t – test for independent data

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Hemoglobin change after administration of two EPO drugs

Test	Reference
0.38	0.8
0.37	0.7
0.24	0.39
0.08	0.06
0.04	0.49
0.03	0.07
0.07	0.63
0.19	0.83
0.35	0.62
0.38	0.95
0.27	0.92
0.2	0.81

Suppose we want to compare the hemoglobin change after administration of two erythropoietin (EPO) drugs, Test (T) and Reference (R)

Null hypothesis (H_0): There is no difference in the mean hemoglobin change between the Test and Reference drug

$n = 12$ patients received the Test drug and $n = 12$ patients received the Reference drug

Descriptive Statistics

Test	0.38	0.37	0.24	0.08	0.04	0.03	0.07	0.19	0.35	0.38	0.27	0.2
Reference	0.8	0.7	0.39	0.06	0.49	0.07	0.63	0.83	0.62	0.95	0.92	0.81
n			12	12								
$\bar{x}$ (mean or average)			0.22	0.61								
s or SD (variability)			0.14	0.30								
CV% (=SD/mean)			64%	49%								
SE (=variability/√size)			0.04	0.09								

Null hypothesis

Test drug	Hypothesis;	Reference drug
μ_t	***The mean difference in hemoglobin change is zero***	μ_r
$s_t = 0.1366$	$\mu_t - \mu_r = 0$ **or** $\mu_t = \mu_r$	$s_r = 0.3017$
	Independence;	
	These two drugs are independent of each other	

Null hypothesis

The null hypothesis is that there is no difference in the mean change in hemoglobin levels between the Test and Reference drug. The difference in the mean change in hemoglobin levels between the two drugs is 0.

$H_0: \mu_t - \mu_r = 0$ **or** $\mu_t = \mu_r$

$H_a: \mu_t - \mu_r \neq 0$ **or** $\mu_t \neq \mu_r$

Significance level: $a = 0.05$

Since $\boldsymbol{\sigma}$ is not known we will use in our test the t-distribution with $(\boldsymbol{n_T} - \mathbf{1}) + (\boldsymbol{n_R} - \mathbf{1}) = (\mathbf{12} - \mathbf{1}) + (\mathbf{12} - \mathbf{1}) =$ 22 degrees of freedom

Decision rule:

Since $a = 0.05$ and we are using the t-distribution, the H_0 will be rejected if the test statistic is > 2.07 (**5% point of the t-distribution with 22 degrees of freedom**) or < -2.07

Evaluating the Statistical Significance of Mean Value Differences

In order to test the **significance** of the difference between the mean values ($\boldsymbol{\bar{d} = 0.22 - 0.61 = -0.39}$),

i.e., to determine if this **difference is significantly different from zero** or **just a random occurrence**,

we need to consider the **difference** ($\bar{d}$) along with the **standard error** of the difference (SE).

This involves taking into account the **overall variability** (standard deviation, SD, of the two treatments) and the **sample size** ($n = 12 + 12$)

Statistical Testing of Mean Differences with the t-test

Then, we could test **statistically** whether the difference between the two means deviates from zero (i.e. is significant) using the **t-test**

$$t = \frac{(\bar{x}_t - \bar{x}_r)}{SE}$$

$$SE = \sqrt{s_{pooled}^2 \left(\frac{1}{n_t} + \frac{1}{n_r}\right)}$$

s_t = Standard deviation of observations for drug T

s_r = Standard deviation of observations for drug R

n_t = the number of patients who received drug T

n_r = the number of patients who received drug R

$$s_{pooled}^2 = \frac{(n_t - 1)s_t^2 + (n_r - 1)s_r^2}{n_t + n_r - 2}$$

Calculating the t-Value for Testing Mean Differences

$$t = \frac{(\bar{x}_t - \bar{x}_r)}{SE}$$

$$SE = \sqrt{\left(\frac{(11)0.1366^2 + (11)0.3017^2}{12 + 12 - 2}\right)\left(\frac{1}{12} + \frac{1}{12}\right)} = \sqrt{0.055(0.167)}$$

$$SE = \sqrt{0.0092} = 0.096$$

	T	R
n	12	12
$\bar{x}$ (mean or average)	0.22	0.61
s or SD (variability)	0.14	0.30

Calculating the t-Value for Testing Mean Differences

$$t = \frac{(\bar{x}_t - \bar{x}_r)}{SE}$$

$$SE = 0.096$$

$$t = \frac{(0.22 - 0.61)}{SE} = \frac{-0.39}{0.096} = -4.06$$ The sign is ignored

	T	R
n	12	12
$\bar{x}$ (mean or average)	0.22	0.61
s or SD (variability)	0.14	0.30

Significance of the difference

The t-value of the t-test ($\boldsymbol{t = 4.06}$) is larger than the 5% point of the **t-distribution** with $(n_T - 1) + (n_R - 1) = (12 - 1) + (12 - 1) =$ **22 degrees of freedom** which is $\mathbf{2.07}$ (see table)

Thus, the $t = 4.06$ value is **significant** with a probability error $\boldsymbol{P < 0.05}$

(i.e. small error probability)

	Percentage points of the t distribution		
	p-value		
df (=n-1)	0.05	0.01	0.001
1	12.71	63.66	636.62
2	4.3	9.92	31.6
3	3.18	5.84	12.92
4	2.78	4.6	8.61
5	2.57	4.03	6.87
6	2.45	3.71	5.96
7	2.36	3.5	5.41
8	2.31	3.36	5.04
9	2.26	3.25	4.78
10	2.23	3.17	4.59
20	2.09	2.85	3.85
22	2.07	2.82	3.79
30	2.04	2.75	3.65
120	1.98	2.62	3.37
∞	1.96	2.58	3.29

