Statistics in biomedical research, 2<sup>nd</sup> session: Classical distributions; generalities about statistical tests.

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This slideshow is accessible at: http://www.igh.cnrs.fr/equip/Seitz/en\_Stats2.pdf Statistics session 2

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Factors determining t-test *p*-value

t-test conditions of applicability

t-test variants

Classical distributions

Everything else being equal:: difference between means  $\uparrow \implies p$ -value  $\downarrow$ 



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R commands used to generate these graphs: [link]



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Everything else being equal:: standard deviations  $\downarrow \implies p$ -value  $\downarrow$ 



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Everything else being equal:: number of replicates  $\uparrow \implies p$ -value  $\downarrow$ 



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Reconsider the importance of a *p*-value !



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### Factors determining t-test *p*-value

Reconsider the importance of a *p*-value ! Increasing replicate number will tend to decrease it.



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Reconsider the importance of a p-value ! Increasing replicate number will tend to decrease it.

Amplitude of the effect (measured by the fold-change) and its significance (measured by the *p*-value) are both important.

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Reconsider the importance of a p-value ! Increasing replicate number will tend to decrease it.

Amplitude of the effect (measured by the fold-change) and its significance (measured by the *p*-value) are both important.

The effect is measured by its amplitude; the *p*-value measures how much confidence we can have in that fold-change.

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 $1^{st}$  condition: each of the two sampled populations is distributed according to a normal distribution (="Gaussian distribution").

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 $1^{st}$  condition: each of the two sampled populations is distributed according to a normal distribution (="Gaussian distribution").

Evaluating if our samples (= our replicate datasets) can realistically be sampled from a normally distributed population.

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The Shapiro-Wilk test

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Evaluating if our samples (= our replicate datasets) can realistically be sampled from a normally distributed population.

The Shapiro-Wilk test (*p*-value: probability that the replicate series could have been sampled from a normally distributed population).

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 $2^{nd}$  condition: variances in both conditions are homogeneous ( $\approx$  equal)



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 $2^{nd}$  condition: variances in both conditions are homogeneous ( $\approx$  equal) ("homoscedasticity" condition).



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Levene's test

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 $2^{nd}$  condition: variances in both conditions are homogeneous ( $\approx$  equal) ("homoscedasticity" condition).

Levene's test (*p*-value: probability that the two series of replicates were sampled from populations having the same variance).



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What should we do if at least one of these conditions is not met  $? \end{tabular}$ 



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What should we do if at least one of these conditions is not met  $\ensuremath{?}$ 

Homoscedasticity: use Welch's t-test (= "t-test with heterogeneous variances") instead of Student's t-test (= "t-test with homogeneous variances").

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If Student's t-test can be used, then it is preferrable: it is more powerful.

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Normality: use a mathematical transformation (logarithm, square root, ...; preferably if it makes physical sense).

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If Student's t-test can be used, then it is preferrable: it is more powerful (detects significance with better sensitivity).

Normality: use a mathematical transformation (logarithm, square root, ...; preferably if it makes physical sense). If the log of calues appears normally distributed, then perform the t-test on the log of values.

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If even a mathematical transformation is not enough: use Wilcoxon's test (= Mann-Whitney test).



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If even a mathematical transformation is not enough: use Wilcoxon's test (= Mann-Whitney test). Analyze the rank of data points (robust to deviations to normality).



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If data points appear normally distributed: t-test is preferrable (more powerful).

Example: comparing two series of 3 replicates each: by construction, Wilcoxon test p-value cannot be lower than 0.1 whatever the distribution of the two series !

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Is there a particular link between each replicate of series #1 and one (and only one) replicate in series #2 ?



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Is there a particular link between each replicate of series #1 and one (and only one) replicate in series #2 ?

Example: it is the same individual, analyzed in two conditions (and each replicate is a different individual).

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Is there a particular link between each replicate of series #1 and one (and only one) replicate in series #2 ?

Example: it is the same individual, analyzed in two conditions (and each replicate is a different individual).

Implication: the two datasets have the same number of replicates.

