

BIOSTATISTICS WITH

SUMMER WORKSHOP
(MITGEST network)

24-27 JULY 2024

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WORKSHOP SCHEDULE

- 4 days
 - 1. Intro to R and data analysis
 - 2. Statistical inference & hypothesis testing
 - 3. Modeling correlation and regression
 - 4. Intro to Machine Learning
- Each day will include:
 - Frontal class (MORNING)
 - Practical training with R about the topics discussed in the morning. (AFTERNOON)

DAY 2 – LECTURE OUTLINE

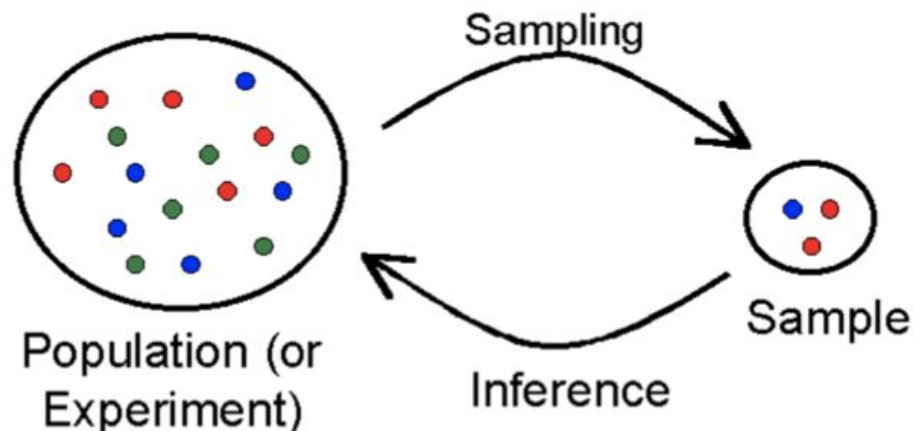
- Purpose and foundations of inferential statistics
 - Probability and random variables &
 - Meaningful probability distributions
 - Sampling distributions and Central Limit Theorem
 - Confidence Intervals
- Getting to know the “language” of hypothesis testing
 - The null and alternative hypothesis
 - The probability of error? (α or “significance level”)
 - The p-value probability and tests interpretation
 - Effective vs statistical significance
 - Types of errors (Type 1 and Type 2)
- Hypothesis tests **examples**
 - Comparing sample mean to a hypothesized population mean (Z test & t test)
 - Comparing two independent sample means (t test)
 - Comparing sample means from 3 or more samples (ANOVA)
- A closer look at testing assumptions (with **examples**)
 - Testing two groups that are NOT independent
 - Testing if the data are not normally distributed: non-parametric tests
 - Testing samples without homogeneous variance of observations

Inferential Statistics: population and samples

Gathering all data is not always possible due to barriers like time, accessibility, or cost.

Therefore, we often gather information from a smaller subset of the population: a **SAMPLE**.

- **POPULATION** = the universe of all possible observations we are interested in
- **SAMPLE** = a subset of the population from which information is actually collected
- **INFERENTIAL STATISTICS** = a collection of methods for using sample data to make conclusions about a population



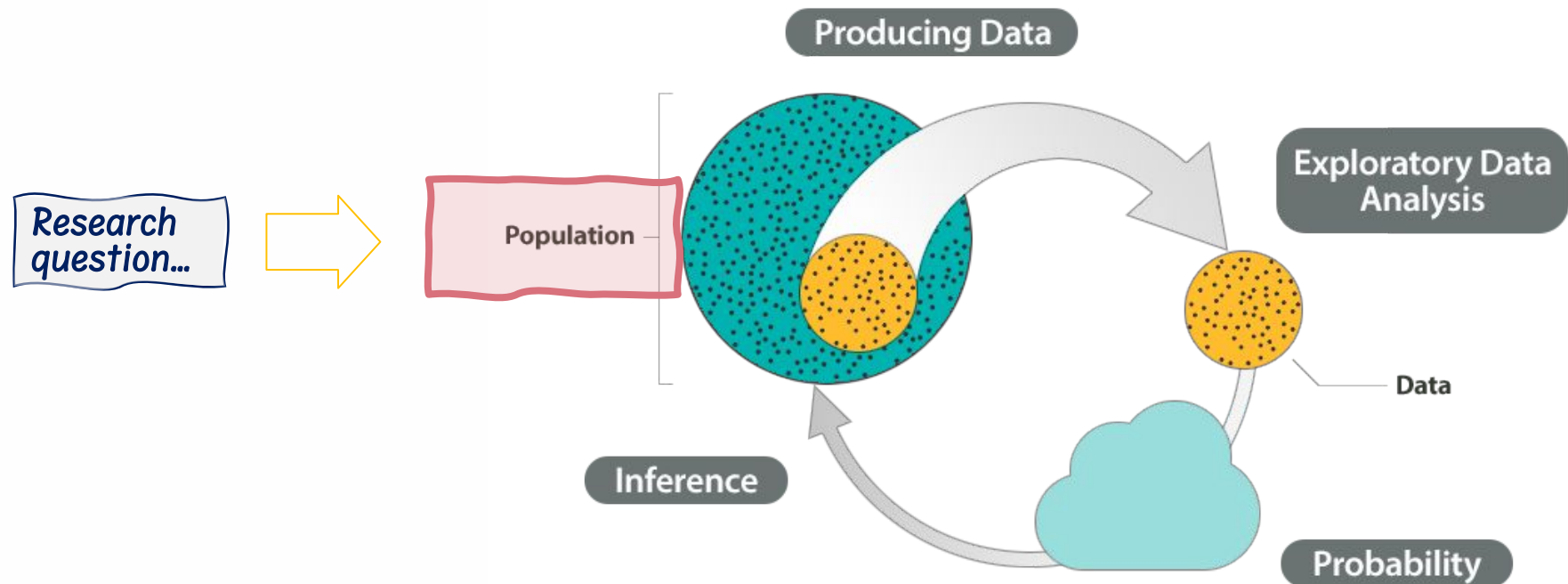
Inferential statistics workflow: defining a population

Decide which population(s) is (are) important

- We may need 2 of them—**control** and **treated** groups—according to the experimental design
- **Statistical populations** are something the investigator defines

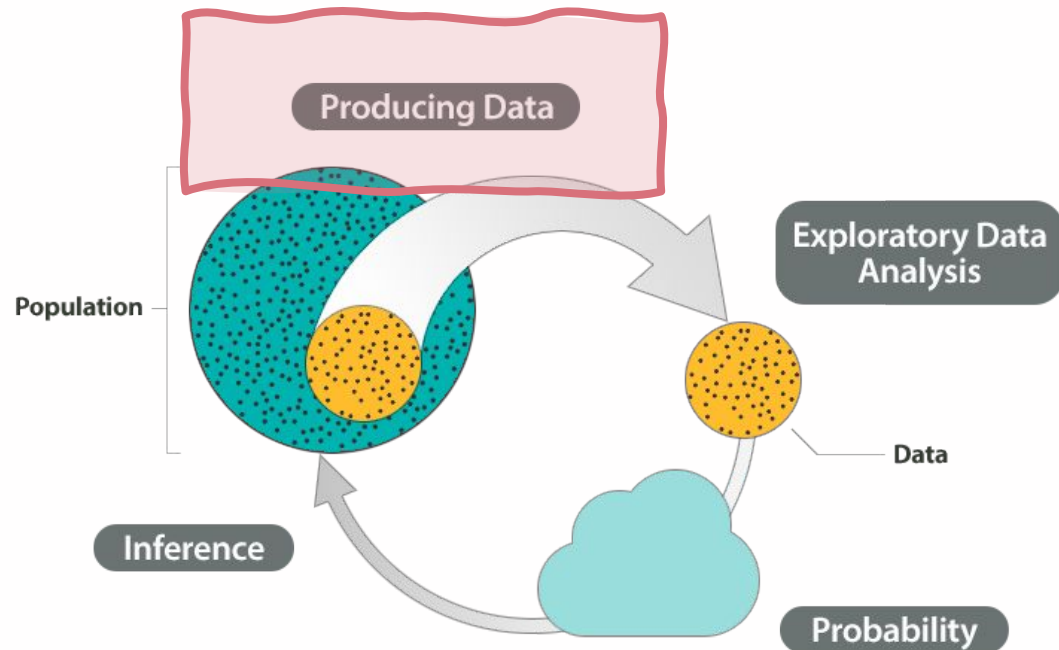
Decide which attributes of the population(s) need to be measured

- **Variable(s)** to measure
- (... not all relevant information are MEASURABLE)
- often the literature can provide information about the general population we are studying



Inferential statistics workflow: producing data

- Pick a **representative sample**
 - A sample is a subset of a population that has been selected to be **representative** (or **unbiased**), i.e. it REFLECTS the characteristics of the entire population
 - ideally, a **random sample**, where each individual has a known, non-zero probability of being selected into the sample
- Estimate the population parameter
 - From a representative sample, we can calculate a **point estimate** of the population parameter (unknown)
- Estimate **uncertainty**
 - **Sampling error**: any point estimate from the sample will be imperfect (it won't exactly match the true population value)



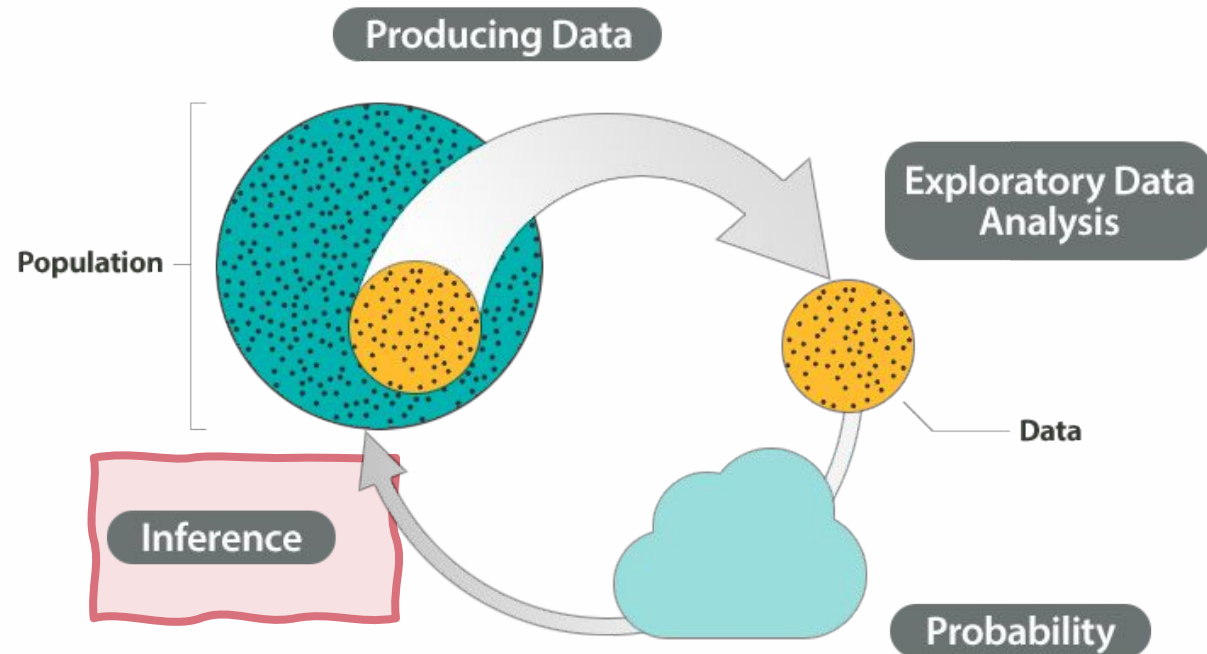
Inferential statistics workflow: making inference based on sample

Formulate **hypotheses** to test through experiments:

- features of a population
- interaction of **dependent** and **independent** variables
- degree of **uncertainty** and **error**

Generalize **findings** to population of interest, assessing:

- **construct validity**
- validity of **causal relationship**
- **generalizability**



Source image: https://stats.libretexts.org/Courses/Lumen_Learning/

Population versus sample: terms

Measurement	Sample statistic	Population parameter
Mean	\bar{x}	μ (mu)
Standard deviation	s	σ (sigma)
Variance	s^2	σ^2 (sigma squared)
Proportion	p	π (pi)
Correlation	r	ρ (rho)
Regression coefficient	b	β (beta)

Source: <https://www.statology.org/statistic-vs-parameter/>

Probability and random variables

Probability & statistical models

- Data are **observed values** («realizations») of **random variables**.
- The **probability distribution** of these random variables can be used to reason about *properties* ('**parameters**') of the unobserved universe (**inference**).
- Statistical models are probability distributions for observable data constructed to enable inferences to be drawn or decisions made from data
- From **observation** to theoretical **probability**:
 - **Absolute frequency (af)** **Relative frequency (rf)**
- Relative frequencies also serve as "empirical probabilities", [between 0 and 1].
 - **Relative frequency** **Probability of an event (p)**
- The relationship between **Absolute Frequency** and **Probability** is further reinforced by the **Law of Large Numbers**

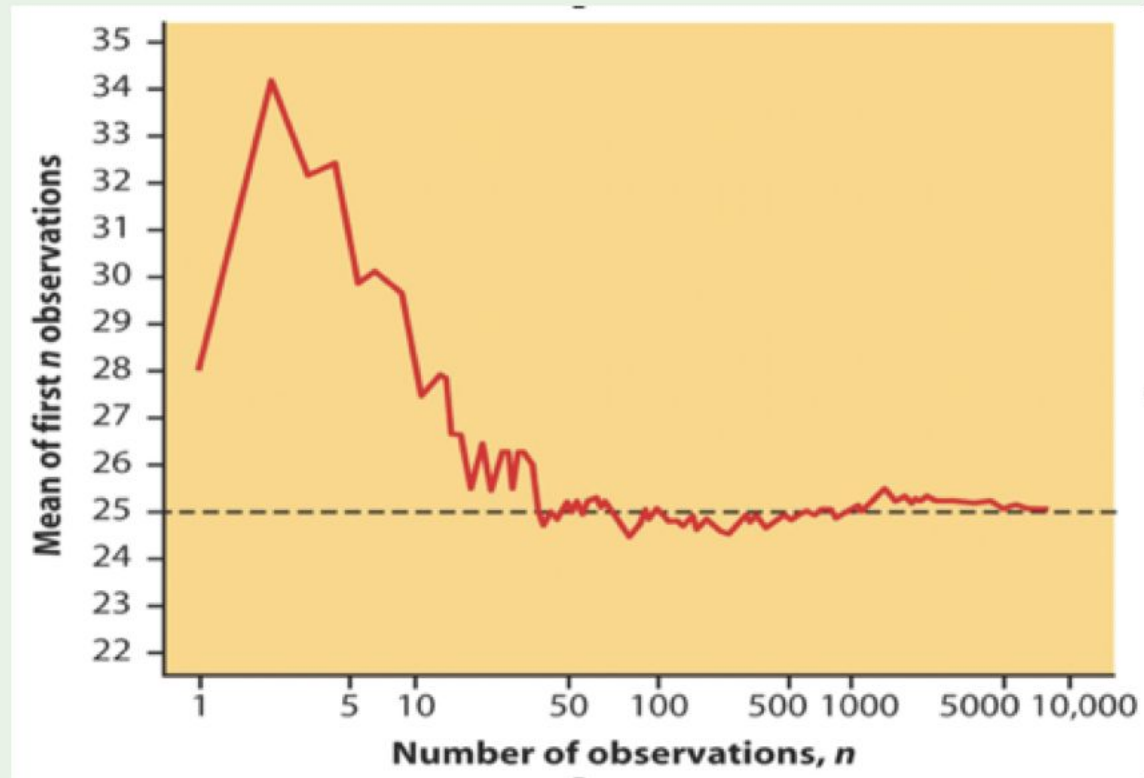
Relationship (af - probability) p

(By conducting a larger number of trials or observations)
we can **derive probabilities from absolute frequency!**

The Law of Large Numbers allows us to use probability to predict absolute frequency and viceversa

Draw observations at random from any population with finite mean μ . As the number of observations drawn increases, the sample mean of the observed values \bar{x} gets closer and closer to the mean μ of the population.

Example: How sample means approach the population mean ($\mu = 25$).



Random Variables and probability distributions

Discrete Random Variables

- can take a finite number of distinct values
 - e.g. # of children per family

Continuous Random Variables

- can take an infinite (or impossible to count) number of possible values
 - e.g. weight of a person

2 types of probability distributions

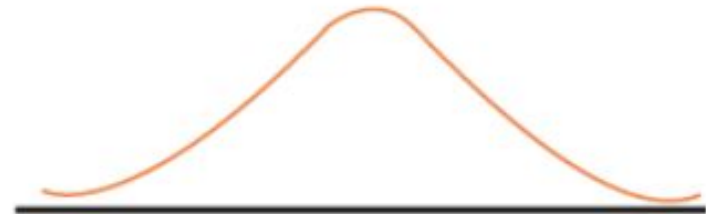
PROBABILITY DISTRIBUTION



The **probability distribution** describes the likelihood of the variable to take a possible individual value.

It is called: **Probability Mass Function (PMF)**

PROBABILITY DENSITY FUNCTION



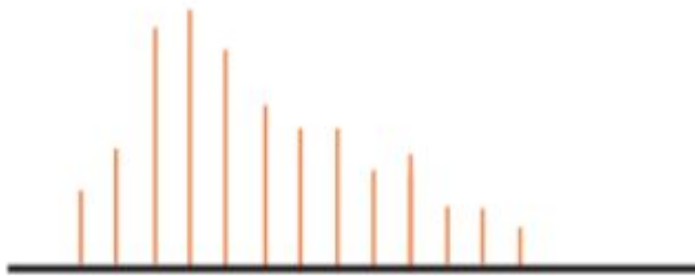
(Since $= 0$) the **probability distribution** describes the likelihood of the variable to fall within an interval of values.

