Module 1AB

Reviewing Hypothesis Testing (Biostatistics I)

Applied Epistemology:

A Framework for how we know things scientifically.

A Refresher on Module 3 (Biostats I): Hypothesis Testing

Agenda:

1. H_O/H_A: Our model of the test universe (the distribution of the variable)
2. Test & assumptions: are the assumptions met? Is the test valid?
3. Quantitative evidence: **p-value**, or critical value.
   - False positive =Type I (α), False Negative = Type II (β), Type III errors
   - Sensitivity, Specificity, Power → confusion matrix, ROC/AUC curve
   - Confusion Matrix
4. Conclusion & uncertainty/estimation

Perspectivism: https://hdsr.mitpress.mit.edu/pub/qasl4fza/release/2

What is “Statistical Thinking”?

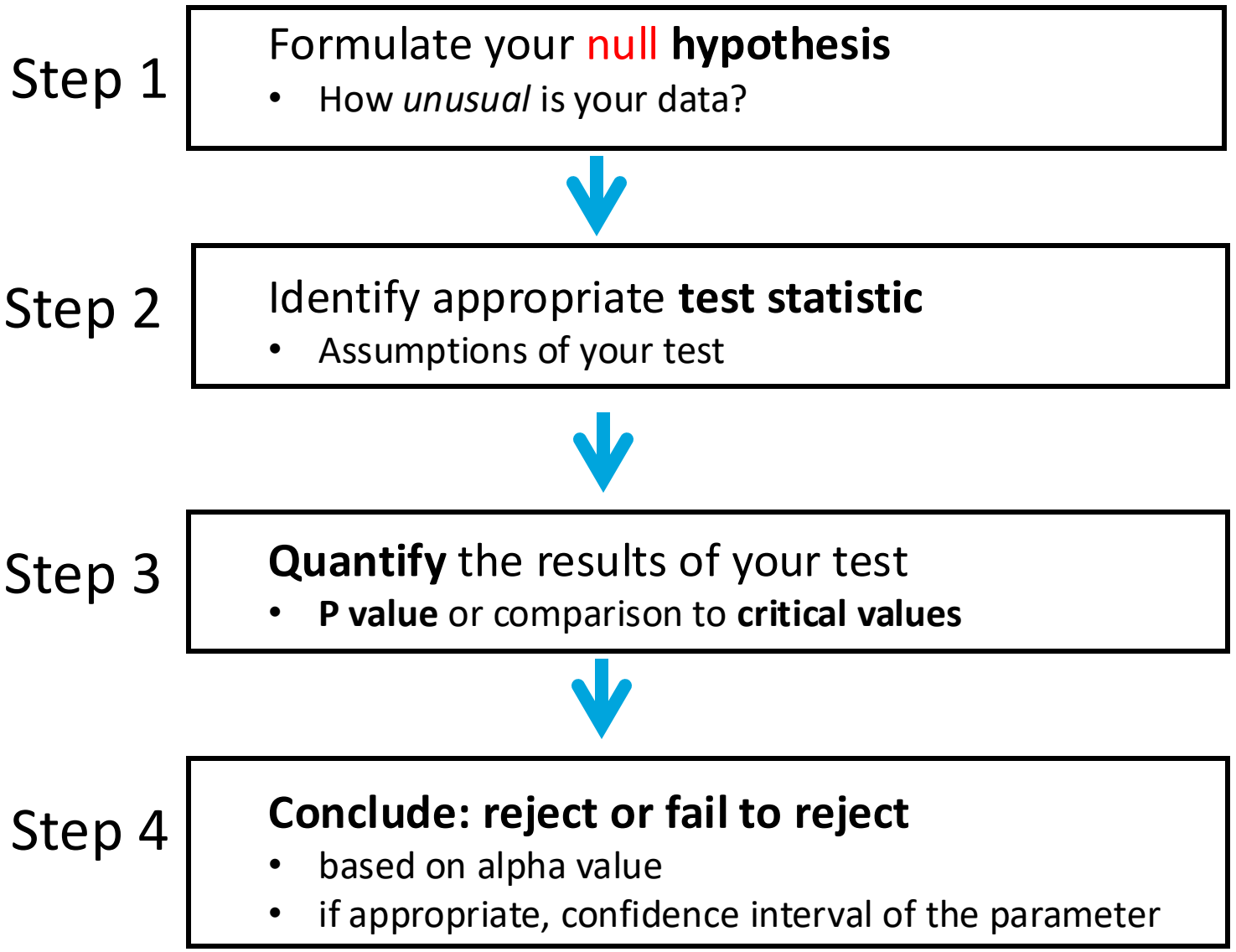
Understanding complexity via:

- Understand Distributions;
- Models and their assumptions;
- Quantify uncertainty;
- Think in probabilities;
- Utilize systematic criteria – that is: automate - for decision making.

Retraining our brains to not rely on heuristics/shortcuts and bias.

- *We see patterns (i.e., faces) when there are none. Funny examples: https://www.boredpanda.com/objects-with-faces/*

Your pipeline for hypothesis testing in statistics



- Most of the work involved in statistics is clearly stating your null hypothesis

What is your expectation? Can you quantify it? What is the sampling distribution?

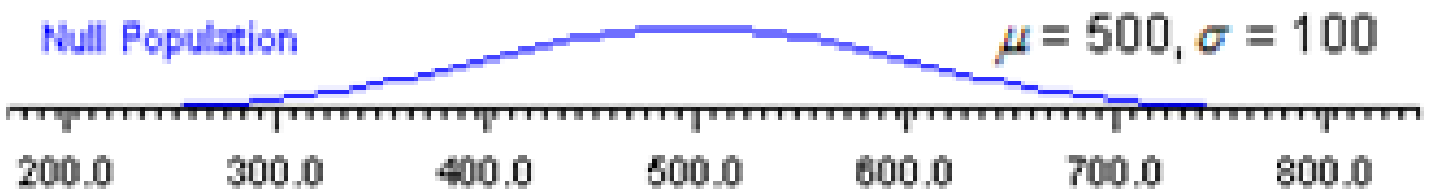
- Hypothesis testing allows you to ask if a parameter ***significantly*** differs from the ***null*** expectation

It quantifies how unusual the data are *if you assume that the null hypothesis is true.*

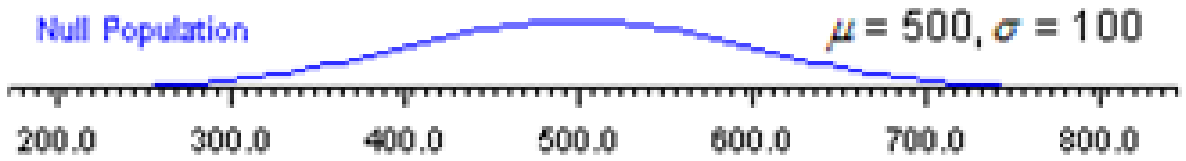
- Hypotheses are about populations but are tested with data from samples

Assumes that the sampling is random & the sampling distribution follows a normal distribution (Thanks, Central Limit Theorem)

Example: The Verbal Ability and Skills Test (VAST) is a standardized test with a range of **0** to **1000**. VAST scores are normally distributed within the population of test takers with a mean score, ***m*,** of **500** with a standard deviation, ***s*,** of **100**. The blue curve shows the distribution of this population of scores.



The ACE training program boasts that their graduates score higher on average than the population of individuals who do not participate in a training course (the null population). That is, ACE claims test takers who first complete the ACE training program on average score higher than 500 on the VAST.

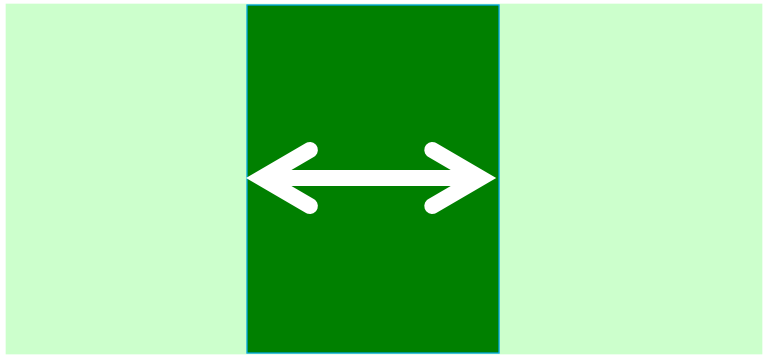


That is, ACE claims test takers who first complete the ACE training program on average score higher than 500 on the VAST.

Step 1: Constructing the null hypothesis

Formulate Hypothesis

- Quantifies how unusual data is *if you assume that the null hypothesis is true*
- H_0 and H_A - mutually exclusive



What is the best Null Hypothesis to test this claim?