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R commands used to generate these graphs: [link]

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### "One-tailed" or "two-tailed" t-test ?

Am I only interested in a single sense of variation ?



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Am I only interested in a single sense of variation ?

If I am looking for a difference, whatever its orientation: two-tailed (= two-sided) t-test.

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If I only care about one sense of variation (*e.g.*, are mutant mice lighter than wild-type mice ?): one-tailed (= one-sided) t-test.

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Be careful ! Ignoring one of the two possible orientations is rare in research, and one-tailed t-test tends to give lower *p*-values.

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 $\implies$  Possible abuse, in order to decrease a *p*-value in an illegitimate manner.

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If I am looking for a difference, whatever its orientation: two-tailed (= two-sided) t-test.

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Be careful ! Ignoring one of the two possible orientations is rare in research, and one-tailed t-test tends to give lower *p*-values.

 $\implies$  Possible abuse, in order to decrease a *p*-value in an illegitimate manner. If there is no imperative reason to neglect one sense of variation, then choose the two-tailed t-test.

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### Decision tree for t-test usage





A few common examples:

Normal (= "Gaussian") distribution: continuous, symetrical, from −∞ to +∞.





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A few common examples:

- ▶ Normal (= "Gaussian") distribution
- Binomial distribution (probability of getting a given number of successes in independent random drawing events): discontinuous, only defined for positive values.



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A few common examples:

- Normal (= "Gaussian") distribution
- Binomial distribution
- Hypergeometric distribution (idem but draws without replacement).



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A few common examples:

- Normal (= "Gaussian") distribution
- Binomial distribution
- Hypergeometric distribution
- Poisson distribution (probability of getting k successes in a given time interval, if there are on average λ independent successes in such an interval): discontinuous, only defined for positive values.







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The t-test estimates the probability of a hypothesis (equality of the means of the two sampled populations).

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The t-test estimates the probability of a hypothesis (equality of the means of the two sampled populations).  $\longrightarrow$  the "null hypothesis".



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The t-test estimates the probability of a hypothesis (equality of the means of the two sampled populations).  $\longrightarrow$  the "null hypothesis".

Null hypothesis in Shapiro-Wilk's test: the sampled populations follows a normal distribution; of Levene's test: both sampled populations have the same variance. Statistics session 2

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Null hypothesis in Shapiro-Wilk's test: the sampled populations follows a normal distribution; of Levene's test: both sampled populations have the same variance.

Measurement of uncertainty: by a probability (the *p*-value) or by a confidence interval ("the difference between means of sampled populations lies in interval [...; ...] with a probability  $\ge 0.95$ ").

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Measurement of uncertainty: by a probability (the *p*-value) or by a confidence interval ("the difference between means of sampled populations lies in interval [...; ...] with a probability  $\ge 0.95$ ").

Be careful with the wording ! Say "I did not see any significant difference", rather than "there is no difference" (it could come out significant with more replicates). Statistics session 2

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For each test: significance threshold determines the expected percentage of false positives.

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For each test: significance threshold determines the expected percentage of false positives. For example with the t-test with a 0.05 threshold: even samples drawn from populations with the same mean will appear significantly different  $\approx\!5\%$  of the time.

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For each test: significance threshold determines the expected percentage of false positives. For example with the t-test with a 0.05 threshold: even samples drawn from populations with the same mean will appear significantly different  $\approx\!\!5\%$  of the time.

Vocabulary: a false positive is also called "Type 1 error".

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Vocabulary: a false positive is also called "Type 1 error".

False negative ("Type 2 error"): does not appear significant while the sampled populations have different means.

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For each test: significance threshold determines the expected percentage of false positives. For example with the t-test with a 0.05 threshold: even samples drawn from populations with the same mean will appear significantly different  $\approx\!\!5\%$  of the time.

Vocabulary: a false positive is also called "Type 1 error".

False negative ("Type 2 error"): does not appear significant while the sampled populations have different means. Its frequency depends on the amplitude of the difference, on each dataset's variance, on the number of replicates, and on the chosen significance threshold. Statistics session 2

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