It is called: **Probability Density Function (PDF)**

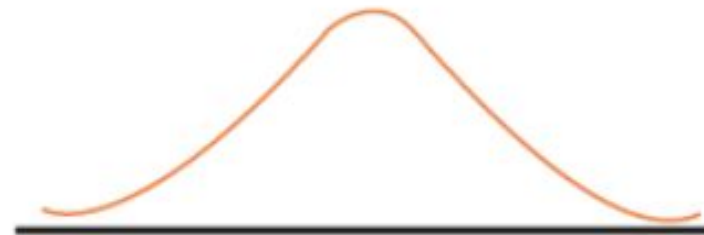
$P[a]$

Random Variables and probability distributions

Discrete Random Variables



Continuous Random Variables



SOME “FAMOUS” DISTRIBUTIONS

- **Bernoulli** Random Variable $X \sim \text{Bernoulli}(p)$

- applicable to random experiments that can only have 1 trial and only 2 possible results -like above (e.g. pass/fail, head/tail)

- **Binomial** Random Variable $X \sim \text{Bin}(n, p)$

- applicable to Bernoulli experiments (2 possible results), but here we can have 1 or more trials (e.g. probability that # patients will experience side effects from a new medication)

- **Poisson** Random Variable $X \sim \text{Poisson}(\lambda)$

- used to show how many times an event will occur within a given time period – knowing events occur independently and with a constant mean rate (e.g. # of meteorites striking Earth in a year)

- **Normal** Random Variable $X \sim (\mu, \sigma^2)$

- more on its feature later... (e.g. birthweight of newborn babies, shoe sizes, diastolic blood pressure, ...)

- **Exponential** Random Variable $X \sim \text{Exp}(\lambda)$

- refers to the process in which the event happens at a constant average rate independently and continuously (e.g. , the amount of time until an earthquake occurs, amount of time a car battery lasts)

Meaningful probability distributions for inference

The normal distribution

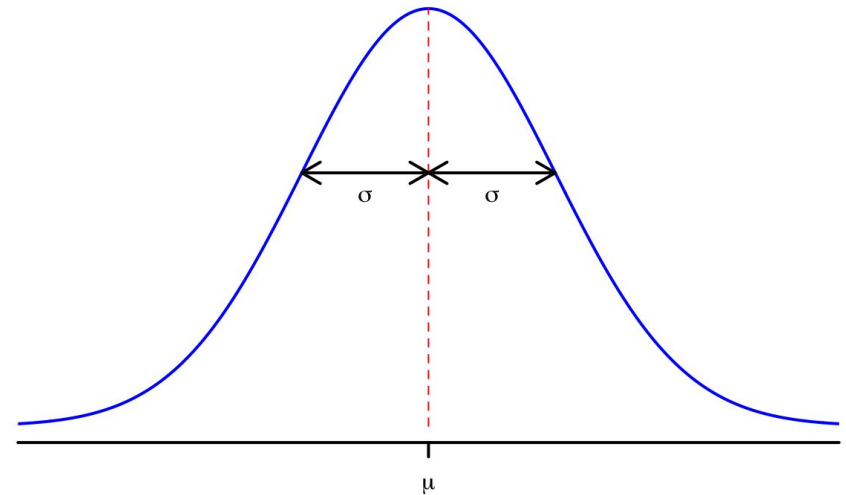
The normal distribution (special case of continuous distr.)

Normally distributed Random Variable

$X \sim \text{Normal}(\mu, \sigma^2)$

with

Probability Density Function

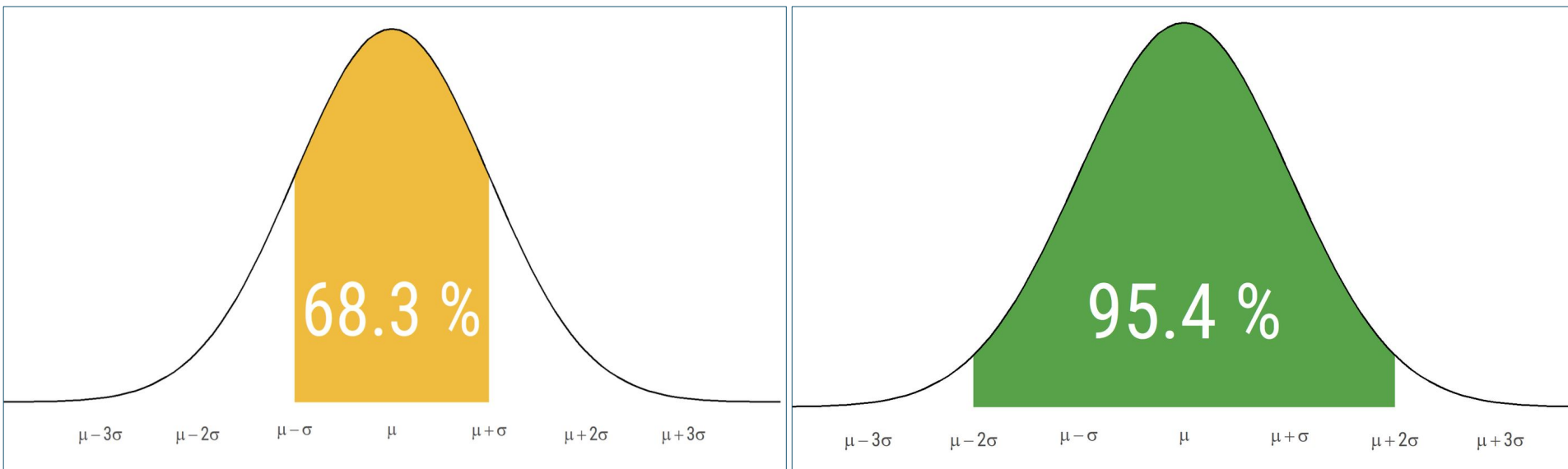


- The **normal distribution** («bell curve» or «Gaussian curve») is extremely important in statistics because:
 - it provides an excellent summary of an empirical distribution providing 2 parameters: the **mean (μ)** and **standard deviation (σ)**
 - many things in real life (and experimental science) can be approximated with it (e.g. blood pressure, height, weight, age children get disease, standardized test scores, etc.)
 - **Measurement error** with scientific instruments is typically modelled as a normal distribution with expectation $\mu=0$. The more precise the instrument, the lower the value of the variance σ^2 .

Features of the normal distribution

- Normal distributions are **symmetric around the mean**
- The **mean, median and mode** of a normal distribution are **equal**
- Normal distributions are **denser in the center** and less dense in the tails
- The area under the normal curve is = 1
- **68% of the area** of a normal distribution is **within 1 standard deviation** of the mean
- Approximately **95% of the area** of a normal distribution is **within 2 standard deviation** of the mean

$X \sim \text{Normal}(\mu, \sigma^2)$



Central Limit Theorem and Sampling Distributions

PROBLEM: How often do we know what the population of values looks like?

- (i.e. what if the observations in our sample are not normally distributed?)

SOLUTION: the **Central Limit Theorem** provides a bridge between (a) the nice properties of the normal distribution and (b) the fact that the distribution of individual elements of many samples are **not** normally distributed

Theorem (Central Limit Theorem)

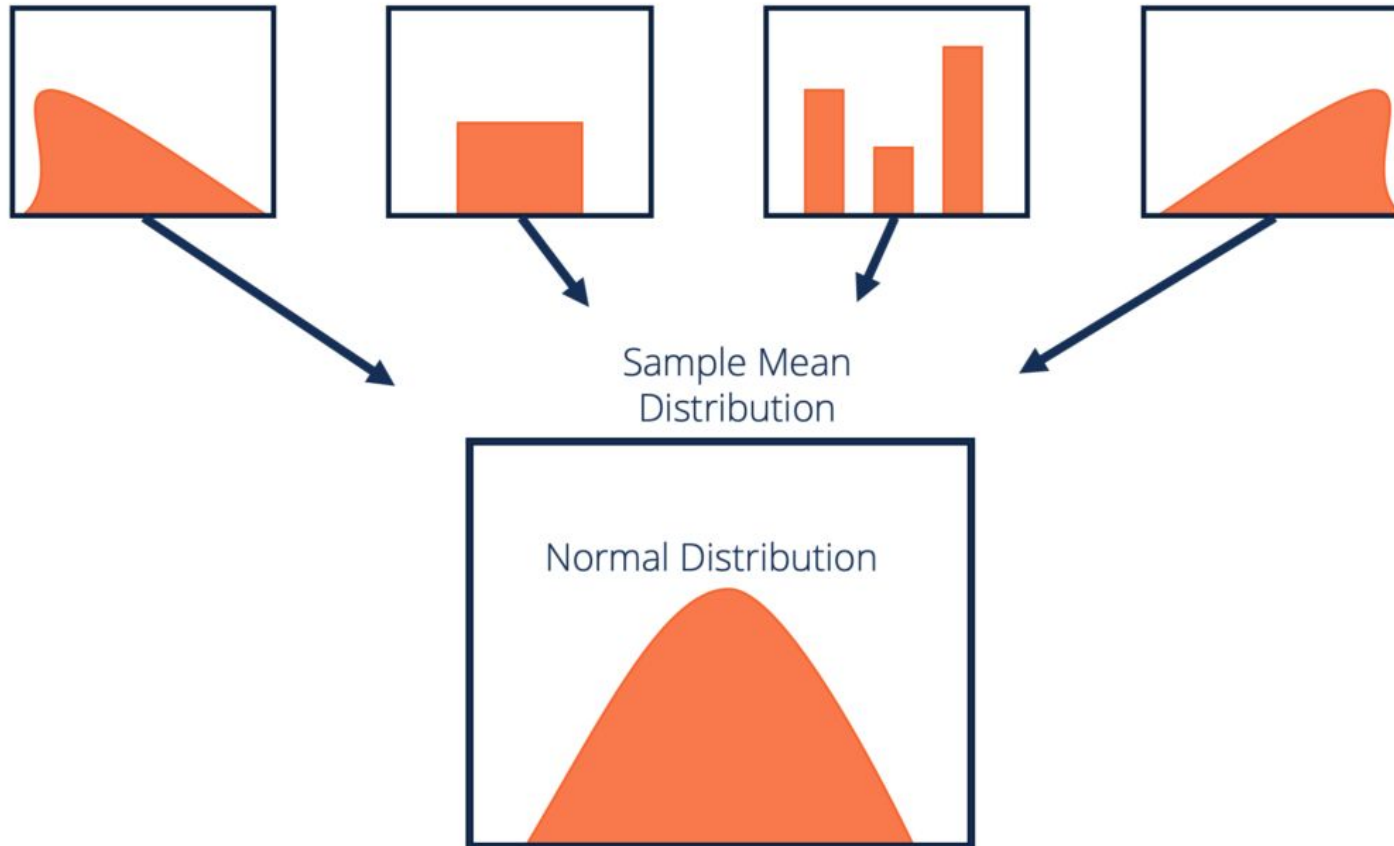
Given a population with a finite mean μ and a finite non- zero variance σ^2 , the sampling distribution of the mean approaches a normal distribution with a mean of μ and a variance of σ^2/N as N , the sample size, increases.

IN OTHER WORDS: regardless of the shape of the population distribution, suppose we take several random samples from a population and see the probability distribution of the sample means (i.e. a “**sampling distribution**”):

- by the CLT, the sample mean will have a normal (or near normal) distribution
 - (provided that N is large enough)
 - (provided that sampling is taken at random)

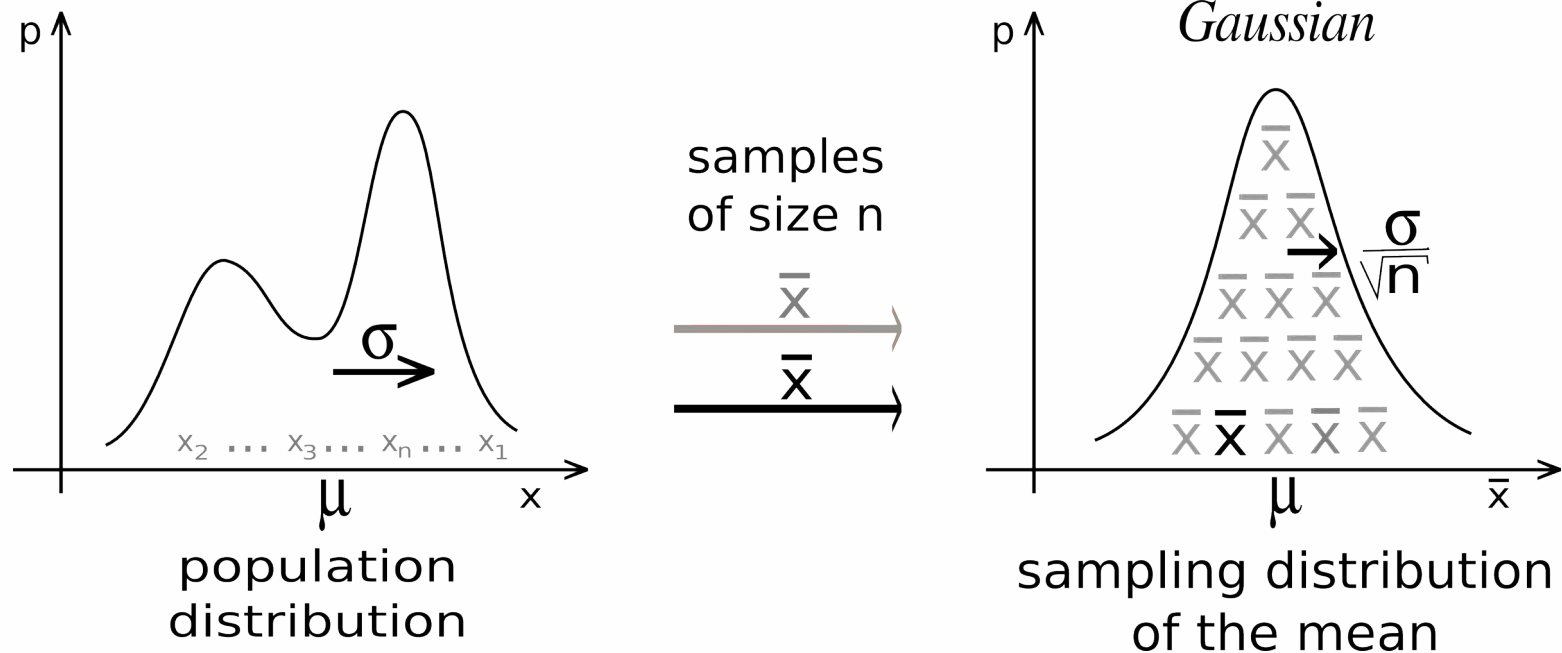
Important implication of the Central Limit Theorem

Calculating (or *simulating*) the mean of multiple samples of the population, and find their average, will result in an estimate of the *population mean*.



Source: <https://corporatefinanceinstitute.com/resources/data-science/central-limit-theorem/>

CLT fundamental for making probabilistic inferences about a population based on a sample



Even if the **objective population** has an **unknown or non-normal distribution**, the mean of all possible casual samples with n sufficiently large ($n > 30$) will have a **normal distribution** with mean and standard deviation = (this is called **Standard Error**)

→ The CLT makes parametric test on sample means (or its linear combination) applicable even if the assumption of population normality is violated, provided that the sample size is $n > 30$.

Confidence intervals and estimate precision

A possible application of the CLT:

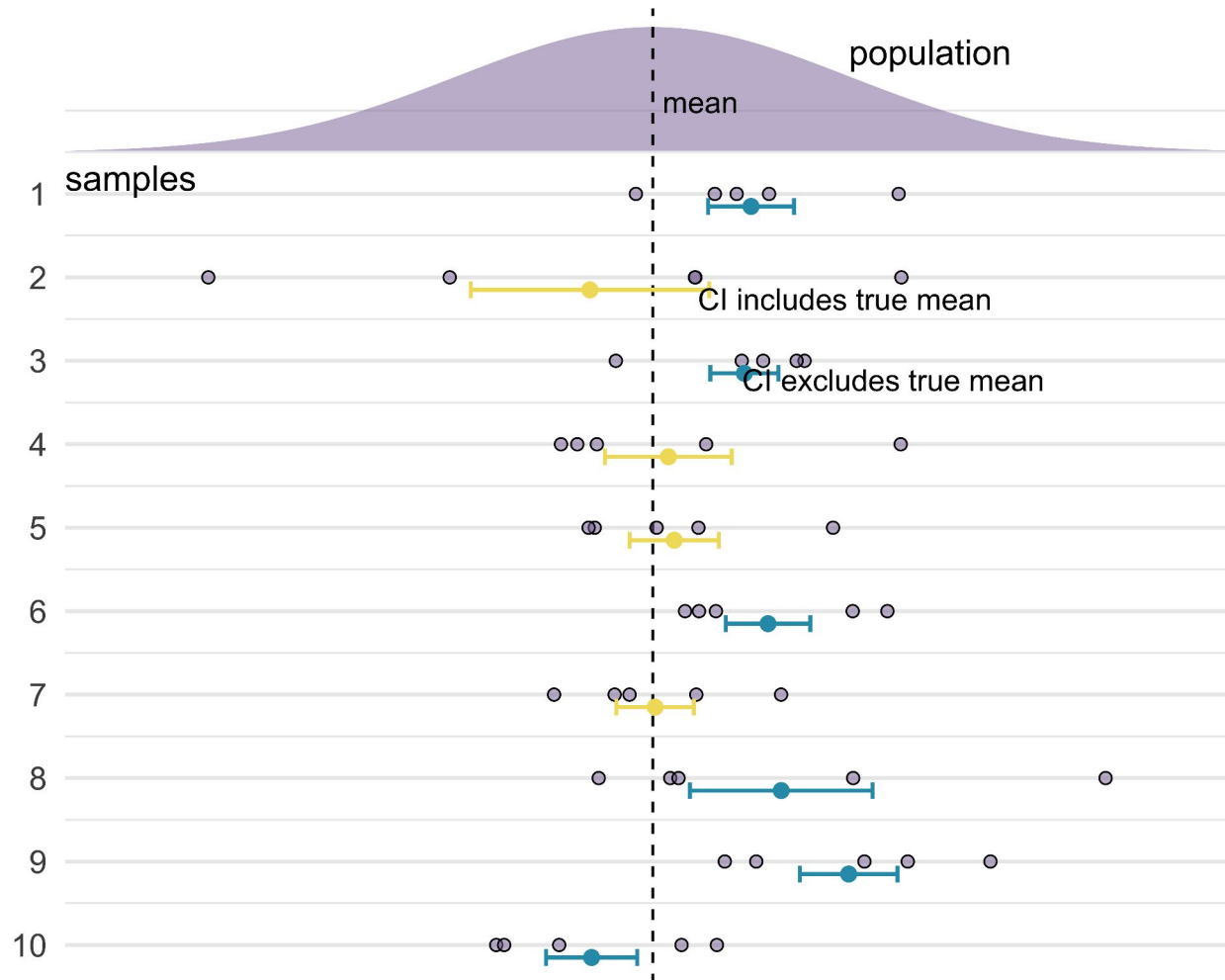
- A **confidence interval (CI)** is a range of values that is likely to contain a population parameter with a certain level of confidence.