A. Ho: ACE mean ≥ 500
B. Ho: ACE mean $= 500$
C. Ho: ACE mean ≤ 500
D. Ho: ACE mean $\neq 500$

Step 1: Making and using hypotheses:

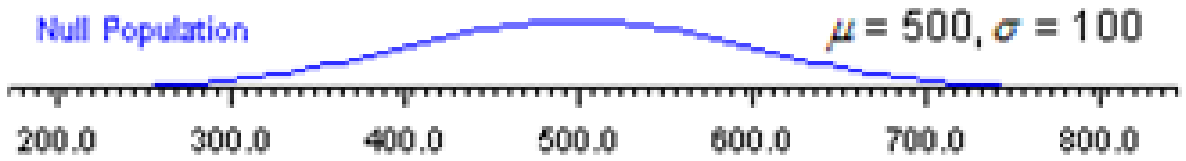
The Null Hypothesis (H_0):

A specific statement about a population parameter made for the purpose of the argument. Usually carefully worded so that it can be rejected (falsified).

The Alternate Hypothesis (H_A):

Represents all other possible parameter values except that stated in H_0. It is often what the researcher hopes is true and remains after the Ho has been rejected.

Falsification of the null hypothesis (Karl Popper, 1959), discussion here: https://pubmed.ncbi.nlm.nih.gov/23249368/

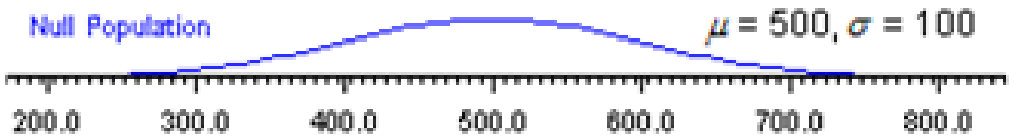


That is, ACE claims test takers who first complete the ACE training program on average score higher than 500 on the VAST.

Other concepts to review:

A. Would you be convinced that the average score for ACE graduates is greater than 500 if you were told that one randomly selected ACE graduate had a score of 550? (What is the probability that a randomly sampled score from the normally distributed population is 550 or greater, given that the population mean is 500 and the standard deviation is 100?)

B. Would it be more convincing if 25 randomly selected ACE graduates had an average score of 550? Why?



A random sample of 30 ACE graduates yields a sample mean of 510 (i.e., $X = 510$) and sd of 90.

Step 2: Identify the test statistic

- We can use a Z-score. (5B, Biostats I)
- Assumptions: Random sample, Normal Distribution
- Mechanics:
- Z = 510 – 500 = 0.609

  16.43

$$Z = \frac{\bar{Y} - \mu}{\sigma_{\bar{Y}}} \qquad \sigma_{\bar{Y}} = \frac{\sigma}{\sqrt{n}}$$

Step 2: Identify a Test Statistic:

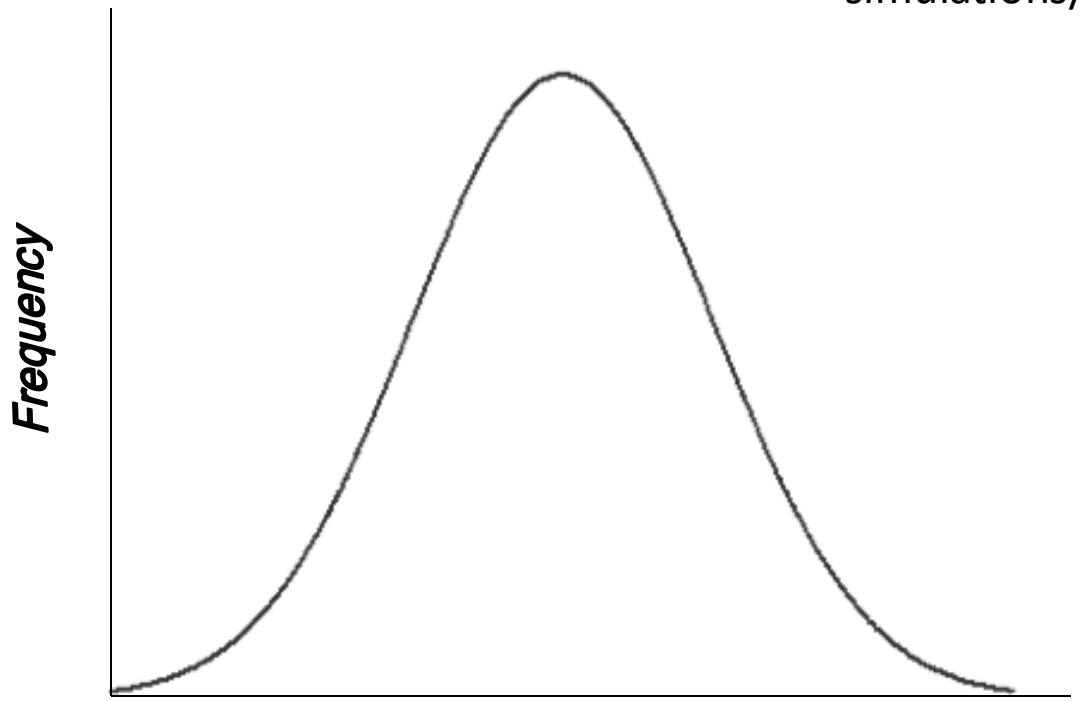
Quantity calculated from the data that is used to evaluate how compatible the results are with those expected the null hypothesis.

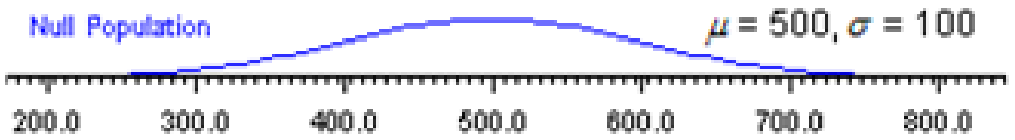
- How 'weird' are your results?
- Do your data support the assumptions of your test statistic?

Null Sampling Distribution:

Probability of the test statistic assuming the null hypothesis

- Usually assume Normal Distribution (for means, we can usually rely on CTL!)
- Null distribution can be acquired via computer simulations/modeling





A random sample of 30 ACE graduates yields a sample mean of 510 (i.e., $X = 510$) and sd of 90.

Z = 0.609

Step 3: Quantify results

Hypothesis testing automates binary decision making:

1. If p-value < α
   - Reject null hypothesis

2. If p-value > α
   - Fail to reject null hypothesis

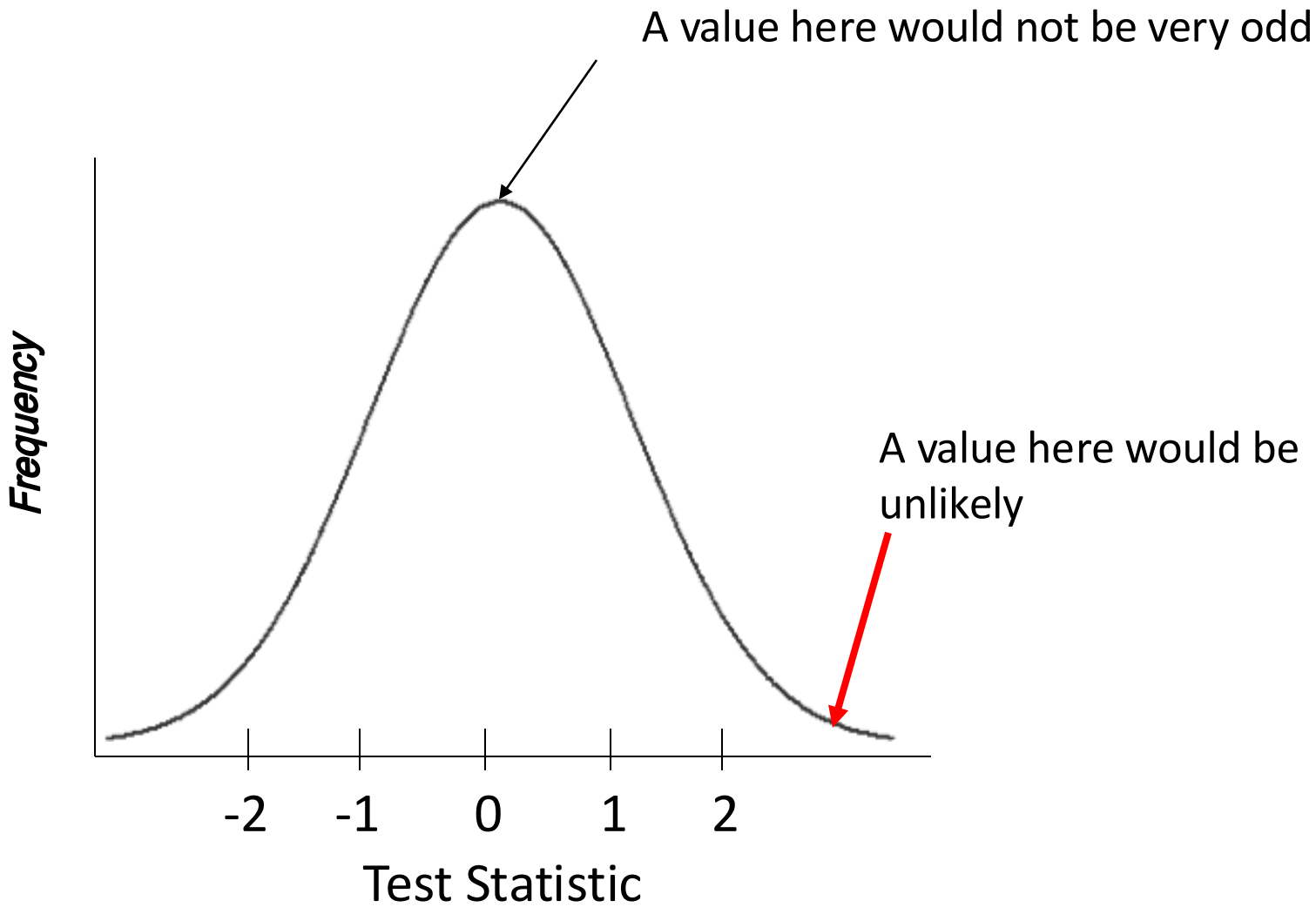
P-Value:

Probability of obtaining data that are equal to or even more extreme than the value assuming the null hypothesis is true

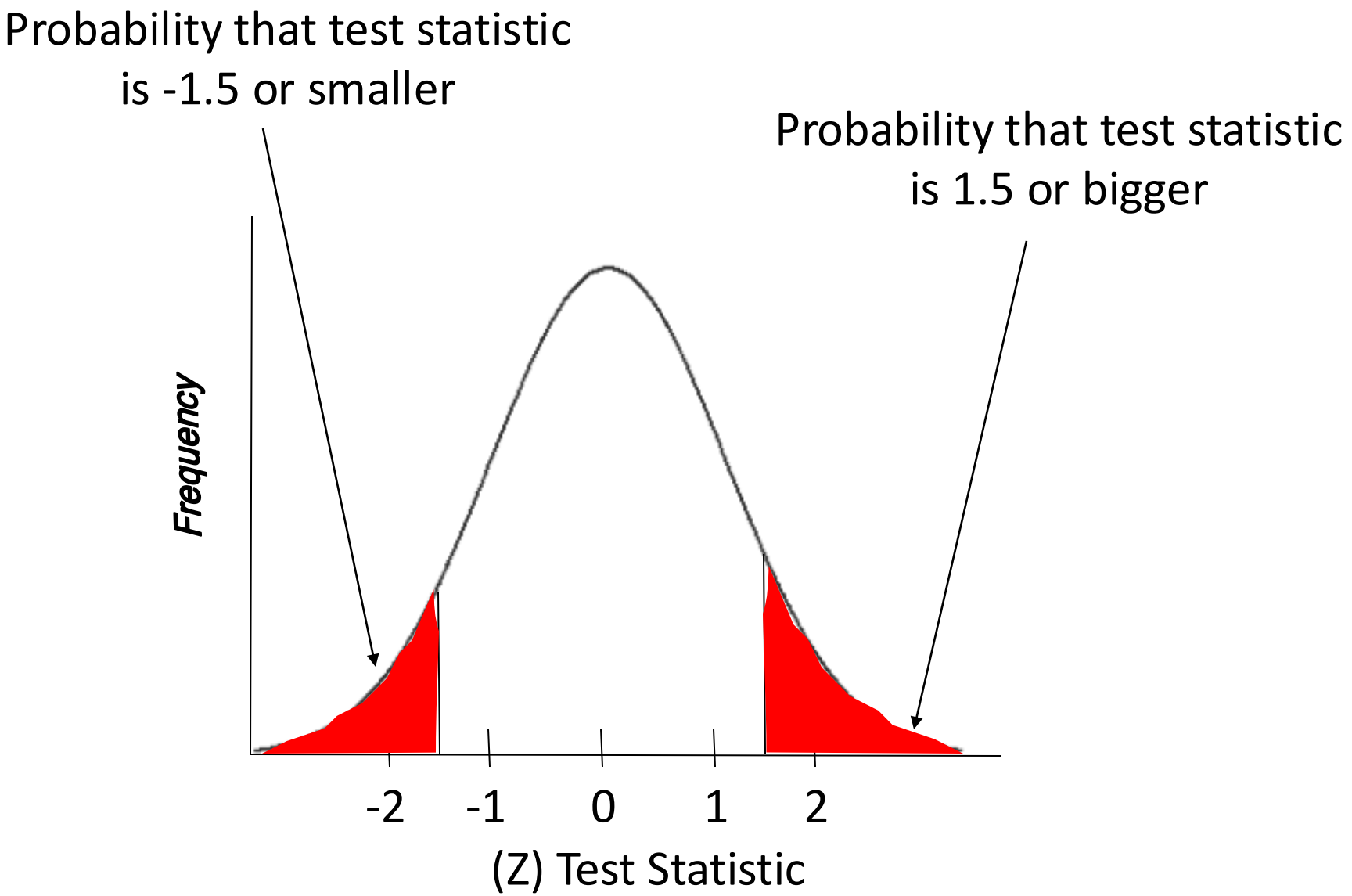
α (significance level):

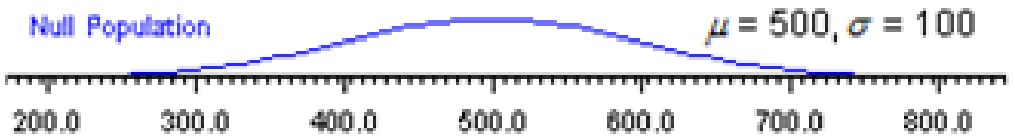
Decided *before* experiment.

Conventional values: α = 0.05 or α = 0.01.



P-value


Probability that test statistic
is -1.5 or smaller
Probability that test statistic
is 1.5 or bigger
Frequency
-2
-1
0
1
2
(Z) Test Statistic



A random sample of 30 ACE graduates yields a sample mean of 510 (i.e., $X = 510$) and sd of 90. **Z = 0.609**

Step 3: Quantify results

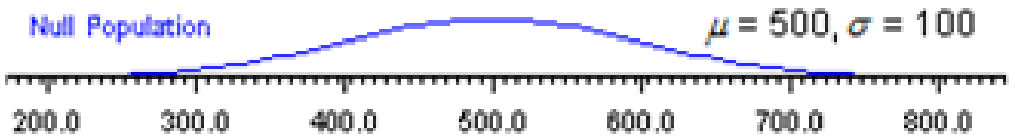
- https://z-table.com/
- We won't calculate a p-value directly, but we can use cut-off values (critical values) that correspond to levels of α. The critical value is defined on the sampling distribution for means of samples of size *N* drawn from the null distribution, where the probability of obtaining a sample mean as large as or larger than the critical value is equal to a pre-defined alpha.
- **P(X > Z) = 0.2451**
- We don't double this, because this is a one-sided test (we would double it for a two-sided test, most will be)
- **0.2451 > 0.05**

We can compute a critical value:

1.Identify the *p*-value for the critical value. Here the *p*-value is .05 on the upper tail.

2.Convert the *p*-value to a *z*-score. Here the *p*–*z* converter (see table) tells us that a *z*-score of 1.645 cuts off .05 on the upper tail.

3. Z=0.609 is << 1.645 (and the probability of 0.2451 is >> 0.05)



A random sample of 30 ACE graduates yields a sample mean of 510 (i.e., $X = 510$) and sd of 90. **Z = 0.609**

Step 4: Quantify results

- **Probability > α** so we Fail-to-reject the null hypothesis
- You will also want to put a confidence interval on your results (we will see this in Module 2 when we introduce the t test).