Result

Therefore, we can conclude that there is a **significant difference** in the **mean hemoglobin change** between the T and R drugs, with a small probability error ($\boldsymbol{P < 0.05}$)

Using statistical packages, such as SPSS or Excel, we can calculate the exact P-value, which in this case is P = 0.001

95% Confidence Interval (CI)

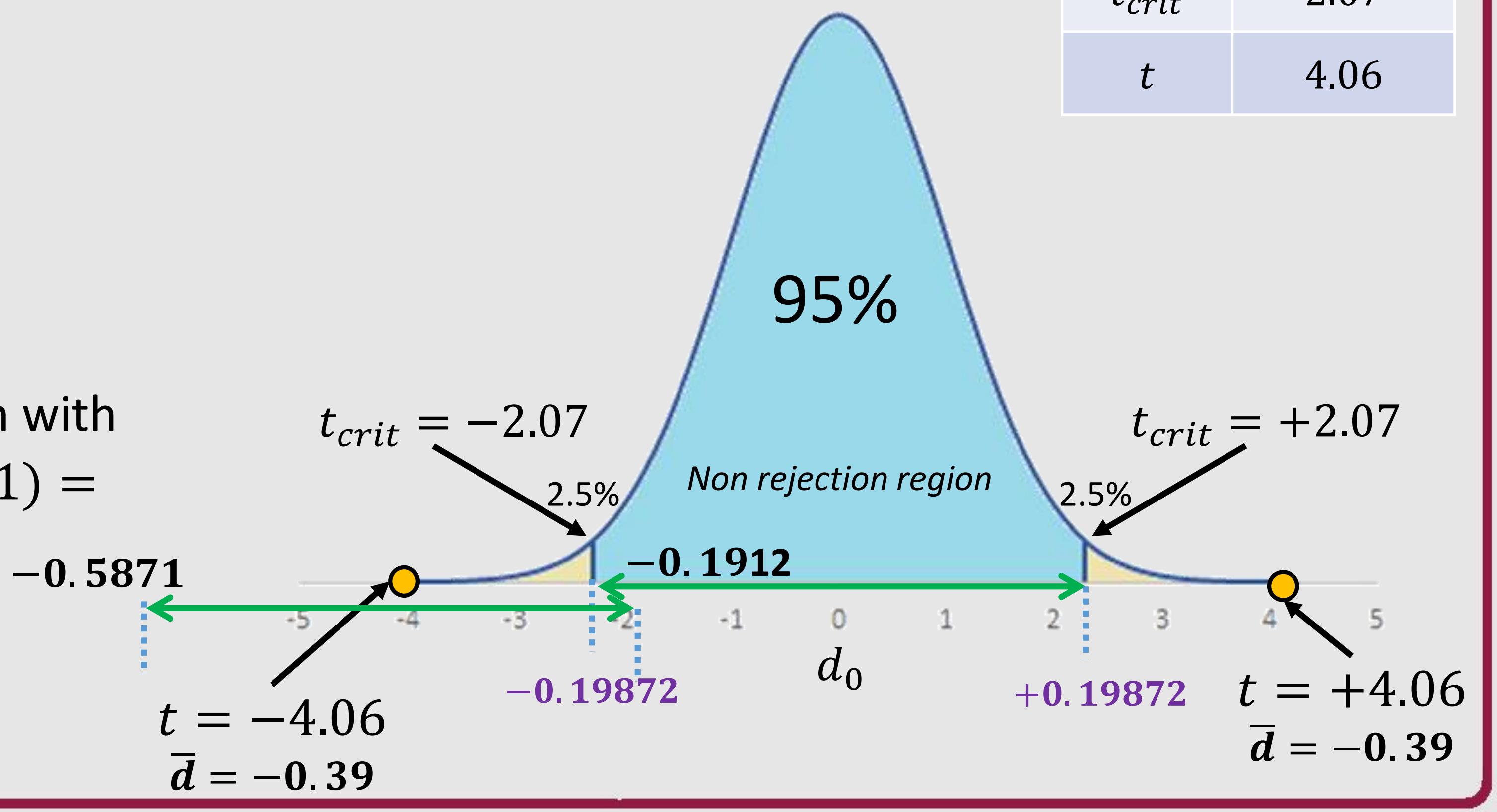
The **significance** of the difference between the two means, $\bar{\mathrm{d}}$, can also be assessed using the **95% confidence interval (CI)**

The 95% CI is defined as:

$$(\boldsymbol{\bar{d} - t * SE, \bar{d} + t * SE})$$

where t is the 5% point of the t-distribution with $(n_t - 1) + (n_r - 1) = (12 - 1) + (12 - 1) =$ 22 degrees of freedom which is 2.07 (see the t-distribution table)

$\bar{d}$	-0.39
SE	0.096
t_{crit}	2.07
t	4.06



95% Confidence Interval (CI)

$$(\bar{d} - \ t * SE, \bar{d} + \ t * SE)$$

$$(-0.39 - 2.07 * 0.096, -0.39 + 2.07 * 0.096)$$

$$(-0.59, -0.19)$$

Thus, with 95% confidence, we can claim that the true difference between the two means lies within the interval **(-0.59, -0.19)**

Zero is not included in the 95 **95% CI**, indicating that there is significance difference between the Test and Reference drug in terms of hemoglobin change