Confidence Interval = point estimate \pm margin of error

Where \pm = (critical value)(**sd** of the statistic)

- The sample mean is a **point estimate** of
 - Each sample mean differs from the next one and from only by chance
 - The smaller n , the more precise will be the sample mean
 - z^* is a critical value that depend upon a test statistic
-
- The **estimate precision** is evaluated with the CI, which is an interval with a lower bound and an upper bound, which likely contains a population parameter with a certain level of confidence

Frequentist interpretation of a confidence interval



Source image: <https://vizdata.org/slides/16/16-uncertainty-I.html#/>

Example of constructing a CI for a mean point estimate

What probability level do we want? (that the interval will contain the population parameter). We choose 95% which implies:

- 5% probability of error =

So, for any single sample we draw, we can calculate a range of values (CI) on either side of the sample mean equal to:

CI(95%) 1.96

such that:

- in 95% of all possible samples of size n , will fall in our confidence interval
- in 5% of all possible samples of size n ,

There is a **trade-off** between the level of confidence (α) and precision in which the parameter is estimated:

- higher level of confidence wider confidence interval
- lower level of confidence narrower confidence interval

Also, increasing n will reduce the margin of error for a fixed α .

Example of constructing a CI for a mean point estimate

Before we were assuming o know the population standard deviation parameter , but we rarely do.

CI(95%) 1.96

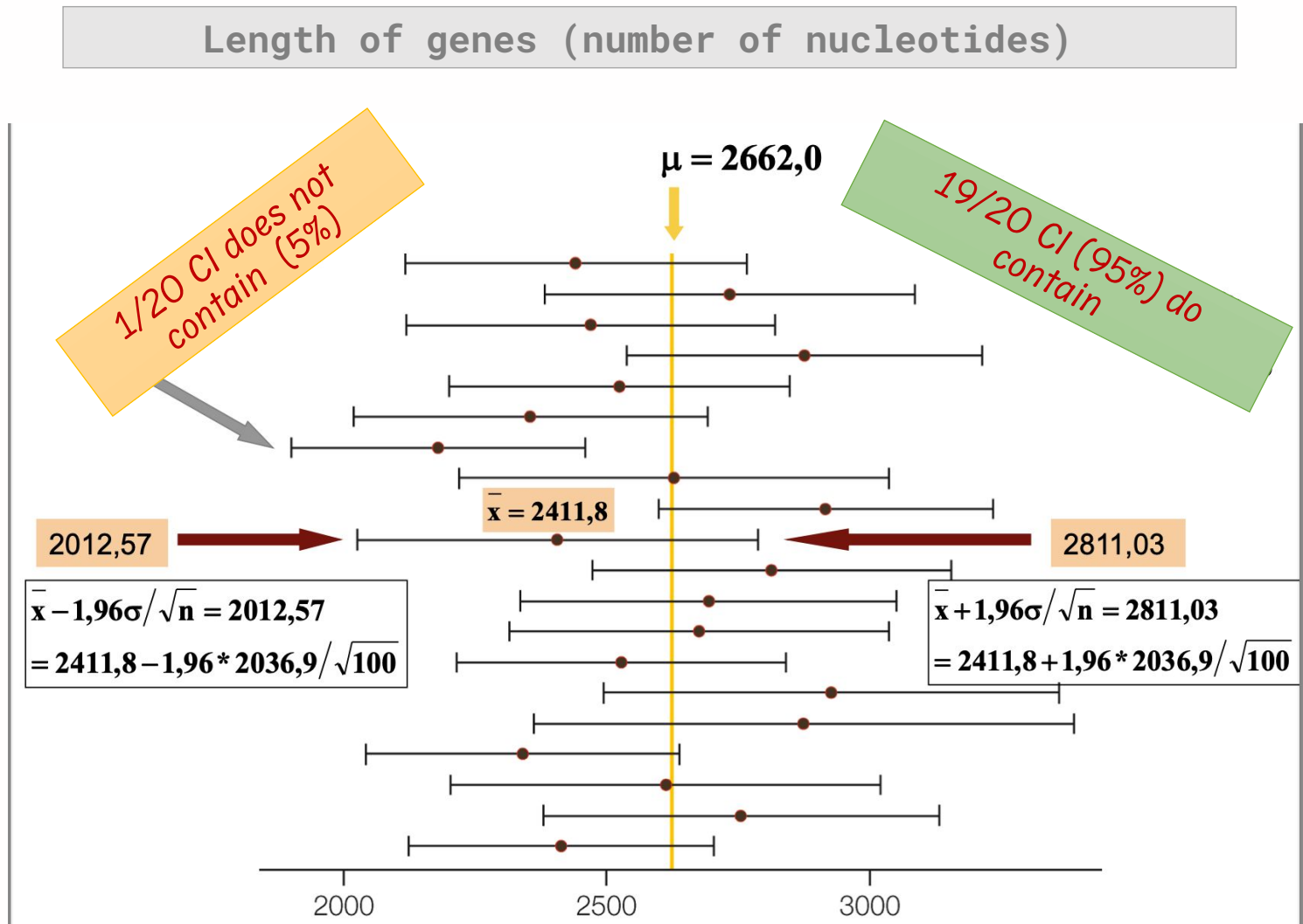
If we don't know the population sd, we will estimate is with the sample :

CI(95%) 1.96

- **NOTE:** we can use this formula (with the statistics) only when the sample size is sufficiently large.

Confidence Interval example

CI (95%) of the mean calculated from 20 random samples of $n = 100$ genes from the human genome



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Statistical Hypotheses

- Any claim made about one or more populations of interest constitutes a **statistical hypothesis**.
- These hypotheses usually involve population parameters, the nature of the population, the relation between the populations, and so on.
- For example, we may hypothesize that:
 - The mean of a population, μ , is 2
 - Two populations have the same variance
 - A population is normally distributed
 - The means in four populations are the same
 - Two populations are independent, etc
- Procedures leading to either the acceptance or rejection of statistical hypotheses are called **statistical tests**.
- There are two approaches to accept or reject hypothesis:
 - The **Bayesian approach** assigns probabilities to hypotheses directly, given the observed data – where population **parameters** are assumed to be a probability distribution
 - The **frequentist approach** (“classical” statistics) works on the assumption of taking many random samples from the same population – where population **parameters** are assumed to be fixed and unknown constants
 - (the one we take for our hypothesis testing review)

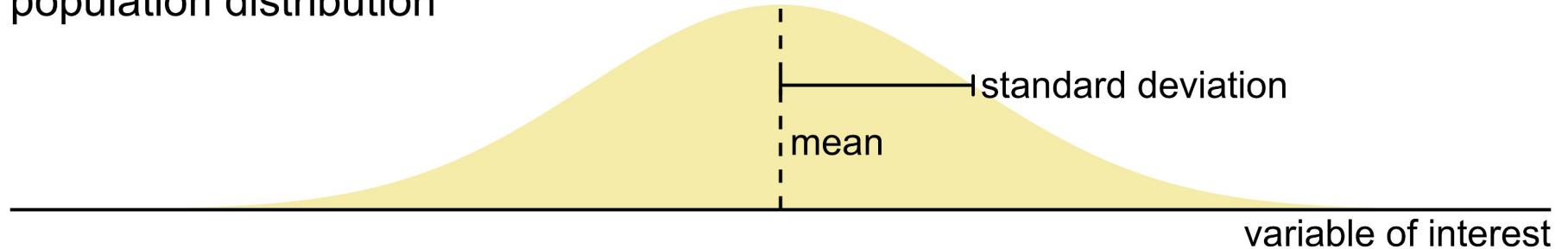
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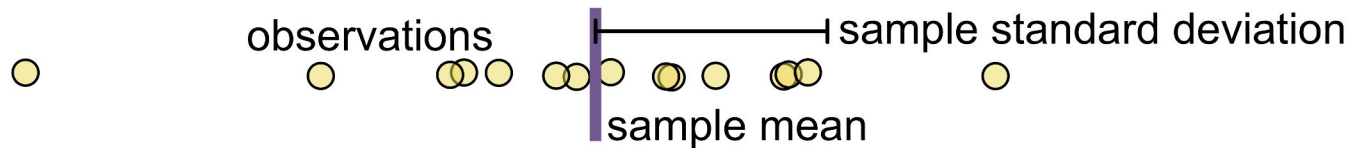
- **Frequentist statistics** works by answering the following question: *what would have happened if we repeated a data collection exercise many times, assuming that the population remains the same each time*
 - we ultimately have to work out what some sort of sampling distribution looks like
- **sampling distributions** = summarise the range of outcomes we can expect to observe when we repeat the same sampling process over and over again
 - **sample size** —often denoted 'n'— has an effect.
- **sampling error** = the variability or 'noise' in some process. Sampling error is the reason why we have to use statistics. Every time we estimate something, there's some sampling error lurking in the estimate. If we ignore it, we risk drawing incorrect conclusions about the world
- **standard errors** = quantitative measure of sampling error variation (the standard deviation of the estimate's sampling distribution)
- **in the absence of multiple samples, WE USE THE ONE SAMPLE TO APPROXIMATE THE POPULATION SOMEHOW, THEN WORK OUT WHAT THE SAMPLING DISTRIBUTION OF OUR ESTIMATE SHOULD LOOK LIKE BY 'TAKING SAMPLES' FROM THIS APPROXIMATION. How?**
 - *pretend the sample is the true population and then draw new samples from this pretend population to get at a sampling distribution (**bootstrapping**)*

Uncertainty of point estimates is expressed as **standard error**, **confidence interval**, or **credible interval**

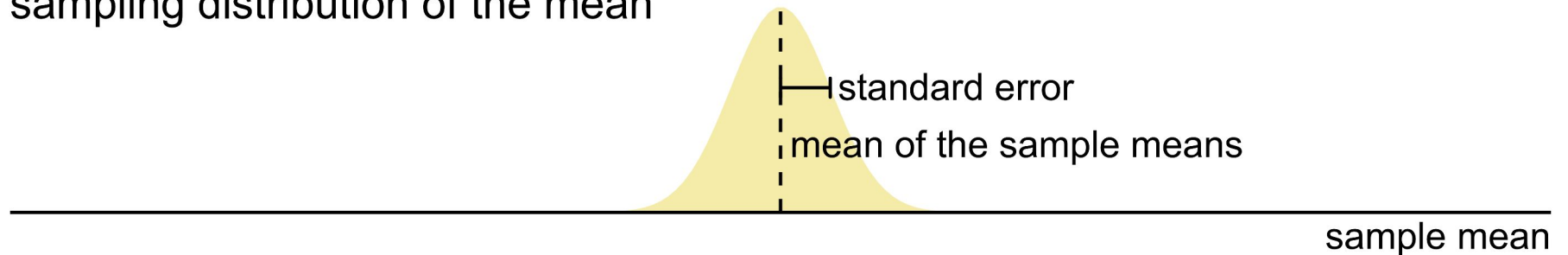
population distribution



sample



sampling distribution of the mean



Source image: <https://vizdata.org/slides/16/16-uncertainty-I.html#/>

Getting to know the “language” of hypothesis testing

(classical approach)

Hypothesis testing steps

1. State the hypotheses (the null hypothesis and an alternative hypothesis)
2. Formulate an analysis plan (e.g. the significance level is 0.05, the test method one-sample z-test)
3. Analyze sample data
4. Interpret result and make decision

What are the Null and Alternative hypotheses?

Null Hypothesis

- is the hypothesis that a sample data statistic occurs purely from chance
 - e.g. there is no difference between the mean pulse rate for people doing physical exercise and the normal pulse rate
- Must contain condition of equality ,
- Test the Null Hypothesis directly: reject or fail to reject

Alternative Hypothesis or

- is the hypothesis that a sample data statistic is influenced by some non-random cause
 - e.g. the mean pulse rate for persons doing the physical exercise is higher than the normal
- Must be true if is false (corresponding to , conditions)
- 'opposite' of Null Hypothesis

What is the “significance level”, α ?

“Is the statistic obtained from my sample > or < than the corresponding parameter in the general population?”

- We can never answer a question like this with absolute certainty relying on a sample!

There is always a certain probability of error: that is rejected even though it is actually true.

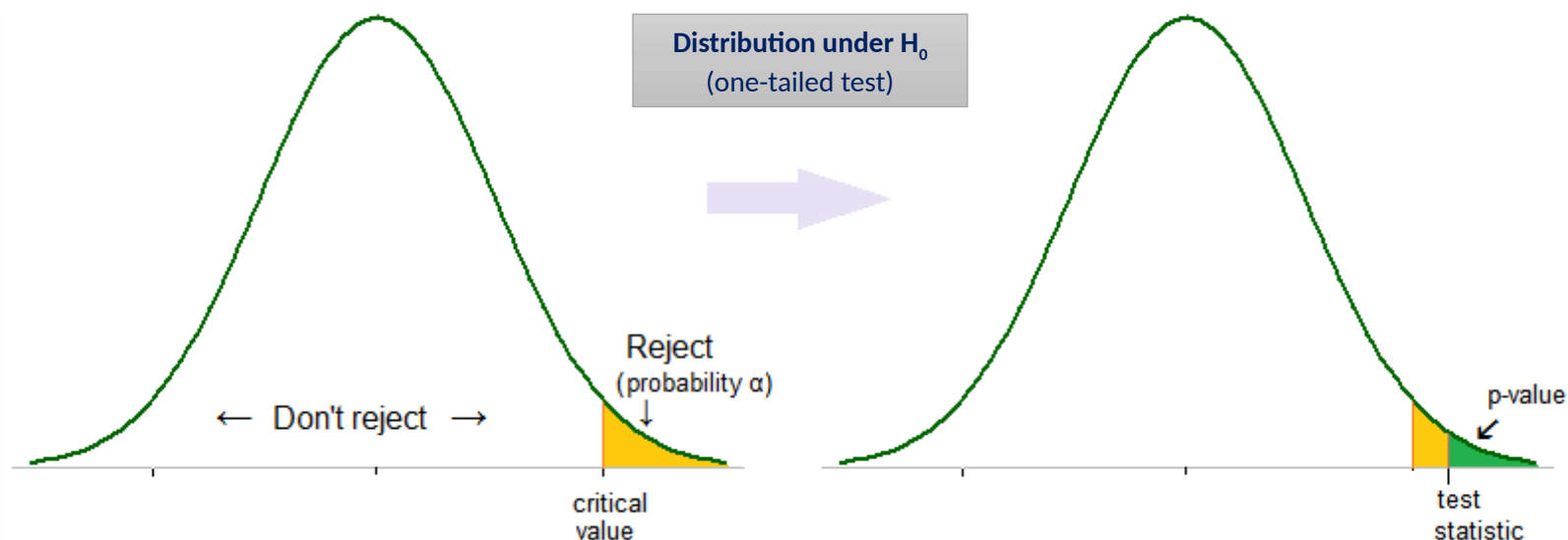
This probability of error is called the **significance level** or α , i.e.:

- α sets at what point the observed sample statistic is “unlikely enough” to reject the null hypothesis
- Usually, a significance level is set at 5% or 1%. For example, a **significance level of 0.05** signifies a 5% risk of deciding that an effect exists () when it does not exist (= FALSE POSITIVE).
 - e.g. To test the hypothesis (there is no difference between the mean pulse rate for people doing physical exercise and the normal pulse rate) a **significance level of 5% is set** and a measurement is conducted over n persons doing physical exercise

Lower significance levels require stronger sample evidence to be able to reject the null hypothesis

What is the p-value?