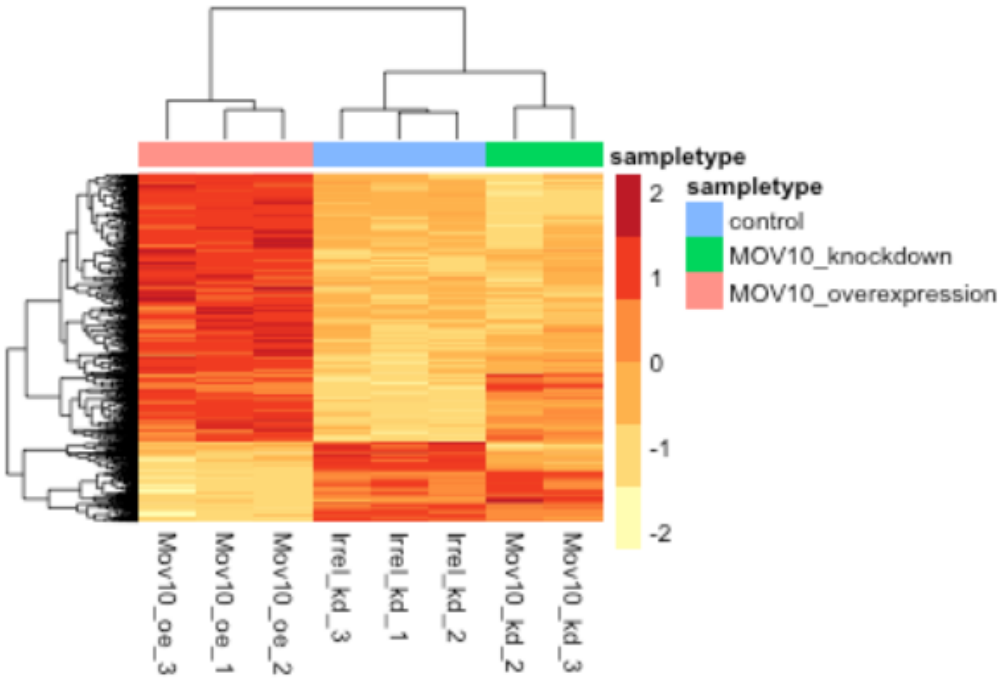
RNA-seq uses Z-scores to create heatmaps:

- In RNA-seq analysis, Z-scores are used to compare expression levels between samples. The Z-score of a gene is calculated by comparing its expression level in a given sample to the expression level of that gene across all samples.
  - A Z-score of **zero** indicates that the gene's expression level is the same as the mean expression level across all samples, while a positive Z-score indicates that the gene is expressed at a higher level than the mean, and a negative Z-score indicates that the gene is expressed at a lower level than the mean.
  - Z-scores are computed on a gene-by-gene (row-by-row) basis by subtracting the mean and then dividing by the standard deviation. It is used as scaling.

- Once Z-scores are calculated, they can be used to identify differentially expressed genes.
  - genes with a Z-score greater than a certain threshold (such as 2 or 3) can be considered as differentially expressed.
  - Z-scores can be used to create a heatmap or volcano plot, which can be a valuable way to visualize the data and identify patterns of expression

Heatmap

In addition to plotting subsets, we could also extract the normalized values of *all* the significant genes and plot a heatmap of their expression using `pheatmap()`.



In this heatmap Z-scores are calculated for each row (each gene) and these are plotted instead of the normalized expression values; this ensures that the expression patterns/trends that we want to visualize are not overwhelmed by the expression values.

> *Z-scores are computed on a gene-by-gene basis by subtracting the mean and then dividing by the standard deviation. The Z-scores are computed **after the clustering**, so that it only affects the graphical aesthetics and the color visualization is improved.*

Which statement(s) is true about p-values?

---

a. p-value is the probability that the null hypothesis is true or false

b. p-value reflects the weight of evidence against the null hypothesis

c. p-value measures the size of the effect

d. if p value is less than or equal to the significance level, then the null hypothesis is not rejected.

Errors in hypothesis testing:

Type I (α) = False Positive=0.05

P[type I]=P[rejecting Ho|Ho is true]

Type II (β) = False Negative

P[type II]=P[Fail-to-reject Ho|Ho is not true]

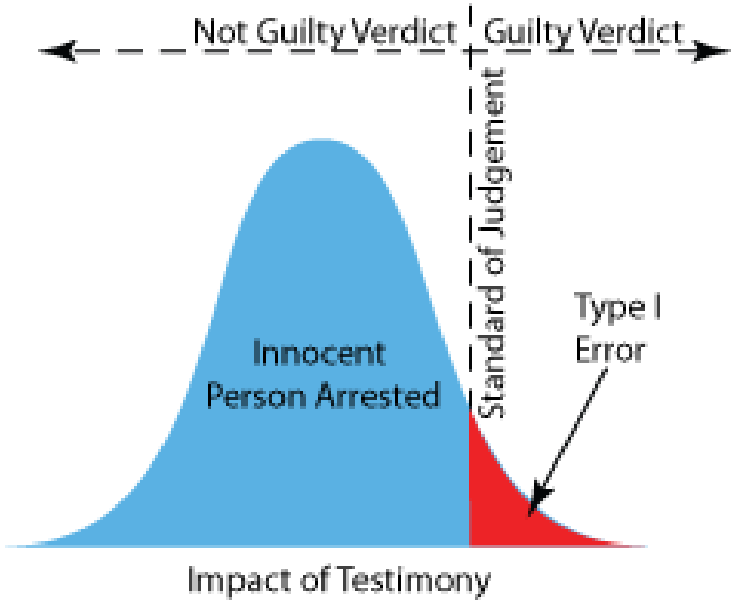
Type III*

- Less consistent definition but is usually correctly rejecting the null hypothesis for the wrong reason (i.e.. mistakenly using the wrong model).
- Right answer to the wrong problem

Type I (α) error:

Rejecting a true null hypothesis

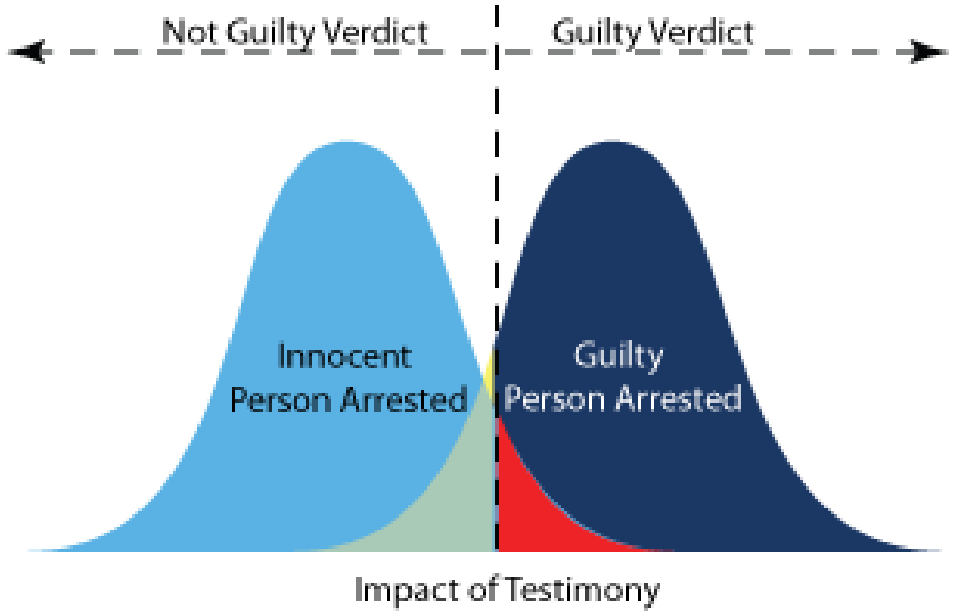
$P(\text{reject } H_0 | H_0 = \text{true}) = \alpha$



Type II (β) error:

Not rejecting a false null hypothesis

$P(\text{Fail to reject } H_0 | \text{Ho=Not True}) = \beta$



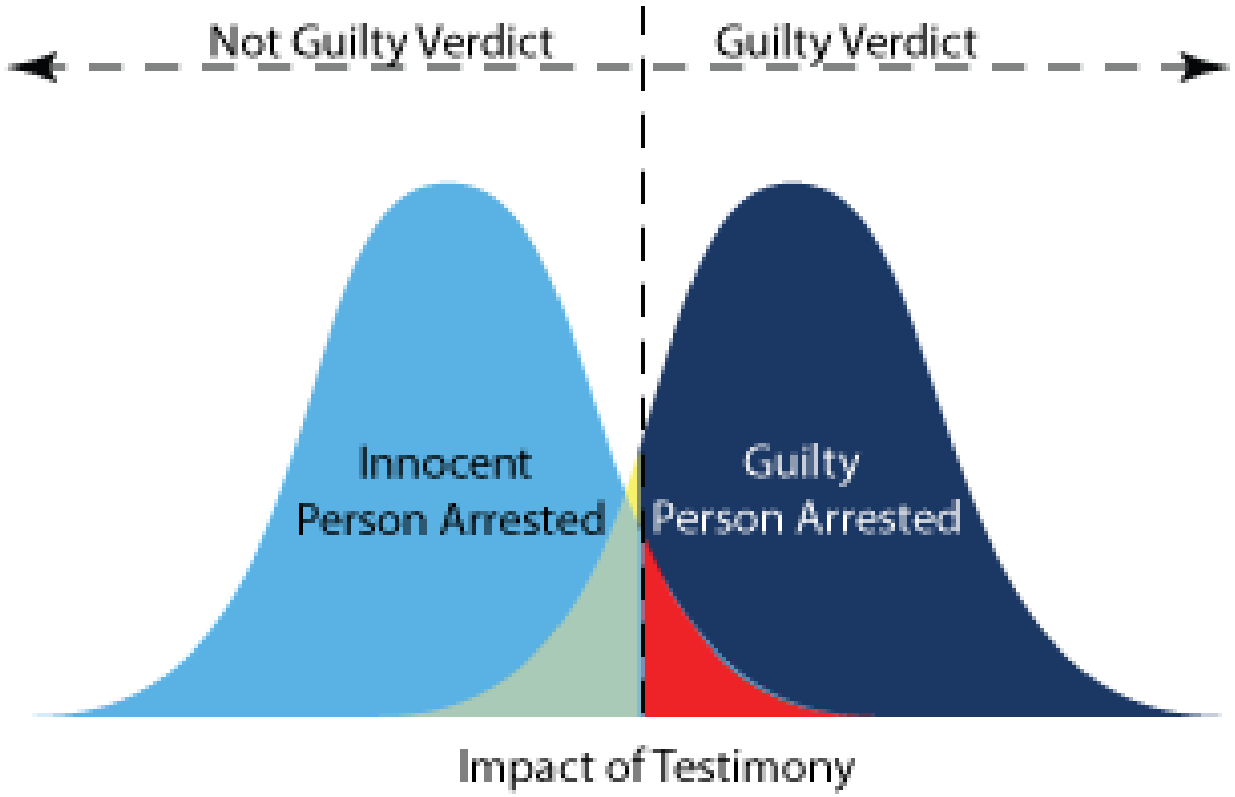
http://www.intuitor.com/statistics/T1T2Errors.html

https://wise.cgu.edu/wise-tutorials/tutorial-hypothesis-testing/type-1-error-rejecting-a-true-null-hypothesis/

	No Disease (H_0 true)	Disease (Ho is not true; H_A true)
Fail To Reject H_0	**No Error** Specificity P[FTR|Ho is true] (True Negative)	**Type II** P[FTR|Ho is not true] (False Negative)
Reject H_0	**Type I** P[reject|Ho] (False Positive)	**No Error** Power/Sensitivity P[Reject|Ho is not true] (True Positive)

$$\text{Sensitivity} = \frac{TP}{TP + FN} \qquad \text{Specificity} = \frac{TN}{TN + FP}$$

https://en.wikipedia.org/wiki/Sensitivity_and_specificity



Generally, type I errors are the ones that we are concerned with in biology.
Although, there are circumstances when we are more concerned with type II errors (i.e.) Medicine

There is a trade-off between type I error and type II error

https://wise.cgu.edu/wise-tutorials/tutorial-hypothesis-testing/type-2-error-fail-to-reject-a-false-null-hypothesis/

Add more data points, n, and you are able to discriminate between smaller differences in the null and alternate hypotheses!

Power is the ability of a test to reject a false null hypothesis

Power = 1 - P(FTR H_0| H_A)= P(Reject H_0| H_A)

Power = 1-β

Sensitivity = 1-Type II = Power = **TP / (TP+FN)**

Specificity = 1-Type I = TN / (TN+FP)

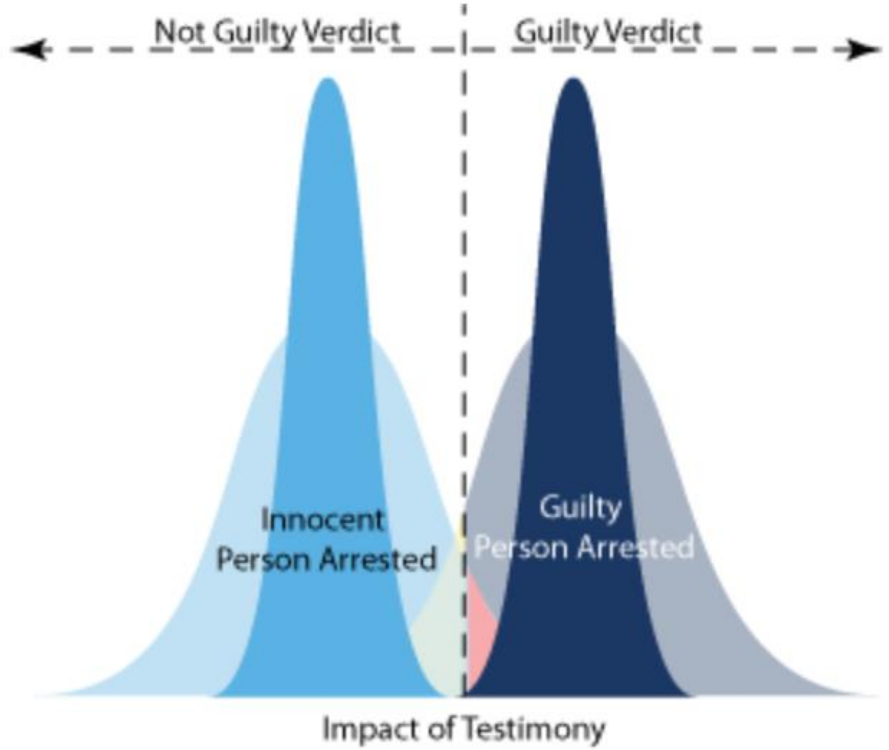


figure 5. The effects of increasing sample size or in other words, number of independent witnesses.

http://www.intuitor.com/statistics/T1T2Errors.html

Two clinical trials are carried out which both test the same null hypothesis under the same conditions with α = 0.05. Trial A has 45 individuals and Trial B has 100 individuals. Power=1-type II (Beta)

Which of the following is true about the two trials described above:

a. Study A has higher probability of type I error than Study B and Study B has a higher probability of type II error than Study A

b. Study A has a lower probability of type I error than Study B and Study B has a lower probability of type II error than Study A

c. Study A has the same type I error as Study B and Study A has a higher probability of type II error than Study B.

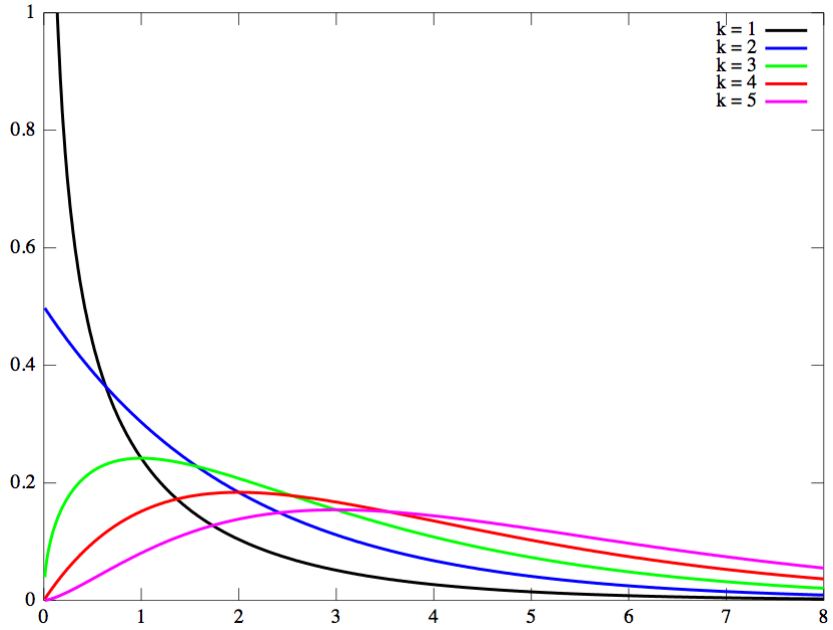
d. Study A has the same type I error as Study B and Study B also has a higher probability of type II error than Study A

You can use the Hypothesis Testing Framework with any sample distribution, not just the Normal Distribution

H_0: *The data come from a particular discrete probability distribution*
H_A: *The data do not come from that distribution*

Step 2: $\chi 2$ Goodness of fit test (or contingency test):

$$\chi^2_{df} = \sum_i \frac{(Observed_i - Expected_i)^2}{Expected_i}$$



Are assumptions met?

