether μ_1 (test group) has the same value as μ_2 (control group).
H_0: $\mu_1 = \mu_2$
sample 1 (n_1) : we calculate $\bar{x}_1$, s^2_1
sample 2 (n_2) : we calculate $\bar{x}_2$, s^2_2

The populations sampled can be different variable – this affects calculation of t-test. Therefore we have to determine **difference between variances** at first (to specify what type of calculation formula we have to use for the following t-test). **F-test:**

$$F = \frac{\text{bigger } s^2}{\text{smaller } s^2} \qquad \begin{matrix} - (\nu_N = n - 1) \\ - (\nu_D = n - 1) \end{matrix}$$

Calculated F is compared with the critical value, that we find according to α and degree of freedom : ν_N (DF of numerator) and ν_D (DF of denominator) .

According to F-test result:

- If $F \le F_{\alpha(\nu_N,\ \nu_D)} \Rightarrow$ **a)** $\boldsymbol{\sigma_1^2 = \sigma_2^2}$:

$$t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\frac{(n_1 - 1) * s_1^2 + (n_2 - 1) * s_2^2}{n_1 + n_2 - 2} * \frac{n_1 + n_2}{n_1 * n_2}}} \qquad \nu = n_1 + n_2 - 2$$

(For $n_1 = n_2 = n$: $t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\frac{s_1^2 + s_2^2}{n}}}$ $\nu = (n-1) .2$)

- If $F > F_{\alpha(\nu_N,\ \nu_D)} \Rightarrow$ **b)** $\boldsymbol{\sigma_1^2 \neq \sigma_2^2}$:

$$t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}} \qquad \nu = \frac{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)^2}{\frac{\left(\frac{s_1^2}{n_1}\right)^2}{n_1 - 1} + \frac{\left(\frac{s_2^2}{n_2}\right)^2}{n_2 - 1}}$$

(for $n_1, n_2 > 30$: $\nu = \infty$)

(For $n_1 = n_2 = n$: $t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\frac{s_1^2 + s_2^2}{n}}}$)

Conclusion:

∗ If $t \le t_{(\alpha,\nu)} \Rightarrow$ statistic. **insignificant** difference between μ_1 and μ_2 at specific α (**H_0: $\mu_1 = \mu_2$** is true; it means that the experiment has not been effective)

If $t > t_{(\alpha,\nu)} \Rightarrow$ statistically **significant** difference between μ_1 and μ_2 (at $\alpha = 0.05$) or **highly significant** difference (at $\alpha = 0.01$) (it means that the experiment has been effective and caused a change of mean value in treated variable compared to the untreated control: **$\mu_1 \neq \mu_2$).**