- A **p-value** describes **the probability to obtain our test statistic calculated from the sample (or a more extreme value) when we assume the null hypothesis is true**
 - i.e. the **p-value** shows how strongly your sample data contradict the null
 - we can also say that the **p-value** gives us the smallest α at which the **would be rejected**
- Conventionally, we use p-values in conjunction with α to determine whether our data favor the null or alternative hypothesis:
 - $p < 0.05$ means we 'reject the null hypothesis' / the 'effect is statistically significant at 5% level'



Lower p-value represents stronger evidence against the null hypothesis

Another way to think of tests' accuracy and precision

- **Accuracy**

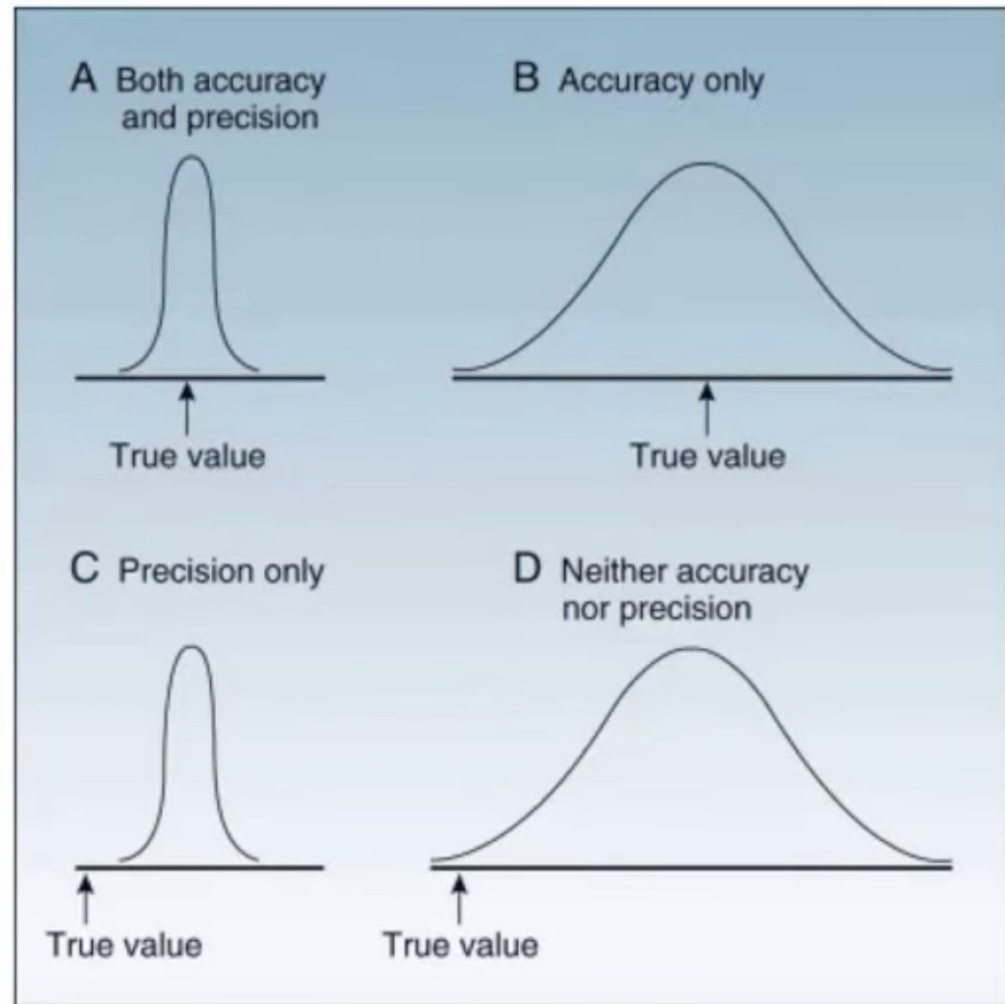
«**accurate tests**» capture what you want them to measure in an unbiased way.

- E.g. if your lab measurement gives you a 3.2 kg for an object that actually weights 10 kg , it is NOT ACCURATE

- **Precision**

«**precise tests**» give reproducible, or reliable results (although *not necessarily accurate*)

- E.g. ... if you weight your object 5 times, and you get 3.2 kg each time, your measurement is VERY PRECISE



Source image: https://www.youtube.com/watch?v=1Q6_LRZwZrc&t=22249s

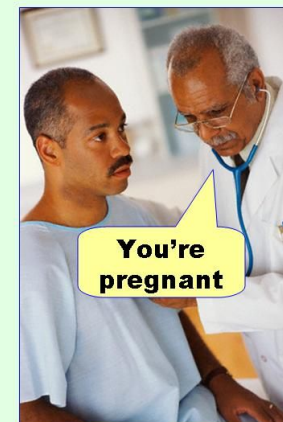
What are Type I and Type II errors?

There are two ways of getting hypothesis tests wrong:

- 1. FALSE POSITIVE** The null hypothesis is true but we conclude it isn't
 - Even if the p-value is less than your significance level ($p < \alpha$), the supposed effect might not exist in the population.
 - This occurs due to sample error (your random sample has overestimated the effect by chance). **Type I error** has a **probability = α** .
- 2. FALSE NEGATIVE** The null hypothesis is false but we fail to reject it
 - The p-value is greater than your significance level ($p > \alpha$), hence your sample provides insufficient evidence for concluding that the effect exists in the population
 - This can depend on small effect sizes, small sample sizes, or high data variability. **Type II error** has a **probability = β** .
 - The inverse of beta is called **statistical power of a test = $1 - \beta$**

		After test we reject H_0	After test, we fail to reject H_0
“State of the world” ~ the Truth	H_0 is true	Type I error	Test OK 👍
		FALSE POSITIVE	(no effect)
	H_0 is false	Test OK 👍	Type II error
		(effect exist)	FALSE NEGATIVE

Type I error
(false positive)



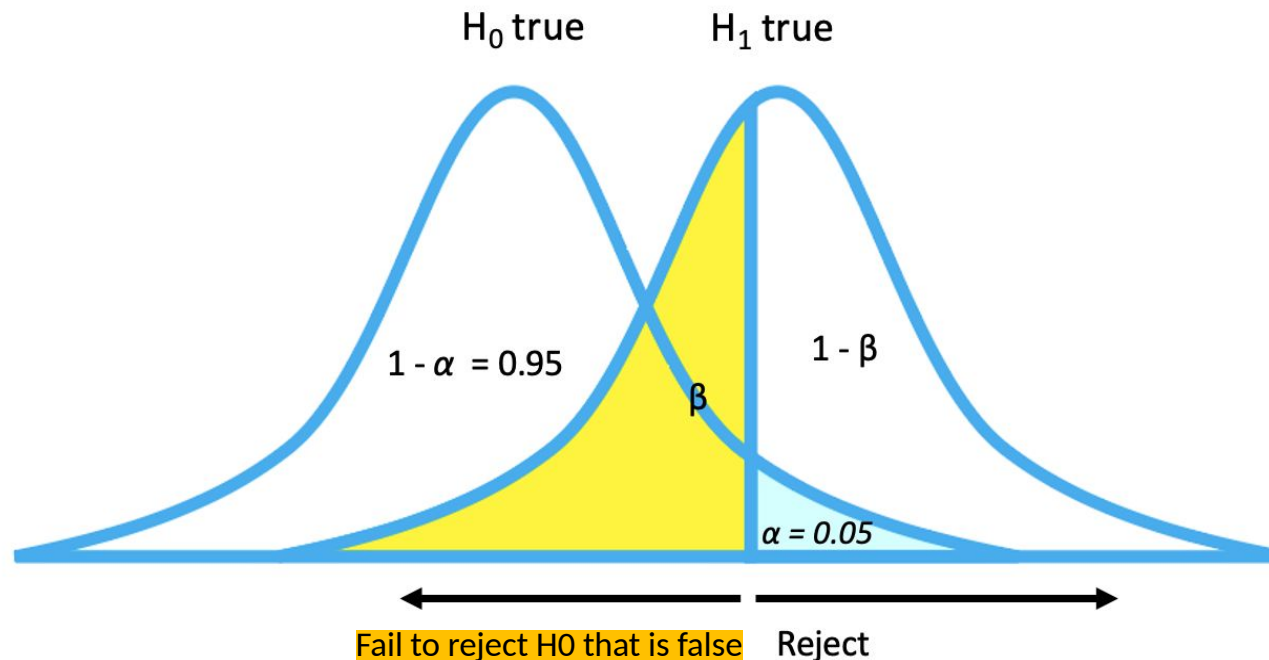
Type II error
(false negative)



What is β (graphical representation)?

bello ma non so
se mostrare

- β = **Type II error** = probability of a False Negative
- $(1 - \beta)$ = **Power** = probability of a True Positive, i.e. the probability to detect an effect when it is there (*a.k.a. the probability that the test says we are right!*)
 - the probability that we reject and therefore accept when is indeed true
 - we only talk about “power” when is true.



What is the Z-score?

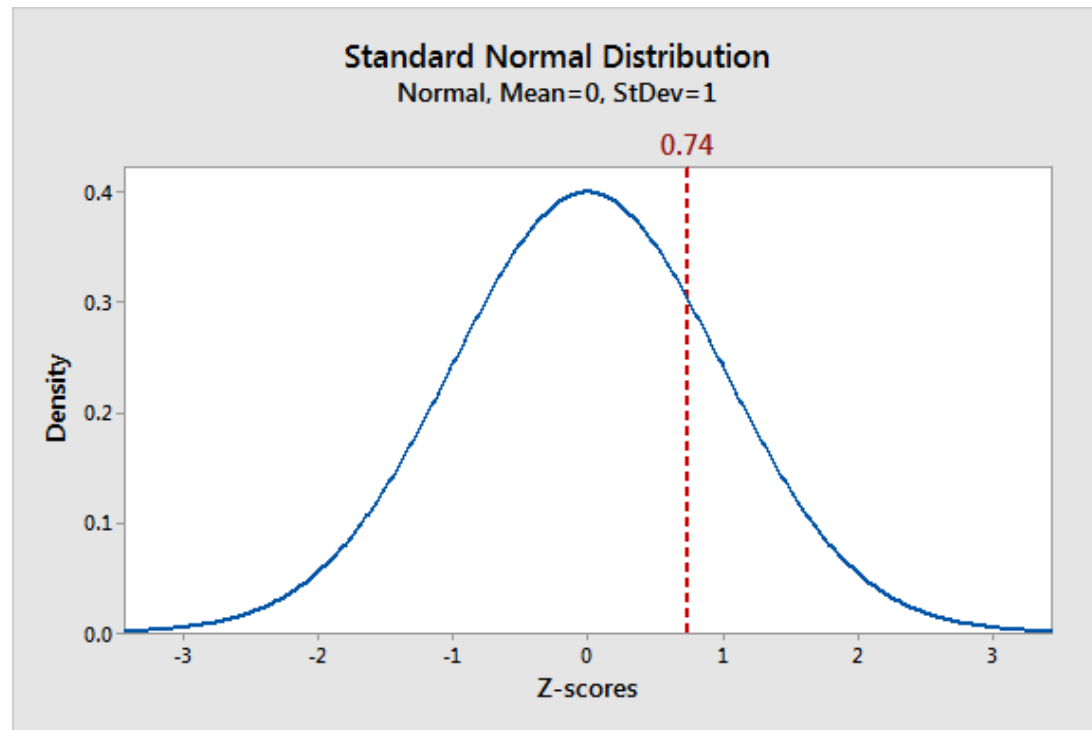
It helps us understand where a specific observation falls within a distribution

- **Z-score** helps us understand where a specific observation falls within a distribution
- Consider the **Z-distribution**, a standardized Normal distribution with $\mu = 0$ and $\sigma = 1$
- The formula for finding z-scores is the following:
$$Z = \frac{X - \mu}{\sigma}$$
- Where:
 - X represents the data point of interest
 - μ and σ represent the mean and standard deviation for the population from which you drew your sample

EXAMPLE

Using the *standard normal distribution* $N(0, 1)$, if a one-month-old baby girl weighs 5 kilograms, how does she compare to others?

A 5 kg weight equals a **Z-score of 0.74**

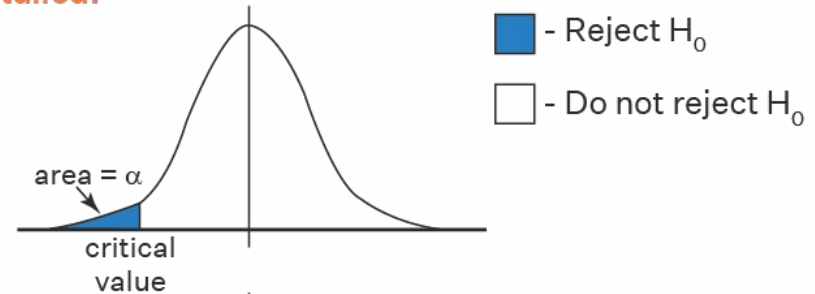


One-tailed and two-tailed tests of hypothesis

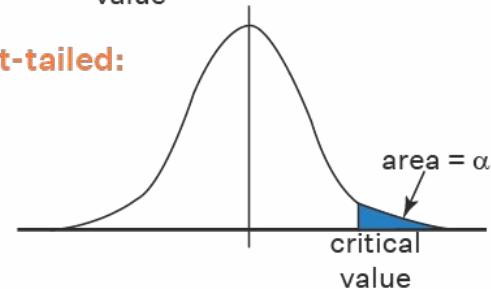
- The choice between a one-tailed and two-tailed test depends on our expectations about the reference population:
 - one-tailed test** → We know in which direction the estimate diverges compared to the population (e.g. there is no difference between the mean pulse rate for people doing physical exercise and the normal pulse rate)
value | value
 - two-tailed test** → We don't know in which direction the estimate diverges compared to the population
value

Rejection Region for Null Hypothesis

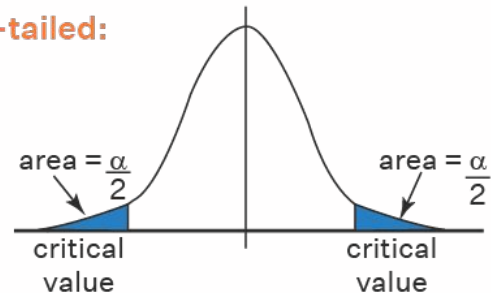
left-tailed:



right-tailed:



two-tailed:



Source image: <https://www.cuemath.com/data/z-test/>

Effective vs statistical significance

- **effective significance** (effect size) is the actual *magnitude of the difference between groups* (e.g. outcome variable mean for treated v. control groups)
 - *absolute effect size* = raw difference between group means
 - *standardized effect* = difference transformed into an easily understood scale
- **statistical significance** (p-value) is the probability that the observed difference between two groups is due to chance
 - if the *P* value is larger than the level chosen (eg, 0.05), any observed difference is assumed to be explained by sampling variability
 - with a sufficiently large sample, a statistical test will *almost always* demonstrate a significant difference

RECOMMENDATION: *don't lose sight of your actual research question: a statistically significant difference – when very small – can still be meaningless for your purposes*

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Comparing a sample mean to a hypothesized population mean

EXAMPLE A: Z-test one-sample hypothesis (for large samples with known population's variance)

Our dataset for the day

- We will be working on a datasets described in 2 recent [open access articles](#) on **cardiovascular heart diseases**
- The original authors (Ahmad, et al., 2017) released also the [open access dataset](#) containing the **medical records of 299 heart failure patients collected at a Hospital in Faisalabad (Punjab, Pakistan), in April–December 2015**
 - all patients > 40 years old, having left ventricular systolic dysfunction
 - age, serum sodium, serum creatinine, gender, smoking, Blood Pressure (BP), Ejection Fraction (EF), anemia, platelets, Creatinine Phosphokinase (CPK) and diabetes were recorded and considered for potentially explaining mortality caused by Cardiovascular Heart Disease (CHD)



RESEARCH ARTICLE

Survival analysis of heart failure patients: A case study

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Chicco and Jurman *BMC Medical Informatics and Decision Making*
<https://doi.org/10.1186/s12911-020-1023-5>

(2020) 20:16

BMC Medical Informatics and
Decision Making

RESEARCH ARTICLE

Open Access

Machine learning can predict survival of patients with heart failure from serum creatinine and ejection fraction alone

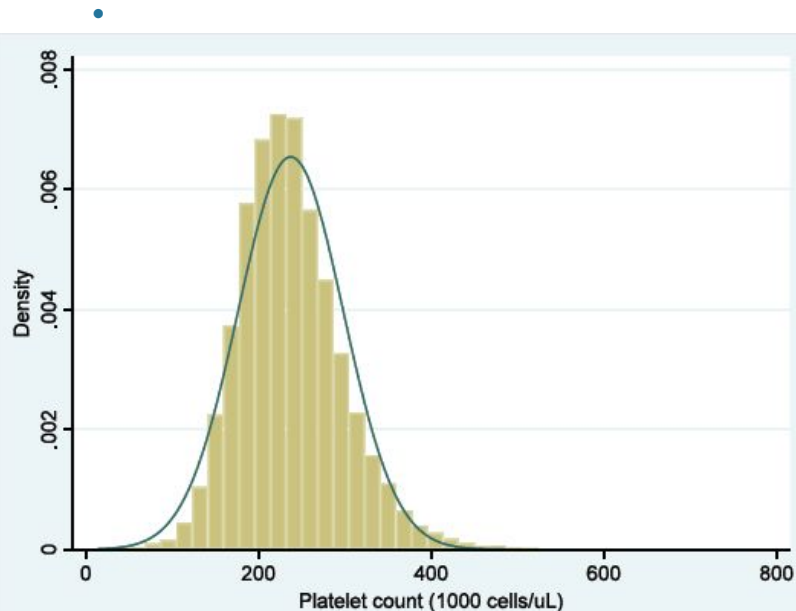
Davide Chicco^{1*} and Giuseppe Jurman²



QUESTION: Compare the mean *platelets count* in the patients' sample against a reference distribution - with known mean (μ) and standard deviation (σ)

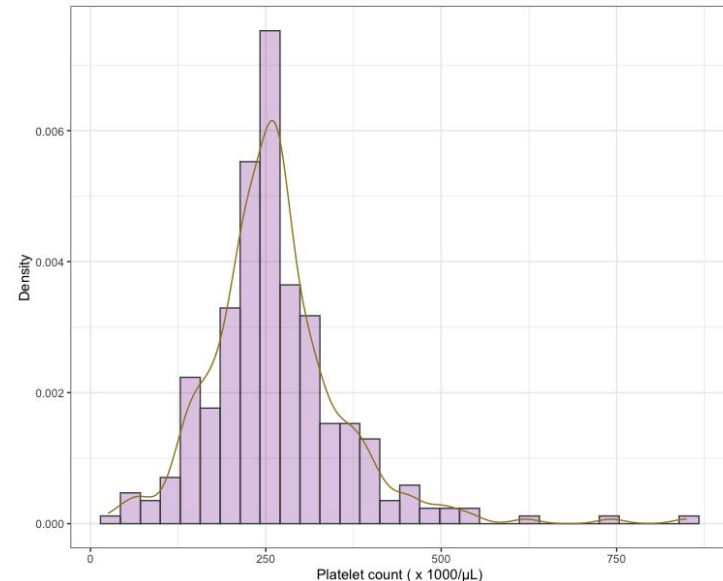
Total Platelet Count distribution (in *Reference* general Population)

- Large population study conducted in the US between 2011–2016 on 17,969 adults.
- **Total Platelet Count (TPL)** measurement showed a Normal distribution with



Total Platelet Count distribution (in *Sample* of heart failure patients)

- Sample of 299 patients population study collected in Pakistan in 2015
- **Total Platelet Count (TPL)** measurement showed a Normal distribution with



Source: <https://pulmonarychronicles.com/index.php/pulmonarychronicles/article/view/558/1223> (left) & author calculations (right)

Example A (step 1): Expressing the research question in the form of hypotheses

- I have a **sample mean** () collected measuring **Total Platelet Count (TPL)** for heart failure patients and I wonder if such population differs from the general population (*which I know have mean*) simply by chance (), or sampling variability, OR if () the sample mean is different from the population's because of some specific effect related to having heart disease.
- Stating the above hypotheses more formally:
 - ***What is the population Total Platelet Count (TPC) mean for all people who suffered of heart failure (?)***
 - : there is no difference in mean TPC between patients who suffered heart failure and the general population
= 236 → hypothesis of no effect or (“no difference”)
 - : there is a difference in mean TPC between patients who have suffered heart failure and the general population (“some effect”). This can be formalized as either:
236 (one-sided test), or
236 (one-sided test), or
236 (two-sided test)

Example A (step 2): Formulating an analysis plan

I start by the analysis plan, i.e. how to use sample data to evaluate

- The evaluation often focuses around a single test statistic
- The analysis plan should specify the following elements
 - Significance level (α): conventionally significance levels are chosen equal to 0.01, 0.05, or 0.10; but *any value between 0 and 1 can be used*.
 - Test method to determine in which direction the hypothesized mean differs significantly from the observed sample mean. Alternative options are:
 - the one-sample z-test
 - the two-sample t-test
 - the two-sample z-test
 - etc.