H_0: *The data come from a particular discrete probability distribution*

H_A: *The data do not come from that distribution*

Step 2: $\chi 2$ Goodness of fit test (or contingency test):

$$\chi^2_{df} = \sum_i \frac{(Observed_i - Expected_i)^2}{Expected_i}$$

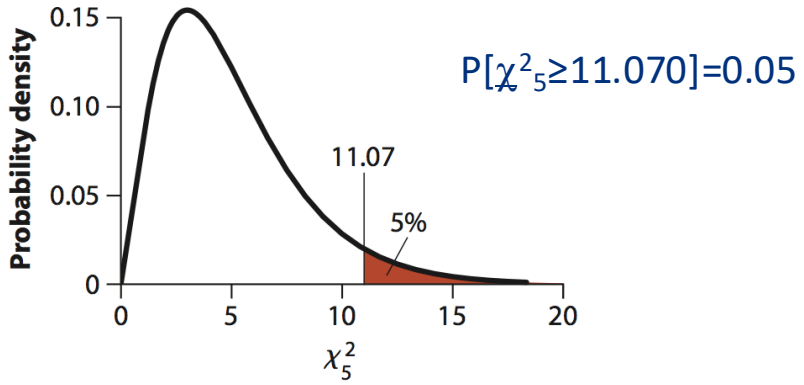
Step 3:

DF= # categories – 1- # estimated parameters*

Finding **critical values for χ^2 distr^n**:

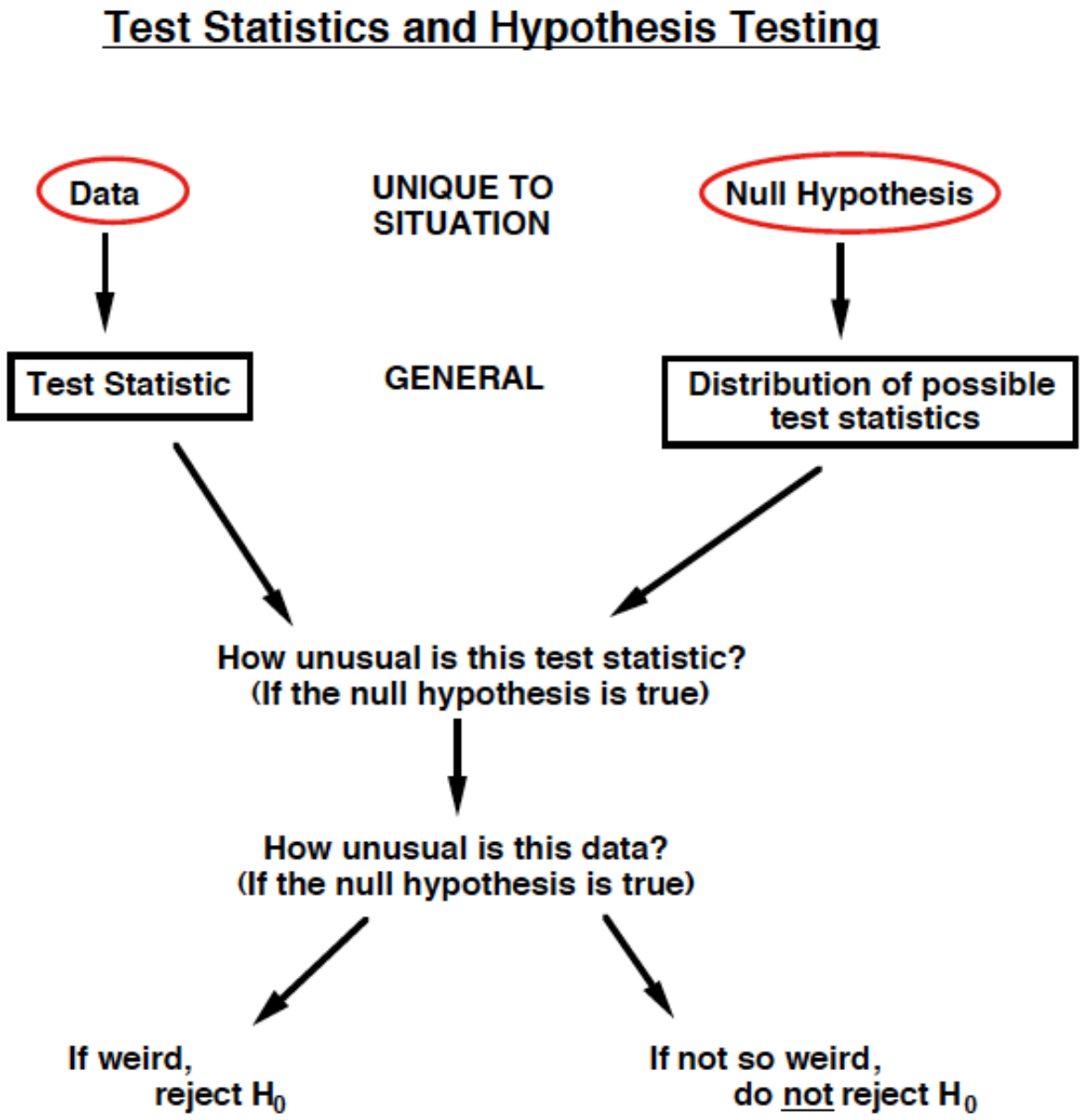
Statistical Table: https://www.math.arizona.edu/~jwatkins/chi-square-table.pdf

df	0.995	....	0.950	0.05
1	0.000		0.00393	3.841
...				
5	0.412		1.145	11.070
6	0.676		1.635	12.592



$P[\chi^2_5 \geq 11.070]=0.05$

* df are calculated differently for contingency test



"A model is a mathematical tool that mimics how we *think* a natural process works.."

Life is interesting when a model doesn't fit the data because it suggests that at least one of the major assumption about how we think about the process is wrong

All models are wrong, but some are useful
- George E.P. Box

Example: the results of a **Monohybrid cross** between a (heterozygous) yellow pea plant **(Yy)** and a green pea **(yy)** plant are as follows: **8 yellow** and **12 green**. Are these results consistent with Mendel's first law (**segregation)** which should a 1:1 ratio in this case? (alpha = 0.05). Punnet square shown:

Yellow (Hetero)\Green (Homo)	y	y
Y	Yy	Yy
y	yy	yy

8

12

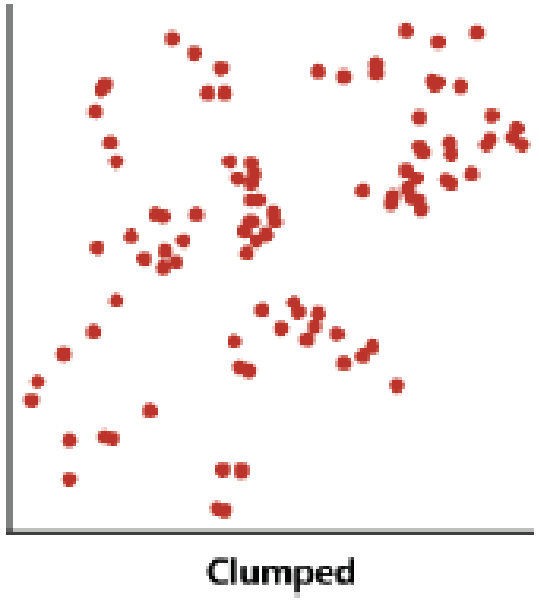
A. Yes – the results FTR the null hypothesis
B. No – the results Reject the null hypothesis
C. Yes – the results reject the null hypothesis
D. No – the results FTR the null hypothesis

* We could do similar queries with Hardy-Weinberg Equilibrium (if you're into that sort of thing…)

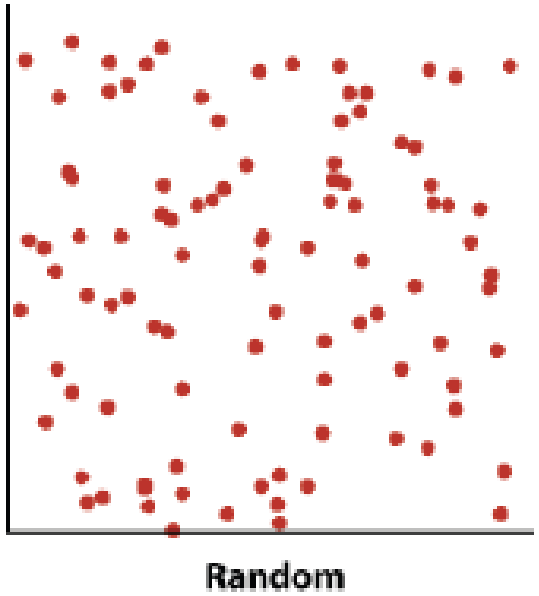
https://PollEv.com/multiple_choice_polls/dFGljMrrl5KEEqYPrPqjX/respond

Fitting the Poisson Distribution:

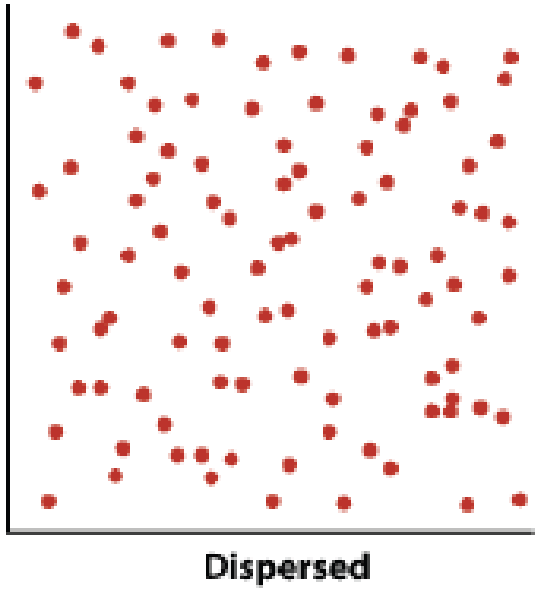
The Poisson Distribution describes the probability of getting X successes in a block of time or space when the successes happen independently of each other and occur with equal probability at every point in time or space.