- Z-tests are closely related to t-tests, but t-tests are best performed when an experiment has a small sample size, less than 30.
- Also, t-tests assume the standard deviation is unknown, while z-tests assume it is known.

Example A (step 3a): Analyze sample data: Test Statistic

- Find the value of the **test statistic** described in the analysis plan. Here:
 - Test statistic → sample mean
 - Significance level → 0.05
- Given the assumptions below:
 - Patients in the HEART FAILURE were independently sampled
 - Large sample with $n = 30$
 - The level of measurement of TPL is interval-ratio
 - the sampling distribution of sampling means for heart failure patients is normally distributed

non capisco se la definizione di sMALL n è 30 o 100 o 130 (come avevano insegnato a me)

Test method → the one-sample z-test

One-sample test of a mean

- Take a sample of the size and a standard error ;
- Compute the **z-statistic** for the population mean assuming is true.

Where :

IF $n = 30$ and	Standard Error	Calculated Z score
known		=
UNknown		=

Example A (step 3b): Analyze sample data: Test Statistic

In this case, we have:

- a large sample ($n > 100$)
- and an **known** (of the reference population)

We obtained the sample mean and sample standard deviation

Hence, we compute the a standard error and the **Z-statistic** for the hypothetical Heart Failure Population mean population mean assuming is true, with :

$$= =$$

One-sample hypothesis test of Total Platelet Count mean in a Heart Failure affected population

Assuming is true:

- Heart Failure population mean would be:
- Given our random sample has size = **299** with sample mean , and we have a known the population standard deviation, we can compute:

$$=$$

$$= =$$

Example A (step 4): make a decision using the critical region

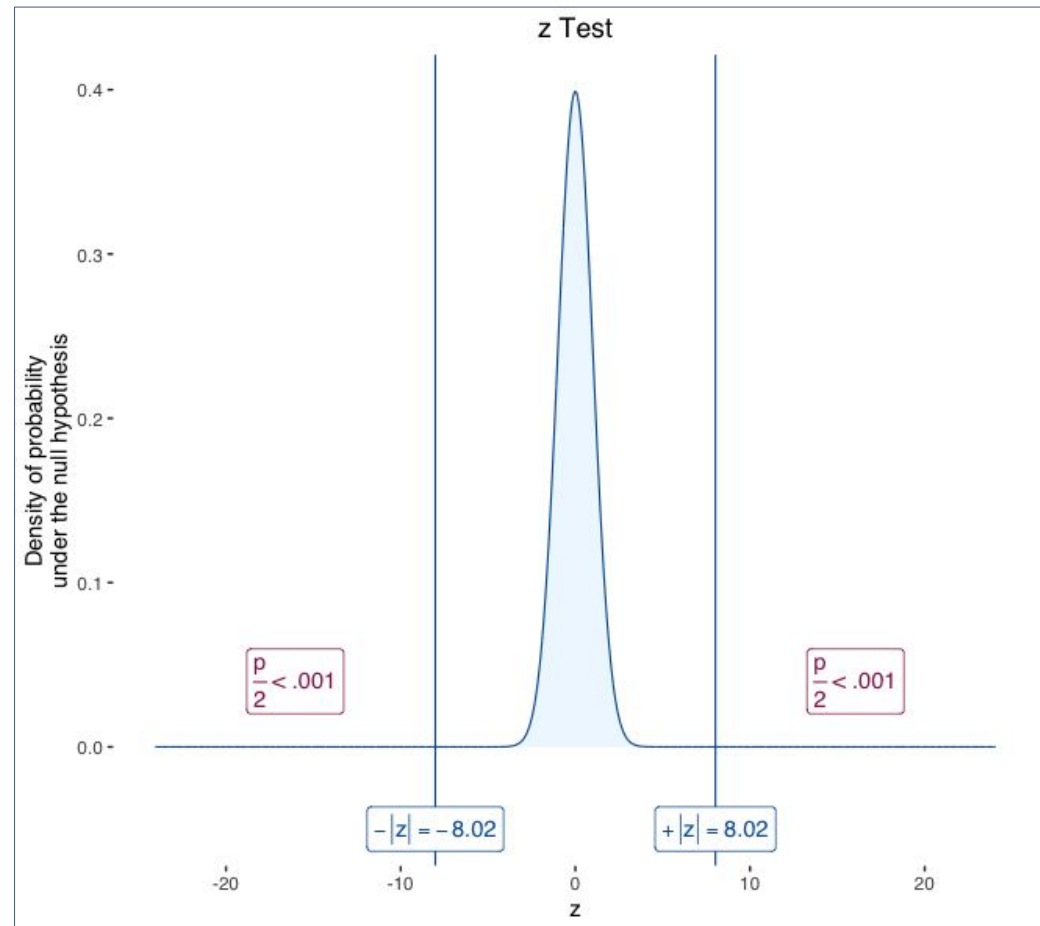
The **critical region** (or **rejection region**) consists of the areas under the sampling distribution of sample mean that include **UNLIKELY SAMPLE OUTCOMES**;

and given that (Z score corresponding to = 0.05);

= **8.0180** actually falls in the **CRITICAL REGION** (well beyond the critical point)

DECISION: we reject the Null Hypothesis (basically we conclude that *it is extremely unlikely that the sample we drew could have occurred just by chance*). So the test indicates that indeed **there is a difference between heart failure patients and the general population in terms of average platelets count**

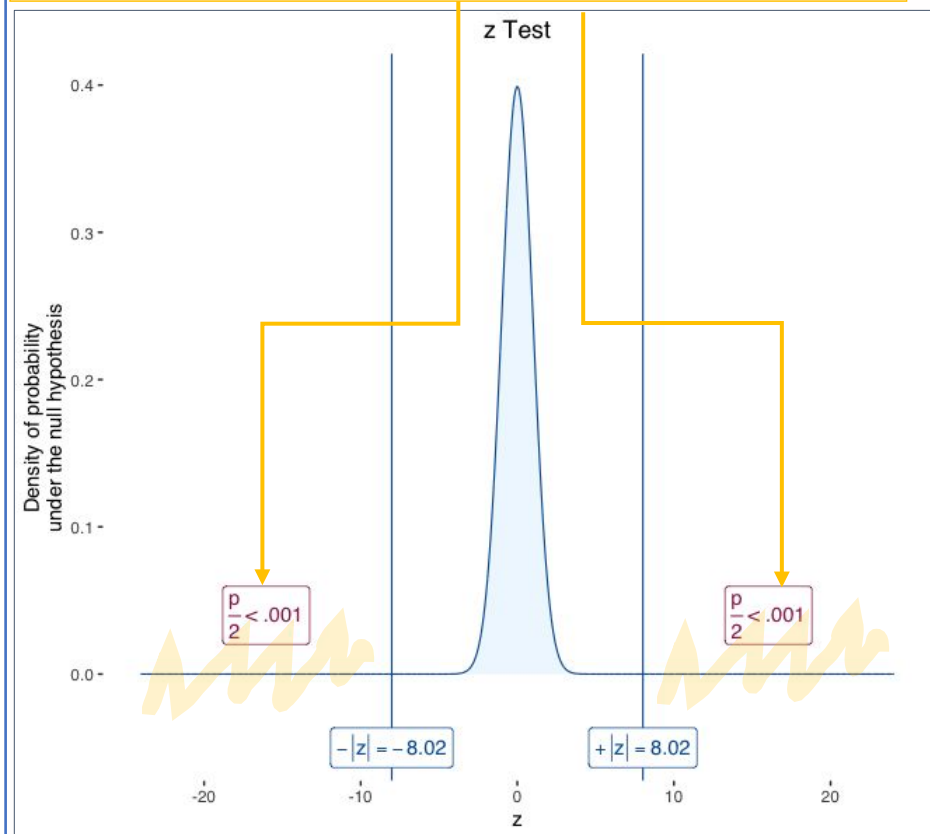
Sampling distribution of sample means with noted in **Z scores**



Example (step 4a): make a decision using the p-value

- The **P-value** corresponds to the answer the question: "What is the probability of the observed test statistic or one more extreme when H_0 is true?"
- This is the area under the curve of the Standard Normal distribution beyond the z .
- Convert z statistic to P-value:
 - For $H_a : > \Rightarrow p = P(Z > z) = \text{area under right-tail beyond } z$
 - For $H_a : < \Rightarrow p = P(Z < z) = \text{area under left-tail beyond } z$
 - For $H_a : \Rightarrow p = 2 \times \text{one-tailed P-value}$

p-value = 0.000000000000000107443 (two-tailed)



DECISION: highly significant evidence against the Null Hypothesis

Interpretation: Thus, smaller and smaller P-values provide stronger and stronger evidence against H_0

(Variation on the theme)

Comparing a sample mean to a hypothesized population mean

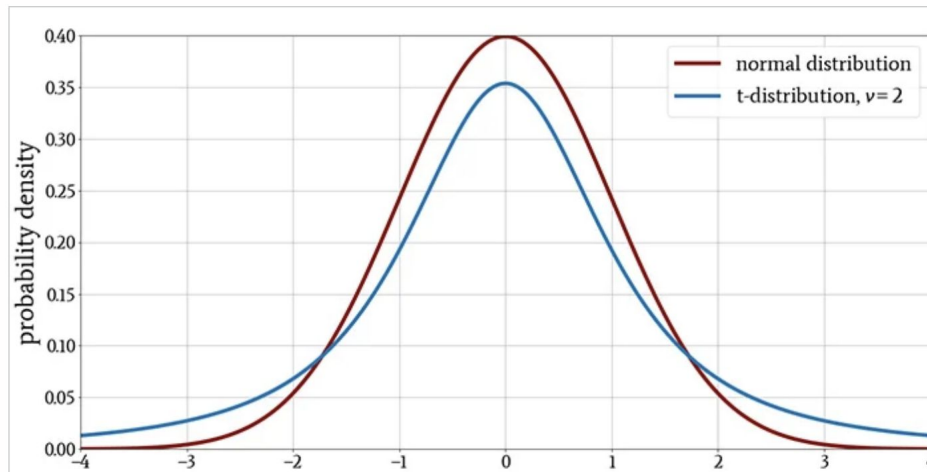
EXAMPLE B: t-test for small samples ($n < 30$) with unknown population's variance)

[Necessary digression] Student's t-Distribution

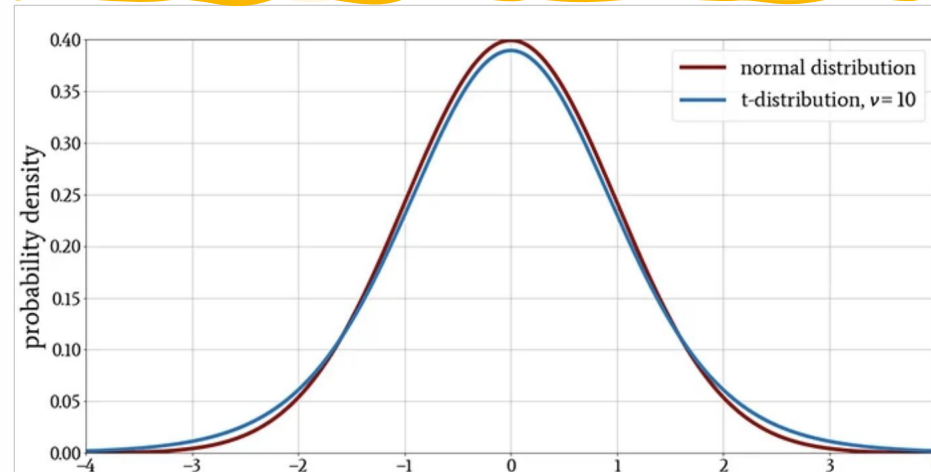
- In some cases we use the **t-distribution** instead of the **normal distribution** to model the null hypothesis because:
 1. it provides a more accurate representation of the variability in sample means and
 2. it enables making reliable inferences, even in situations where the standard normal distribution fails
- In particular in cases when **dealing with small sample sizes** or **when the population variance is unknown**
- The shape of the t-distribution changes according to the parameter ν , which denotes **degrees of freedom** and is determined by the sample size (denoted by n):

$$\nu = n - 1$$

For small n the **t-distribution** is a “flattened” version of normal



For larger n , the **t-distribution** and the normal distribution is increasingly close



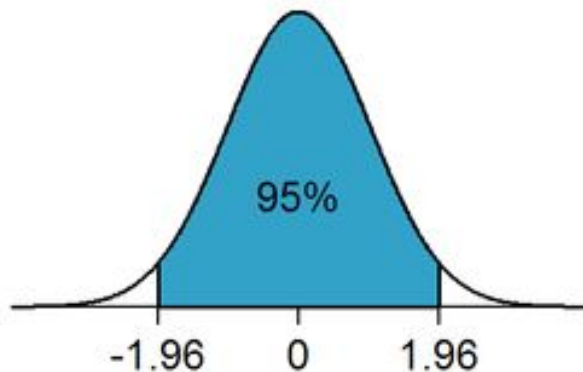
Critical value for the t-distribution v. the normal distribution

The critical value

- Recall that the critical values for normal distribution are

$$Z_{.95} = 1.96$$

$$Z_{.99} = 2.58$$



95% of the area under a normal curve lies within 1.96 standard deviations of the mean

Critical value for the t-distribution v. the normal distribution (cont.)

- The critical values for t-distribution t_{CL} depend on the degrees of freedom:

Table 1. Abbreviated t table.

df	0.95	0.99
2	4.303	9.925
3	3.182	5.841
4	2.776	4.604
5	2.571	4.032
8	2.306	3.355
10	2.228	3.169
20	2.086	2.845
50	2.009	2.678
100	1.984	2.626

The higher is N
→
The closer are t critical
values to Z critical values

Example B (step 1): Expressing the research question in the form of hypotheses

*[Let's consider a situation in which the available **sample is smaller ($n = 23$)**, e.g. there are records only for patients who had a follow-up visit in 21 days or less.]*

- GOAL: make inference on a “**hypothetical**” **population** of patients who suffered heart failure & who have had a follow-up visit within 30 days →
- QUESTION: if the **sample mean** () collected measuring **Total Platelet Count (TPL)** for heart failure patients differs from the expected mean of a general population (*which I know to be*) simply by chance () OR if () the sample mean is different from the population's because of some specific effect of having heart failure.
- MORE FORMALLY:
 - : there is no difference in mean TPC between patients who suffered heart failure (visited in 30 days) and the mean TPC the general population
= 236 → hypothesis of no effect or (“**no difference**”)
 - : there is a difference in mean TPC between patients who have suffered heart failure and the general population (“**some effect**”). This can be formalized as:
236 (two-sided test)

Example B (step 2): Analyze sample data: Test Statistic

- Find the value of the **test statistic** described in the analysis plan. Here:
 - Test statistic → sample mean
 - Significance level → 0.05
- Given the assumptions below:
 - Patients in the HEART FAILURE with follow-up visit in 30 days or less were **independently sampled**
 - “Small” sample with **n 30**
 - The level of measurement of **TPL is interval-ratio**
 - The **sampling distribution** of sampling means for heart failure patients is **normally distributed**

Test method → the one-sample t-test

One-sample test of a mean

- Take a sample of the size and a standard error (let's pretend we don't know the general population variance)
- Compute the **t-statistic** for the population mean assuming is true.

Where :

IF n 30 and	Standard Error	Calculated Z score
known		=
UNknown		=

Example B (step 3): Analyze sample data: Test Statistic

In this case, we have:

- a **small** sample ($n < 30$)
- and an **unknown**

We obtained the sample mean and sample standard deviation

Hence, we compute the a standard error and the **t-statistic** for the hypothetical Heart Failure (<30 d) population mean assuming is true, with :

$$= =$$

One-sample hypothesis test of Total Platelet Count mean in a Heart Failure affected population (<30 d)

Assuming is true:

- Heart Failure population mean would be:
- Our random sample has size = **23**, sample mean , and sample deviation . So we compute:

$$=$$

$$=$$

Example B (step 4a): make a decision using the critical region

The **critical region (or rejection region)** consists of the areas under the sampling distribution of sample mean that include **UNLIKELY SAMPLE OUTCOMES**;

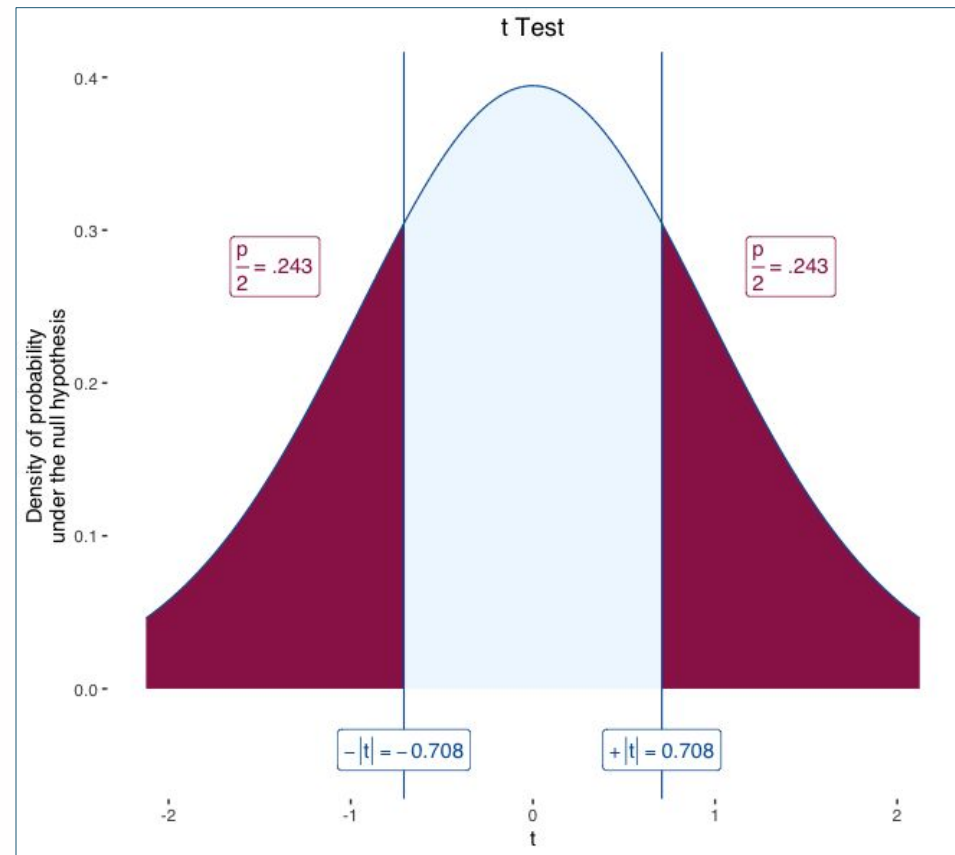
With d.f. = $23 - 1 = 22$ a two-sided test with $\alpha = 0.05$,

the (for Two-tailed t score corresponding to $\alpha = 0.05$);

Hence, actually falls in the **ACCEPTANCE REGION**

DECISION: Since the t statistic is less than the t critical value of on 22 degrees of freedom at 95% level ($P=0.05$): **we FAIL to reject the Null Hypothesis**

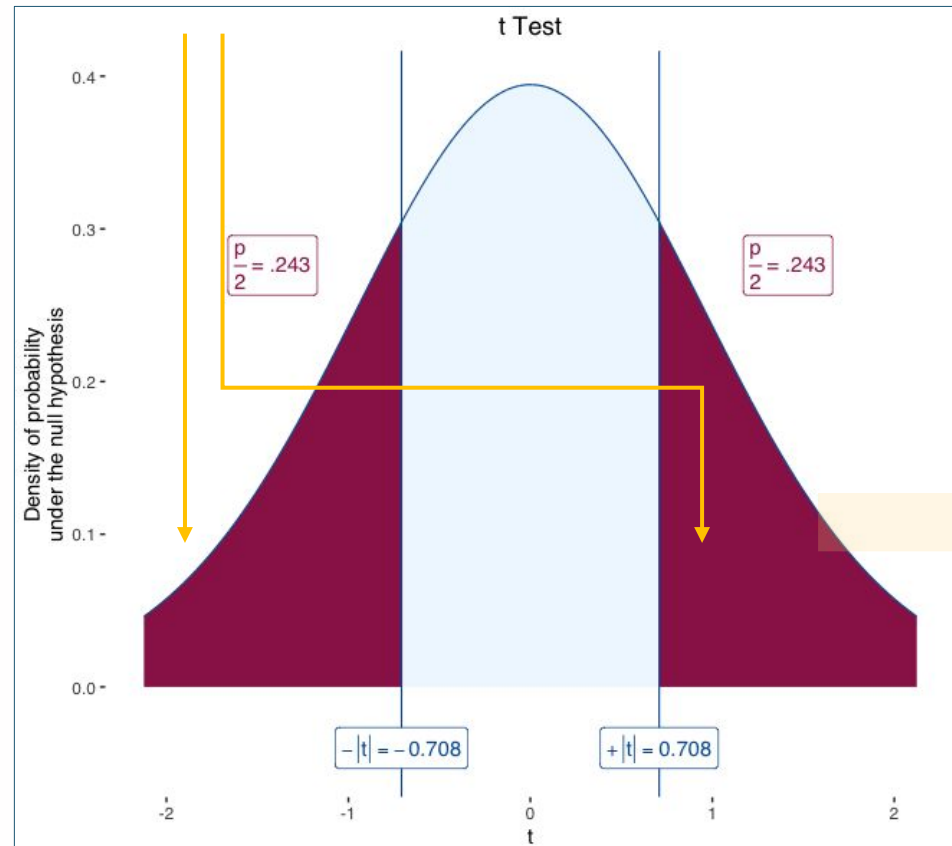
Sampling distribution of sample means with noted in **t scores**



Example B (step 4b): make a decision using the p-value

- The **P-value** responds to the question: “What is the probability of the observed test statistic or one more extreme when H_0 is true?”
- This is the area under the curve of the t distribution beyond the .
- Convert t statistic to P-value:
 - For $H_a : > \Rightarrow p = P(>) = \text{area under right-tail beyond } z$
 - For $H_a : < \Rightarrow p = P(<) = \text{area under left-tail beyond } z$
 - For $H_a : \Rightarrow p = 2 \times \text{one-tailed P-value}$

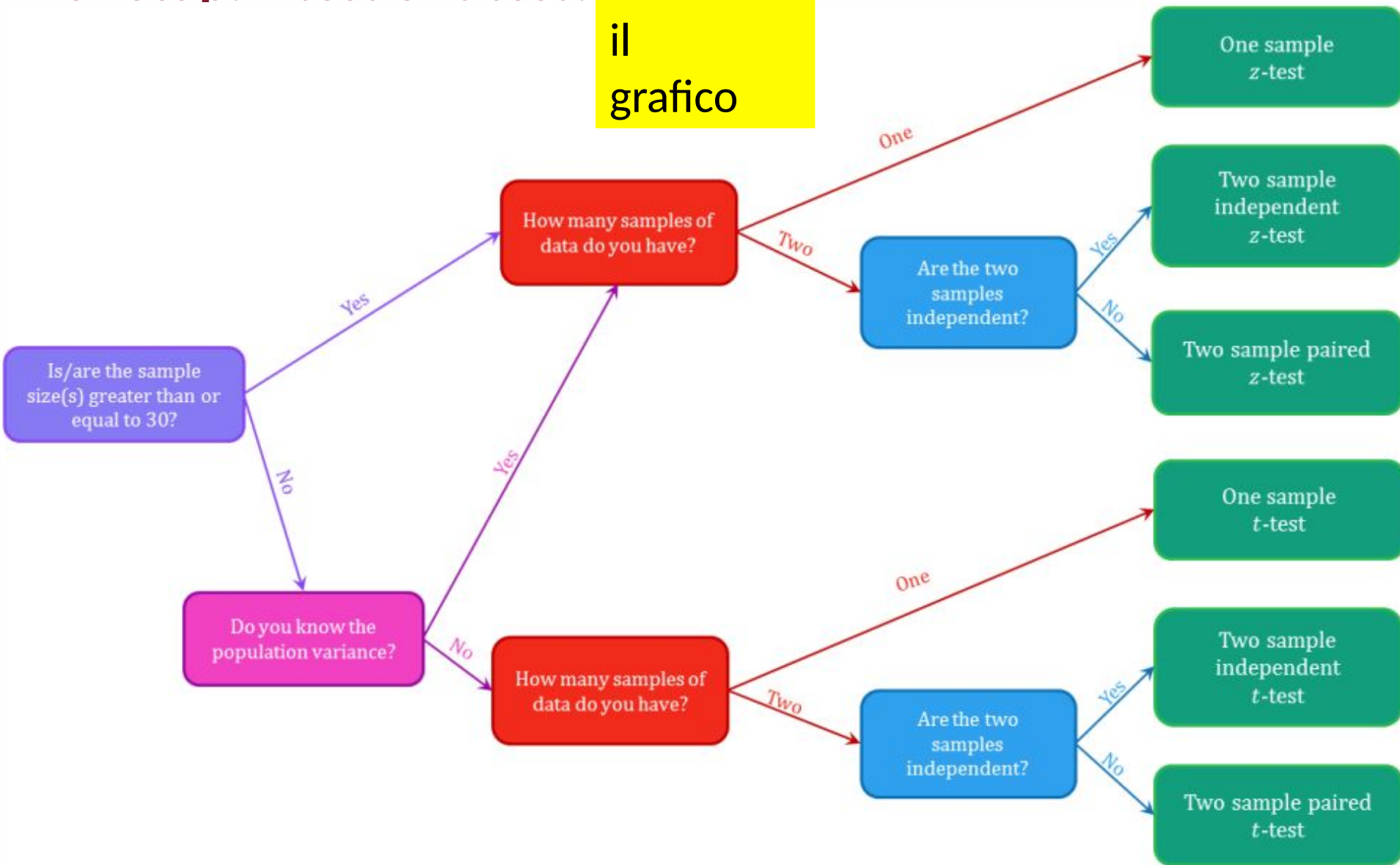
p-value = **0.48** (two-tailed)



DECISION: Since the obtained p-value is (much) higher than our significance level $\alpha = 0.05$, **we FAIL to reject the null hypothesis**. We have insufficient evidence proving the difference between the **general population mean TPC** and **mean TPC** of HF patients visited within 21 days is statistically significant.

To recap: Z test or t test?

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il
grafico



Source image: <https://www.ncl.ac.uk/webtemplate/ask-assets/external/maths-resources/business/hypothesis-tests/>

Comparing 2 sample means

EXAMPLE C: two samples independent t-test

What changes in two-sample tests type of problems?

1 sample

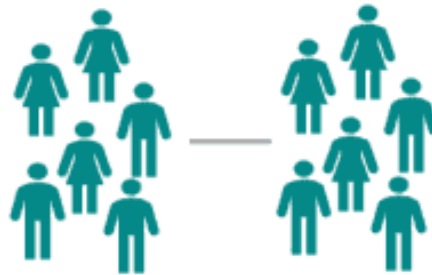
One sample t-Test



Is there a **difference** between a **group** and the **population**

2 samples

Independent samples t-Test



Is there a **difference** between **two groups**

Paired samples t-Test



Is there a **difference** in a **group** between **two points in time**

Independent samples: e.g. patients receiving treatment v. those receiving placebo drug

Dependent samples: e.g. same patients visited twice: before and after surgery

Source image: <https://datatab.net/tutorial/one-sample-t-test>

Example C (step 1): the research question and the test hypotheses

*[This time, I wonder if there a statistically significant difference between the **Total Platelet Count** in the patients who died and the patients who survived heart failure.]*

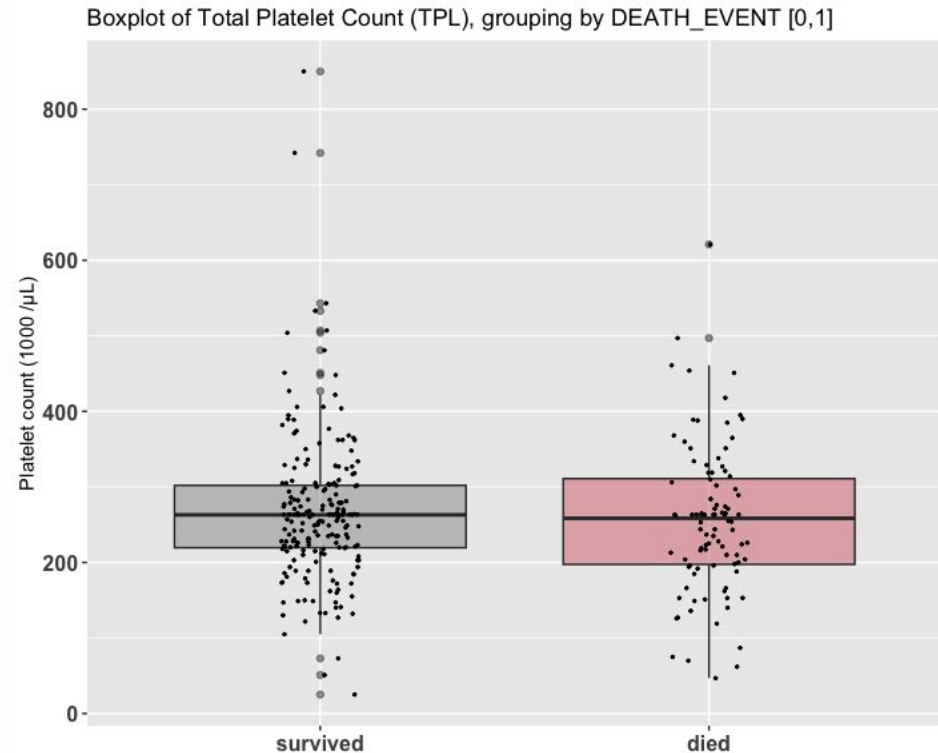
- GOAL: verify if there is a difference between means and
 - where: died = 1 and alive = 0
- QUESTION: Is there a statistically significant difference between the mean values of two groups?
- MORE FORMALLY:
 - : = The two population means are equal
 - : There is a mean difference between the two groups in the population. Possible directional difference formulation (two-tailed, left-tailed, right-tailed) :
 - : (the two population means are not equal)
 - : (population 1 mean is less than population 0 mean)
 - : (population 1 mean is greater than population 0 mean)

Example C (step 2): analyze sample data, test statistic

- ANALYSIS PLAN decisions:
 - Data → there is a categorical variable defining 2 groups (DEATH_EVENT = 1 or 0)
 - Test statistic → two samples' means comparison
 - Significance level → 0.05
 - Direction of equality → two-tailed (I don't have an expectation)
 - Test method → ??
- Verify the **ASSUMPTIONS** for independent (unpaired) t-test:
 1. The 2 samples ("died" and "survived") must be **independent***: i.e. a value in one sample must not influence a value in the other sample ✓
 2. The dependent variable is scaled in **intervals** (Total Platelets Count in) ✓
 3. The dependent variable is **normally distributed** (Total Platelets Count in) ✓
 - If the variables are not normally distributed, the **Mann-Whitney U test** can be used.
 4. The **variance within the two groups** should be similar (**F-test** or **Levene's test**, ...) ✓
 - If the variances are not equal you should instead perform **Welch's t-test** (the R default)
- Test method → **two sample independent t-test**

Example C (step 3): collect data about the two samples

- **Sample 1** (DEATH_EVENT = 1)
 - size = 96
 - mean TPC = 256
 - standard deviation = 9
- **Sample 2** (DEATH_EVENT = 0)
 - size = 203
 - mean TPC = 267
 - standard deviation = 97.5
- Also the last assumption (equal variances) is verified by the **F test of variance equality**
 - with and
 - and : =



F test statistic	df1	df2	p
1.0205	95	202	0.8915

Interpretation

H_0 is “equal variances”

p-value = 0.89 > 0.05 → FAIL to reject H_0

Equal variances between groups ✓

Example C (step 4): compute the test statistic for t-Test for independent samples

- Since we verified the required assumptions, the test method is the independent (two-sample) t-test
- The test statistic is computed with this equation, given:
 - the population standard deviation(s) are unknown, but we can assume = variances in 2 groups
 - large sample (+ > 100)
 - obtained as pooled estimate standard deviation of the sampling distribution of the difference

(corrected bias) or

where:

- and are the sample sizes,
- and are the sample means,
- and are the sample variances
- **df = 2** are the degrees of freedom

Results:

$$= -10.28 = [-34.23, +13.66]$$

p-value 0.3989

Example C (step 5): Interpret the results

RECAP:

An **independent samples t-test** was conducted to compare mean counts of platelets in patients with heart failure who died and patients with heart failure who survived.

RESULTS INTERPRETATION:

To make a statement about the results of the test (whether the of equal populations means holds or not) one of the following two values is used:

- p-value (2-tailed)
 - The **p-value 0.3989** correspondent to the test statistic with **2** degrees of freedom is MORE than our chosen significance level (0.05), so we CAN NOT reject the null hypothesis.
- lower and upper confidence interval of the difference
 - The magnitude of the differences in the means = **-10.28** falls inside the lower and upper bounds of the Confidence Interval = **[-34.23, +13.66]** consistent with the null hypothesis.

DECISION:

We do not have sufficient evidence to say that the mean counts of platelets in between these two populations is different.

Other similar cases we cannot review...

- one-sample Tests on proportions?
- two-sample Tests on proportions?
- two-sample Paired test mean?
 - we would need a dataset with before/after or other matched groups

Comparing sample means from 3 or more samples

EXAMPLE D: using ANOVA test

Extending comparison to 3 or more groups...