Variance > mean



Variance = mean



Variance < mean

Poisson Distribution:

$$P[X] = \frac{e^{-\mu}\mu^{X}}{X!}$$

Example: Mutations are rare events, and we typically model mutations as having the same rate at each position in the genome and that each position in the genome is independent of every other position.

Is the following 100 replicates of spontaneous mutations in HIV consistent with a Poisson distribution of mutations?

Number of Mutations	Observed Frequency
0	1
1	5
2	12
3	10
4	14
5	20
6	19
7	10
8	6
9	1
10	2

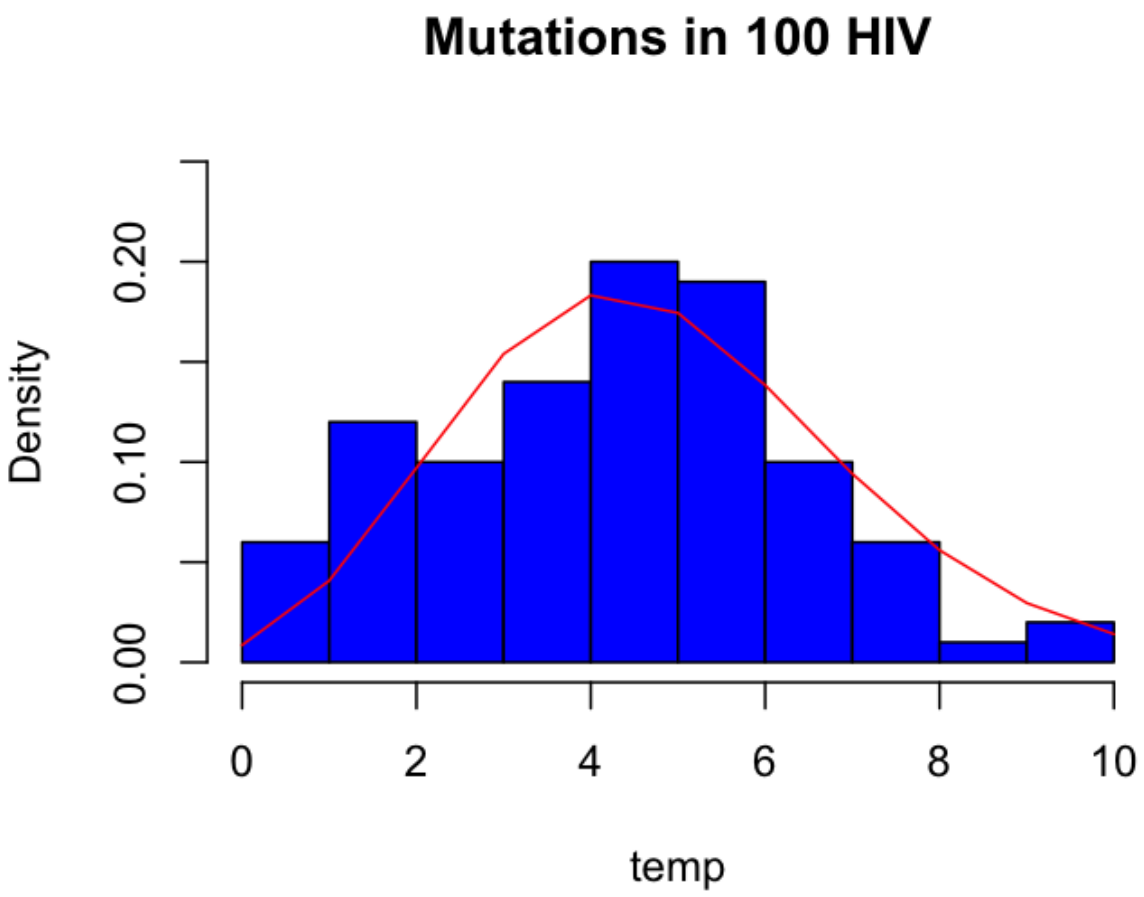
Step 1

H_0: The number mutations per nucleotide per generation has a Poisson distribution

H_A: The number of mutations per nucleotide per generation does *NOT* have a Poisson distribution

Step 2

Estimate μ: $\bar{X} = \frac{1X0+5X1+12X2+10X3+14X4+20X5+19X6+10X7+6X8+1X9+2X10}{100}$ = 4.82



Number of Mutations	Observed Frequency	Expected Frequency
0	1	
1	5	
2	12	
3	10	
4	14	
5	20	
6	19	
7	10	
8	6	
9	1	
10	2	

$$P[X] = \frac{e^{-\mu}\mu^{X}}{X!}$$

Number of Mutations	Observed Frequency	Expected Frequency
0	1	0.009*100=0.9
1	5	0.040*100=4.0
2	12	0.095*100=9.5
3	10	0.150*100=15.0
4	14	0.181*100 = 18.1
5	20	0.175*100=17.5
6	19	0.140=14.0
7	10	0.097=9.7
8	6	0.060=6.0
9	1	0.031=3.1
10	2	0.015= 1.5

These don’t quite add up to 100, due to rounding

Number of Mutations	Observed Frequency	Expected Frequency
0	1	0.008*100=0.8
1	5	0.040*100=4.0
2	12	0.095*100=9.5
3	10	0.150*100=15.0
4	14	0.181*100 = 18.1
5	20	0.175*100=17.5
6	19	0.140=14.0
7	10	0.097=9.7
8	6	0.060=6.0
9	1	0.031=3.1
10	2	0.015= 1.5

These don't quite add up to 100, due to rounding

Number of Mutations	Observed Frequency	Expected Frequency
0 +1	6	4.0+0.8=4.8
2	12	0.095*100=9.5
3	10	0.150*100=15.0
4	14	0.181*100 = 18.1
5	20	0.175*100=17.5
6	19	0.140=14.0
7	10	0.097=9.7
8+	9	6.0+4.6 = 10.6

We had to combine bins in order to met the assumptions:

≥80% *expected* cells must be ≥ 5; no expected cell can have a value <1.0.

This will impact our degrees of freedom calculation!

Step 3:

Calculate your test statistic

$$\chi^2 = \frac{(4.8-6)^2}{4.8} + \frac{(9.5-12)^2}{9.5} + \frac{(15-10)^2}{15} + \frac{(18.1-14)^2}{18.1} + \frac{(17.5-20)^2}{17.5} + \frac{(14-19)^2}{14} + \frac{(9.7-10)^2}{9.7} + \frac{(10.6-9)^2}{10.6}$$

$$= 6.11$$

Step 3:

dof = 8 categories **– 1 – 1 estimate = 6** degrees of freedom

Step 4:

Critical value for χ^2 is given in statistical table found at:
https://www.math.arizona.edu/~jwatkins/chi-square-table.pdf
For alpha=0.05, df=6: 12.592
Therefore, we FTR the Poisson distribution. **BUT THERE IS MORE WE CAN SAY….**

Variance = Mean:

If Variance > Mean, then CLUMPED

- visual hint: histogram is ‘u-shaped’

If Variance < Mean, then DISPERSED

- points are spread uniformly in space or time

- This may be a bit confusing if you are familiar with molecular genetics, because we refer to the “over dispersed molecular clock” which is really saying that variance > mean number of substitutions. Sometimes, terminology is ambiguous!

Rejecting a null hypothesis of a Poisson distribution of successes implies that

A- Success are not independent

B- The probability of a success occurring is constant over time or space.

C-The probability of a success occurring is NOT constant over time or space.