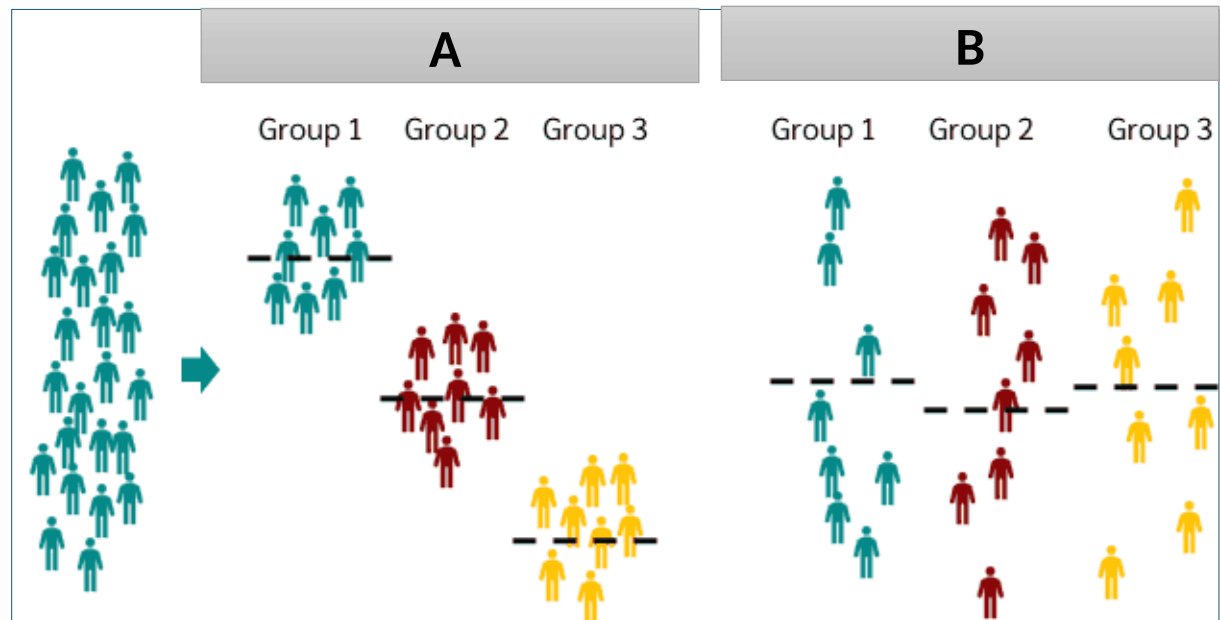
- **ANOVA** (“Analysis Of Variance”) is an extension of the previous hypothesis testing ideas, but examined how means of a variable differ across **three or more groups**
- For this purpose, the means and variances of the respective groups are compared with each other.
- While the **t-test** serves with a categorical explanatory variable that has two levels, (one-way) **ANOVA** looks at quantitative outcomes and a single categorical explanatory variable with any number of levels
- There are different types of ANOVA. The most used are:
 - ‘one-way’ ANOVA (or one-factor ANOVA) if there is only one explanatory variable (“treatment”) with two or more levels, and only one level of treatment is applied for a given subject (e.g. ethnicity)
 - ‘two-way’ ANOVA (or two-factor ANOVA), if the levels of two different explanatory variables are being assigned, and each subject is mapped to one level of each factor (e.g. ethnicity + treatment type)
- Another distinction refers to the selection of groups:
 - **with** repetition (as in the case the same person interviewed at several points in time)
 - **without** repetition (independent groups)

Example: an oncologist may be interested in knowing whether patients with different types of cancer have the same average survival times (‘one-way’ ANOVA) under several different competing cancer treatments (‘two-way’ ANOVA).

Formalizing One-Way ANOVA (“Analysis Of Variance”)

- The **dependent variable** is on a metric scale. In the case of the analysis of variance, the **independent variable (factor)** has at least three levels.
- **Assumptions** for the results of a one-way ANOVA to be valid:
 1. **Independence of observations** – The observations in each group are *independent* of each other and the observations within groups were obtained by a random sample.
 2. **Normally-distributed response variable** – The values of the dependent variable follow a normal distribution.
 3. **Homogeneity of variance** – The variances of the populations that the samples come from are equal.
- Key concept with ANOVA: **“within”** and **“between” variations** in the dependent variable values
 - **Total Variation = Within Variation + Between Variation**

- **Case A** (*small variance within the groups, large between the groups*)
- **Case B** (*large variance within the groups, small between the groups*)?



ANOVA: Research application

Population	Sample Size	Sample Mean	Sample Variance

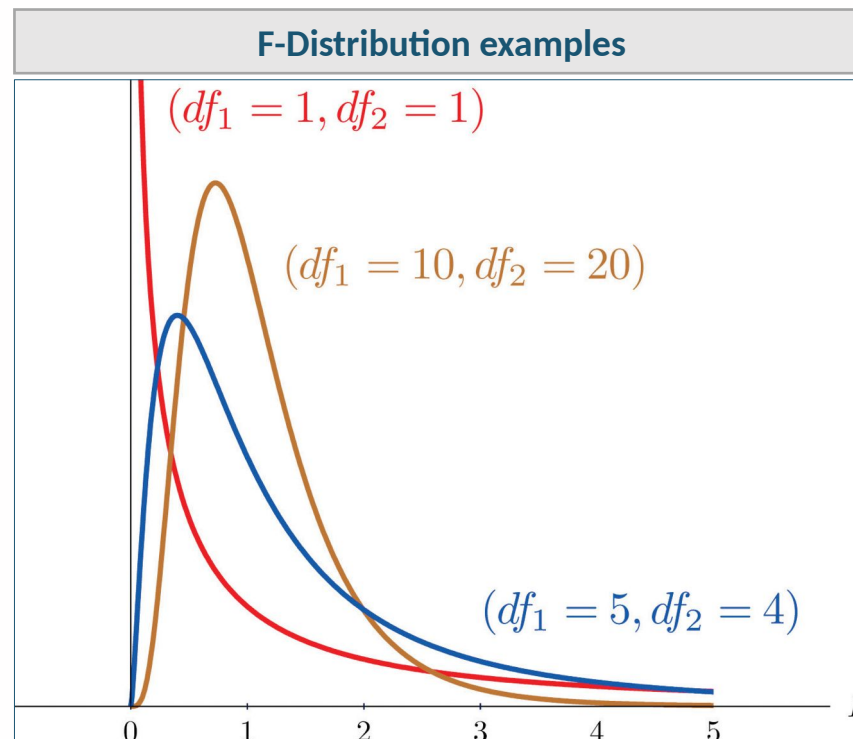
- MOTIVATION: In general, suppose there are K normal populations with possibly different means, $(\mu_1, \mu_2, \dots, \mu_K)$, but all with the same variance σ^2 . To perform the test, K independent random samples are taken from the populations to obtain K sample means.
- QUESTION: Is there a statistically significant difference between the mean values of the k populations?
- MORE FORMALLY:
 - $\mu_1 = \mu_2 = \dots = \mu_K$ all K population means are equal
 - $\mu_1 \neq \mu_2 \neq \dots \neq \mu_K$: not all K population means are equal
- To calculate the variation we use the “sum of squares”, like so:
 - “sum of squares BETWEEN groups”, with $df_1 = K - 1$
 - “sum of squares WITHIN groups”, with $df_2 = n - K$
 - “TOTAL sum of squares”, where
- where, \bar{x}_i = mean of each category, \bar{x} = the “grand mean” of the sample, n = number of observations in each group

Test statistic for ANOVA (“Analysis Of Variance”)

- For ANOVA, the test distribution we use is the **F-distribution**
 - The F test (like the t test) requires the **degrees of freedom** input
- The test is right-tailed: H_0 is rejected at level of significance α if \geq
- With:
with $df1=K-1$ and $df2=n-K$

Each F-distribution is specified by 2 *degrees of freedom* parameters denoted:

- $df1$ (numerator d.f.)
- $df2$ (denominator d.f.)



Example D (problem): research question and test hypotheses

- DATA: A research laboratory developed two treatments aimed at prolonging the survival times of patients with an acute form of thymic leukemia. To evaluate the potential treatment effects 33 laboratory mice with thymic leukemia were randomly divided into 3 groups.
 - The 1st group received Treatment 1, the 2nd groups received Treatment 2, and the 3rd group was observed as a control group (survival times of these mice are given below)
- RESEARCH QUESTION: Is there sufficient evidence to confirm the belief that at least one of the two treatments affects the average survival time of mice with thymic leukemia?

Mice survival times in days by group			
Treatment 1		Treatment 2	Control
71	75	77	81
72	73	67	79
75	72	79	73
80	65	78	71
60	63	81	75
65	69	72	84
63	64	71	77
78		84	67
71		91	

Source example data: https://saylordotorg.github.io/text_introductory-statistics/s15-04-f-tests-in-one-way-anova.html

Example D (step 1): analysis plan and hypotheses

- GOAL: Test, at the 1% level of significance, whether the differences between the samples are large enough to reject the null hypotheses and justify the conclusion that *the populations represented by the samples* are different
- Verify the ASSUMPTIONS for One-way ANOVA
 1. Independence of observations ✓ (assignment to groups was done randomly)
 2. Normally-distributed response variable ✓
 3. Homogeneity of variance ✓
 - (more on this in the practice session)
- ANALYSIS PLAN decisions:
 - Data → there is a categorical variable defining 3 groups
 - Test statistic → F distribution
 - Significance level → 0.01
 - Direction of equality → The test is right-tailed: is rejected at level of significance α if \geq
- HYPOTHESES formalization:
 - : $=$ all K population means are equal
 - : at least one population mean is different from the rest

with $df1=K-1$ and $df2=n-K$

Example D (step 2): compute the test statistic for H_0 (i.e. K Population Means Are Equal)

- Experiment set up:
 - $n = 33$, $K = 3$, so that degrees of freedom $df1 = K - 1 = 2$ and $df2 = n - K = 30$
- with these samples' statistics:

Groups	Sample Size	Sample Mean	Sample Variance
	= 16	= 69.75	
	= 9	= 77.78	
	= 8		

- The overall sample mean (all 33 observations) is = 73.42
- We compute Means Square Between and Means Square Within:

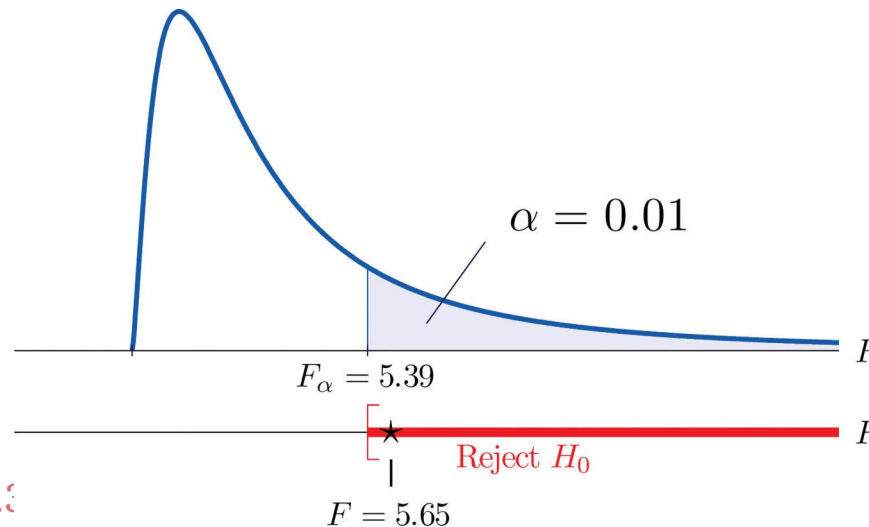
$$= = = 217.31$$

$$= = = 38.45$$

- so that:

Example D (step 3): interpret the results

- The obtained test statistic is:
- The test is right-tailed. The single critical value is **5.39**, thus the rejection region is **$[5.39, \infty)$**



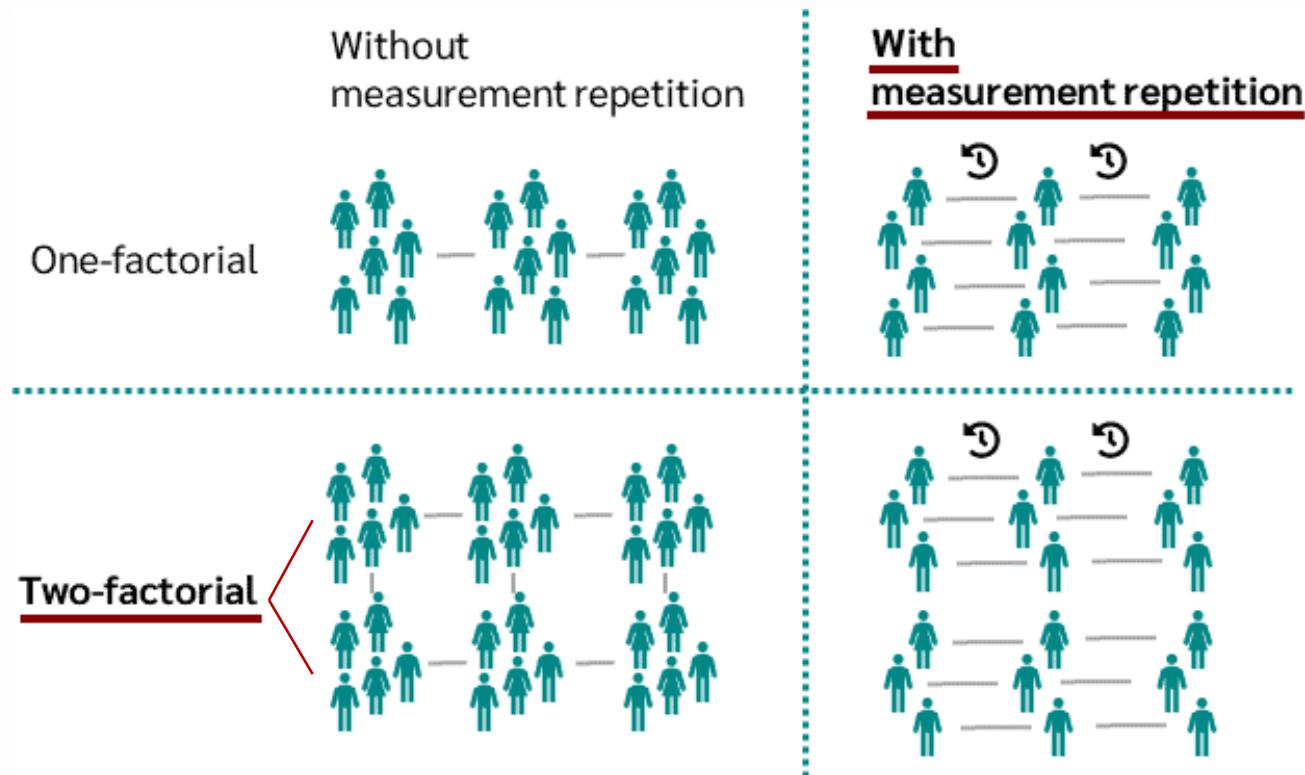
DECISION: Since $5.65 > 5.39$

The data provide sufficient evidence, at the 1% level of significance, to conclude that **a treatment effect exists at least for one of the two treatments** in increasing the mean survival time of mice with thymic leukemia.

NOTE: ANOVA does **NOT** specify *which* population means are different. To determine this, you need to perform **post hoc tests**, also known as “multiple comparisons” tests.

Other types of ANOVA...

- Depending on:
 - how many explanatory variables (“categories”) we consider
 - if samples are assigned with measurement repetition (within-subjects factor) or without measurement repetition (between-subjects factor)



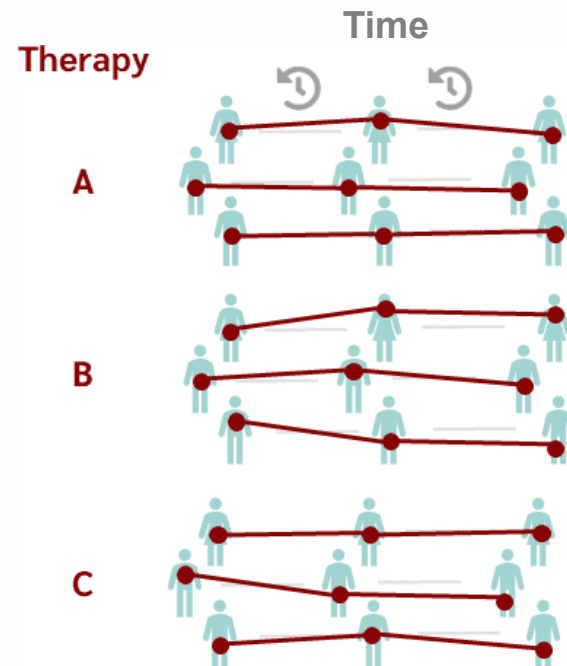
Source image: <https://datatab.net/tutorial/two-factorial-anova-with-repeated-measures>

Two-way, repeated measures ANOVA

- A **repeated measures ANOVA** is used to compare mean scores across multiple observations of the same subjects (**dependency**). It is typically used in two specific situations:
 - Measuring the mean scores of subjects during **three or more time points**
 - Measuring the mean scores of subjects under **three or more different conditions**.
- **Two-factor ANOVA** allows to decompose the total dispersion of the data into four components:
 1. the share attributable to the 1st factor
 2. the share attributable to the 2nd factor
 3. the share attributable to the **interaction** between 1st factor and 2nd factor
 4. the unexplained, or residual portion

EXAMPLE two-factorial ANOVA with repeated measures

Taking samples of people with high blood pressure for each treatment (1st factor = treatment) & repeat their blood pressure measurement over 3 points in time: before, during and after the treatment (2nd factor = time)



Repeated measures ANOVA: applications

- In real life there are two benefits of using the same subjects across multiple treatment conditions:
 1. It's **cheaper and faster** for researchers to recruit and pay a smaller number of people to carry out an experiment since they can just obtain data from the same people multiple times
 2. We are able to attribute some of the variance in the data to the subjects themselves, which makes **it easier to obtain a smaller p-value** («**control of confounders**»)
- One potential drawback of experimental design is that subjects might get bored or tired if an experiment lasts too long («**attrition**»), which could skew the results.

DAY 2 – LECTURE OUTLINE

- Purpose and foundations of inferential statistics
 - Probability and random variables
 - Meaningful probability distributions
 - Sampling distributions and Central Limit Theorem
 - Confidence Intervals
- Getting to know the “language” of hypothesis testing
 - The null and alternative hypothesis
 - The probability of error? (α or “significance level”)
 - The p-value probability and tests interpretation
 - Effective vs statistical significance
 - Types of errors (Type 1 and Type 2)
- Hypothesis tests **examples**
 - Comparing sample mean to a hypothesized population mean (Z test & t test)
 - Comparing two independent sample means (t test)
 - Comparing sample means from 3 or more samples (ANOVA)
- A closer look at testing assumptions (with **examples**)
 - Testing two groups that are NOT independent
 - Testing if the data are not normally distributed: non-parametric tests
 - Testing samples without homogeneous variance of observations

What if tests assumptions do not hold?

What if the assumptions did not hold?