D- A and B

E- A and C

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Contingency Analysis

Contingency: *allows us to determine if two categorical variables are associated (some contingency tests will allow us to quantify the degree of association as well but not all do this).*

Major tests:

- **χ^2 Contingency Test** –> similar but not exactly as the same χ^2 Goodness of fit test. You can think of it as a subset of χ^2 Goodness of fit tests with some calculation differences. Basis of test is Multiplication rule with the assumption of independence. Degrees of freedom are calculated differently!
- **Odds ratio** –> Ho: OR=1. Challenge: transforming the sampling distribution of OR so that it is normally distributed.
- **Relative Risk** -> like OR but accounts for proportion of (rare) event in the population
- **Fisher's Exact test** -> exact calculation. You can think of it as the contingency version of calculating a p-value

Contingency Analysis:

Review prompt: Associations between categorical variables

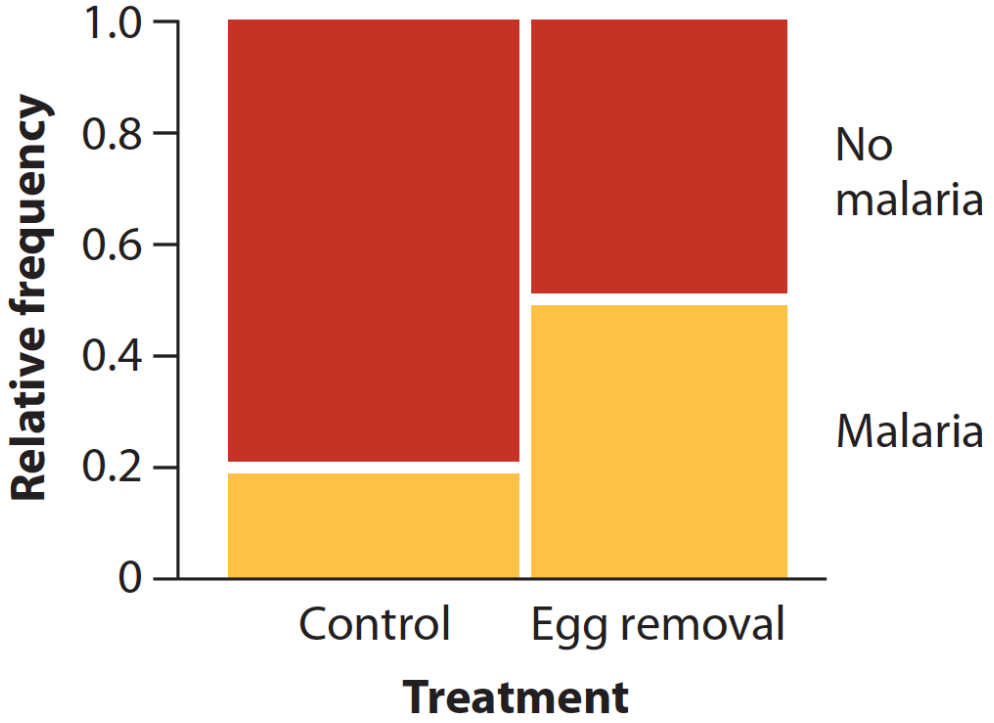
- Test the independence of two or more categorical variables

	Control Group	Egg-Removal Group	Row Total
Malaria	7	15	22
No Malaria	28	15	43
Column Total	36	30	65

Contingency Analysis:

- Associations between categorical variables
- Test the independence of two or more categorical variables

	Control Group	Egg-Removal Group	Row Total
Malaria	7	15	22
No Malaria	28	15	43
Column Total	36	30	65



Reminder: Multiplication Rule

Multiplication rule: $P[A \text{ and } B] = P[A|B]P[B]$

IFF INDEPENDENT, this collapses to: $P[A \text{ and } B] = P[A]P[B]$

χ^2 Contingency Test:

- Tests goodness-of-fit to the data of the null hypothesis of independence of variables

- Two categorical variables but, unlike the Odds Ratio, each variable can have more than 2 categories

- Assumptions:
  - The value of the cell ***expected values*** should be 5 or more in at least 80% of the cells
  - No cell should have an **expected value** of less than one

- Description of χ^2 Contingency Test:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3900058/

When is it appropriate to use Chi-Squared tests?

---

a. When you are determining if two categorical variables are associated.
b. When you are directly comparing proportions
c. When your number of independent data points is less than 5
d. When you are looking for an exact P value.

Example: *Is there a relationship between age at first birth and the development of breast cancer?*

	<20	20-29	30-34	>=35	Row total
Cancer	320	2217	463	220	3220
No Cancer	1422	7325	1092	406	10245
Column Total	1742	9542	1555	626	13465

STEP 1: Formulate null hypothesis

Example: *Is there a relationship between age at first birth and the development of breast cancer?*

	<20	20-29	30-34	>=35	Row total
Cancer	320	2217	463	220	**3220**
No Cancer	1422	7325	1092	406	**10245**
Column Total	**1742**	**9542**	**1555**	**626**	**13465**

Step 1:

H_0: The development of breast cancer is ***independent*** of the age at first birth

H_A: The development of breast cancer is ***dependent*** of the age at first birth

Step 2: Identify the test statistic

χ^2 expectation under independence. Assumptions: no cells less than 5 so both assumptions are met.

With independence,

P[Age at first birth AND breast cancer] = ?

Example: *Is there a relationship between age at first birth and the development of breast cancer?*

	<20	20-29	30-34	>=35	Row total
Cancer	320	2217	463	220	**3220**
No Cancer	1422	7325	1092	406	**10245**
Column Total	**1742**	**9542**	**1555**	**626**	**13465**

Step 1:

H_0: The development of breast cancer is ***independent*** of the age at first birth

H_A: The development of breast cancer is ***dependent*** of the age at first birth

Step 2: Identify the test statistic

χ^2 expectation under independence

With independence,

P[Particular Age at first birth AND breast cancer] = P[Particular Age at first birth]P[Breast cancer]

Calculating the expectations under H_0:

	<20	20-29	30-34	>=35	Row total
Cancer	320	2217	463	220	3220
No Cancer	1422	7325	1092	406	10245
Column Total	1742	9542	1555	626	13465

$$P[Age < 20 Birth] = \frac{1742}{13465} = 0.13$$

$$P[Cancer] = \frac{3220}{13465} = 0.24$$

$$P[NoCancer] = \frac{10245}{13465} = 0.76$$

If H_0 is true, then:

P[< 20 Age at first birth AND breast cancer] = 0.13*0.24 = 0.031

Calculating the expected **COUNTS** under H_0:

EXPECTED values Under Ho	<20	20-29	30-34	>=35	Row total
Cancer	416.6 320	2281.9 2217	371.9 463	149.7 220	3220
No Cancer	1325.6 1422	7260.2 7325	1183.2 1092	477 406	10245
Column Total	1742	9542	1555	626	13465

χ^2 Contingency Test

Step 2:

$$\chi^2 = \sum_i \frac{(Observed_i - Expected_i)^2}{Expected_i} = 104.76$$

$$= \frac{(416.6-320)^2}{416.6} + \frac{(2281.9-2217)^2}{2281.9} + \frac{(371.9-463)^2}{371.9} + \frac{(149.7-220)^2}{149.7} + \frac{(1325.6-1422)^2}{1325.6} + \frac{(7260.2-7325)^2}{7260.2} + \frac{(1183.2-1092)^2}{1183.2} + \frac{(477-406)^2}{477}$$

Step 3:
Degrees of Freedom:

dof = (row - 1)(column - 1)

For the Birth age/cancer example: **dof = (2-1)(4-1)=3**

Step 4, Conclusion:

$$\chi^2 = 104.76 \gg \chi^2_3 = 7.81$$

We reject the null hypothesis of independence with a significance level of <= 0.05 and say that the age of first birth was not independent on whether breast cancer eventually developed.

A chi-squared test statistic on a contingency table that is equal to zero means:

A. The two nominal variables have values consistence with independence.
B. The two nominal variables have values that are consistent with equality.
C. The two nominal variables have the same proportions listed in Ho.
D. All these choices.

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What would a chi-square contingency test resulting in a significance value of P > 0.05 suggest?

A. We cannot reject the hypothesis of independence between the two variables
B. We cannot reject the hypothesis of dependency between the two variables
C. There is a significant relationship between the two variables
D. We can reject the hypothesis of dependency between the two variables

Example: Is there an influence of the following three SES on preterm delivery rates?

Socio-Economic status	Preterm Birth	Normal Birth
Upper/Upper-middle	25	85
Middle	33	64
Lower/Lower-middle	112	149

A. Yes, we reject the null hypothesis
B. No, we fail to reject the null hypothesis
C. Yes, we fail to reject the null hypothesis
D. No, we reject the null hypothesis

https://PollEv.com/multiple_choice_polls/gayNb4tVDJb36c1SNunNy/respond