Revisit the **ASSUMPTIONS** we verified for independent (unpaired) t-test:

1. The **response variable must be expressed through an interval and ratio scale** (quantitative variable → continuous scale)
2. What if the **2 groups/samples are NOT independent** (e.g. taken via before & after surveys)?
 - We use the dependent sample t-test (**or paired t-test**)

with **$df=n-1$**

3. What if **dependent variable is NOT normally distributed**?
 - The normality assumption is more important for small sample sizes than for larger sample sizes (but if it is hard to verify, we rely on our domain knowledge)* we use (**nonparametric tests** that doesn't assume normality)
 - For **INDEPENDENT SAMPLES** we can perform **Mann-Whitney U test** (or **Wilcoxon rank-sum test** as in R) -- best for continuous variables
 - For **PAIRED SAMPLES** we can perform **Wilcoxon signed-rank test** -- OK with ordinal variables
4. What if **variance within the two groups is NOT similar**? (F-test, Levene's test,...)
 - We can perform **Welch's t-test** (the R default)

[We will learn the R code in the PRACTICE SESSION]

UNMET ASSUMPTION II)

The two groups are not independent: paired t-tests

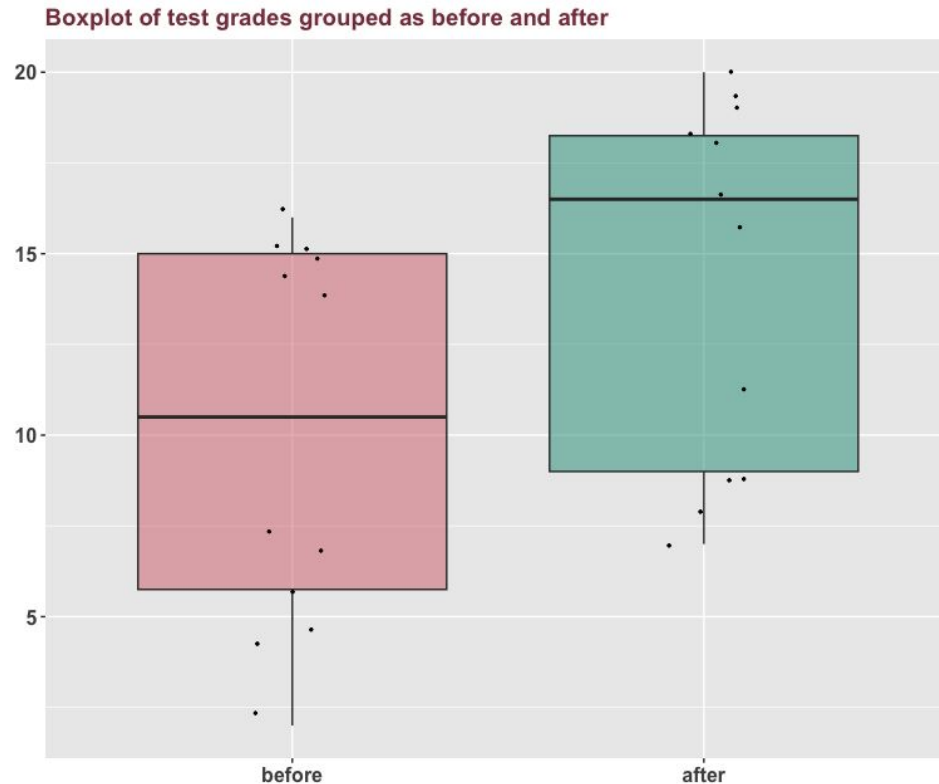
EXAMPLE E: using Wilcoxon signed-rank test

Example E (step 1): Independence of observations

- The samples subjected to the different treatments should be generated by **random selection** from the target population
- The test of sequences for a sample or for alternative answers (Run test) is used in the case of observations collected in a temporal succession or in a geographical succession, starting from an origin point
- It allows you to test whether, in reference to the order, sequence, succession (run) or series (synonymous terms), the sample data is random

Example E (step 2): Question: Is the difference between two **PAIRED** samples statistically significant?

- Let's imagine a statistics test is administered to the *same* group of 12 students **before and after** attending a workshop 😊
- Given the assumptions:
 - independence** ✗ (observations paired by design)
 - normality** ✗
 - small** sample
- We use the **Wilcoxon test for paired samples**, with the following hypotheses:
 - : grades before and after the workshop are equal
 - : grades before and after the workshop are different



Example E (step 3): Interpret the results

- With the non parametric **Wilcoxon test for paired samples**, we have obtained the test statistic $V = 21$, with $p\text{-value} = 0.1692$, where $p\text{-value} > 0.05$
- Therefore, at the 5% significance level, **we do not reject the null hypothesis** that the grades are similar before and after the wokshop 😓.

UNMET ASSUMPTION III)

If the data are not normally distributed: non-parametric tests

EXAMPLE F: using Wilcoxon Rank Sum Test

Example F (step 1): Compare two independent sample when the data are not normally distributed

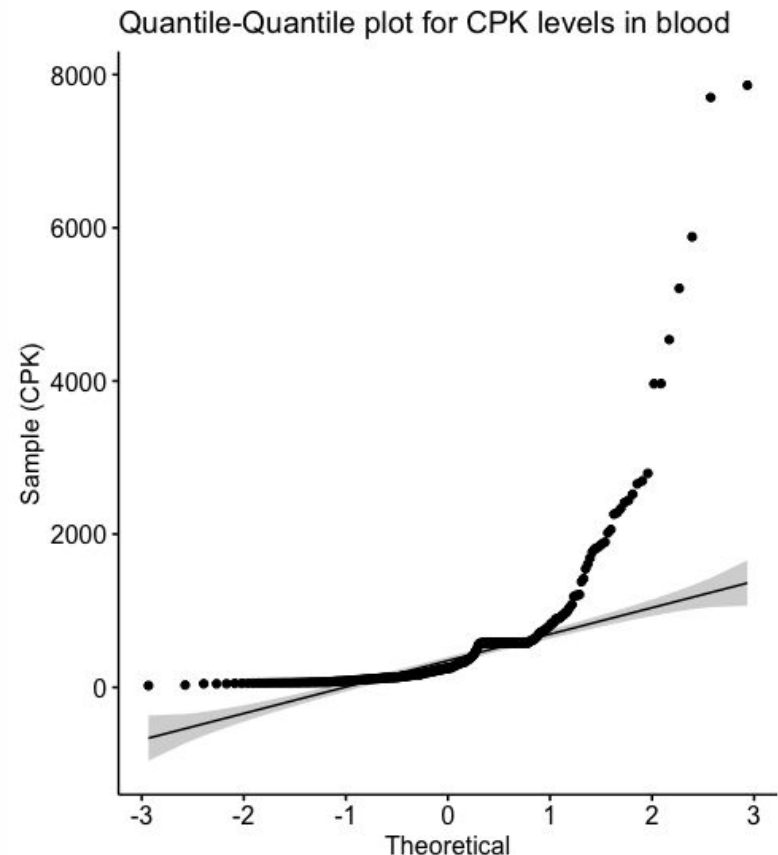
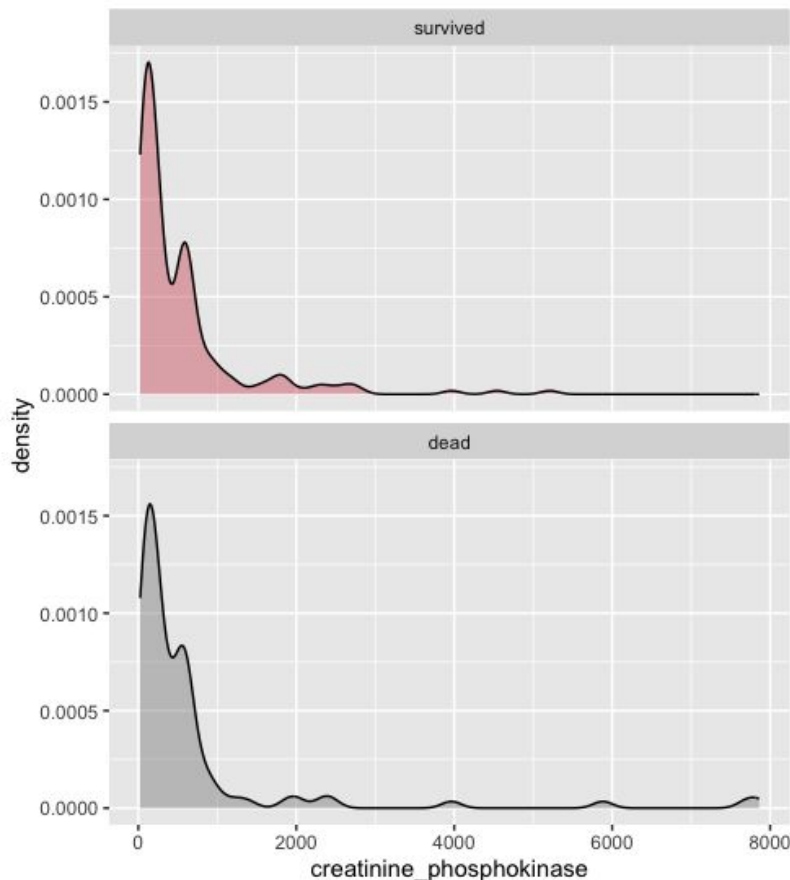
[Let's go back to the HEART FAILURE dataset but looking at the levels of Creatinine Phosphokinase (CPK) in the blood, an enzyme that might indicate a heart failure or injury]

- GOAL: verify if the difference in **CPK levels in the blood** of the survivors versus those who died after heart failure is statistically significant or only due to sampling error.
- From the sample I get:
 - the general sample mean (with)
 - sample mean for group of survived patients (with)
 - sample mean for group of dead patients (with)
- MORE FORMALLY: I want to run a test to verify whether my sample' statistics represent an actual difference in the respective hypothetical populations () or if there is no difference between the two hypothetical populations ()
 - : there is no difference in mean CPK between patients who suffered heart failure and died versus patients who survived after heart failure
= → hypothesis of no effect or (**“no difference”**)
 - : there is a difference in mean CPK between patients who suffered heart failure and died versus patients who survived after heart failure (**“some effect”**).
(two-sided test) or

Example F (preliminary check): visually check the “normality” assumption for parametric testing

We can graphically confirm that CPK is **not normally distributed** by using:

1. the **density plot**, in which we can see the distribution is not bell shaped
2. the **QQ plot** (or Quantile-Quantile plot) for large samples – data points should roughly fall along a straight diagonal line when the dataset follows a normal distribution.



Example F (preliminary check): confirming a “normality” assumption violation with tests

- We can confirm that CPK is **not normally distributed** by using tests for normality:
 1. **Shapiro-Wilk test**
 2. **Kolmogorov-Smirnov test**
- The null hypotheses are defined as:
 - H_0 : The mean value of specific feature is equal regardless if patients died or survived in given period
 - H_1 : The mean value of specific feature is not equal in the two populations of patients who died survived in given period
- The test has been run over each group:
 - Evidently, we reject the H_0 !

Shapiro-Wilk Normality Test results			
variable	DEATH EVENT	statistic	p
creatinine	survived	0.6277141	0.000000000000000000008509006
phosphokinase	died	0.4392427	0.000000000000000000019922527779

Example F (step 2): compare independent samples using a non parametric test

Cocca , in Realta R non lascia fare il Mann-Whitney U test ma l'altro (che e' equivalente.....) dipende dal software che usi

- Since we verified that the explained variable (CPK) is **NOT normally distributed**, we run a **Wilcoxon Rank Sum test** (equivalent to the **Mann-Whitney U test**) to compare independent samples
 - It is considered to be the nonparametric equivalent to the two-sample independent t-test
- ASSUMPTIONS :
 - **Ordinal or Continuous dependent variable**: the variable you're analyzing, e.g. CPK levels ✓
 - **Independence**: All of the observations from both groups are independent of each other ✓
 - **Shape**: The shapes of the distributions for the two groups are roughly the same ✓

Wilcoxon Rank Sum Test (two-sided alternative)	
DATA	creatinine_phosphokinase by DEATH_EVENT_f
Statistic	W = 9460
	p-value = 0.684

RESULTS: since the test statistic is $W = 9460$ and the corresponding p-value is $0.684 > 0.05$, we fail to reject the null hypothesis.

We do not have sufficient evidence to say that CPK levels for dead patients is less than that of survived patients ()

UNMET ASSUMPTION IV)

The variances of the two groups are not homogeneous

EXAMPLE G: using t test with the Welch correction

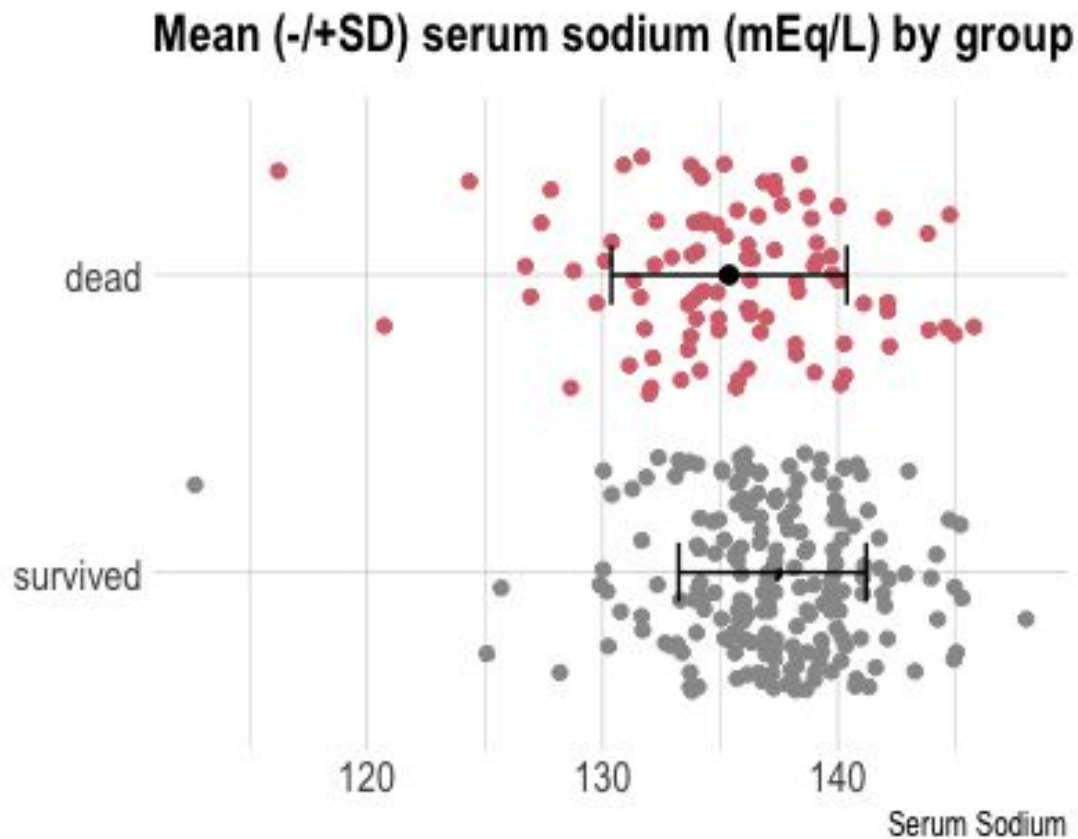
Example G (step 1): Compare two independent sample when the variance is not homogeneous

[Once again we use *HEART FAILURE dataset* but look at the levels of *Serum sodium* in the blood, a mineral that serves for the correct functioning of muscles and nerves]

- GOAL: verify if the difference in **Serum sodium levels in the blood** of the survivors versus those who died after heart failure is statistically significant or only due to sampling error.
- In from the sample I get:
 - milliequivalents per liter (mEq/L) the general sample mean (with)
 - sample mean for group of survived patients (with)
 - sample mean for group of dead patients (with)
- MORE FORMALLY: I want to run a test to verify whether my sample' statistics represent an actual difference in the respective hypothetical populations () or if there is no difference between the two hypothetical populations ()
 - : there is no difference in mean CPK between patients who suffered heart failure and died versus patients who survived after heart failure
= → hypothesis of no effect or (“no difference”)
 - : there is a difference in mean CPK between patients who suffered heart failure and died versus patients who survived after heart failure (“some effect”).
(two-sided test) or

Example G (preliminary check): visually check the “HOV” assumption for parametric testing

- Once again, plotting the data offers a graphical intuition that the variance of observations in the two groups seem not homogenous
 - recall that



Example G (preliminary check): confirming a “HOV” assumption violation with tests

- It is always best to use an actual test to confirm this intuition. So in this case we can use the **Fisher's F test** to verify equal variances of Serum Sodium concentration in the two groups
- The null hypotheses are defined as:
 - H_0 : → (The true ratio of variances is equal to 1)
 - H_1 : → (The true ratio of variances *not* equal to 1)

F test to compare two variances			
variable	groups	statistic	p-value
Serum Sodium	survived	F = 1.5769, num df = 95, denom df = 202	p-value = 0.007646
	died		

- I use the P-value as a decision rule, which leads me to **reject the null hypothesis** since it is lower than 0.05 conventional alpha level

Example G (step 2): compare independent samples using a specification of the t test

- Since we verified that the explained variable (Serum Sodium in the blood) is **NOT homogeneous in variance**, we will run a **t test with the Welch correction** to compare two independent samples
 - Unequal variance is compensated by lowering the degrees of freedom

Welch Two Sample t-test	
DATA	serum_sodium by DEATH_EVENT_f
Statistic	t = -3.1645 df = 154.01
	p-value = 0.001872

RESULTS: since the test statistic is $t = -3.1645$ (with $df = 154.01$) and the corresponding p-value is $0.001872 < 0.05$, we reject the null hypothesis.

We therefore have sufficient evidence to say that the level of serum sodium levels for dead patients is significantly different than that of survived patients

Classification of Hypothesis Test

Type of Test	Level of Measure	Sample characteristics					Correlation
		One Sample	Two Samples		K samples (i.e. >2)		
			Independent	Dependent	Independent	Dependent	
Parametric Test	Interval or Ratio	Z-test or t test	Independent sample t-test	Paired sample t-test	One way ANOVA	Repeated measure ANOVA	Pearson's r test
Non-Parametric Test	Categorical or nominal	Chi square test	Chi square test	McNemar test	Chi-square test	Cochran's Q Test	Spearman (ρ) test
	Rank or ordinal	Chi square test	Mann-Whitney u-test	Wilcoxon signed-rank test	Kruskal Wallis test	Friedman's test	