Module 2: Introduction to Statistics

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Topic

- Estimation
 - Point Estimates, Interval Estimates
- · Hypothesis testing
 - Type I and Type II Error
- Comparing means
 - Parametric test: t-test
 - Nonparametric test: Wilcoxon test
- Power and sample size calculations

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Estimation: Point Estimate

- Point Estimate. Let, $x_1, x_2,, x_n$, be a random sample from a population with mean μ and variance σ^2
 - A point estimate for a parameter of interest θ is a function of the sample, named $\widehat{\theta},$ which is a good approximation of θ
- Good estimates are unbiased and have minimum variance (error)
 - Unbiased
 - $E(\hat{\theta}) = \theta$
 - Minimum variance
 - If $\hat{\theta}_1$ and $\hat{\theta}_2$ are both unbiased estimates of θ , then the one with the smallest error is a better estimate

Example: Unbiased Estimates for μ and σ^2

Estimate of \mu: \overline{x} and \tilde{x} are two unbiased estimates for the mean parameter μ

$$\overline{x} = \frac{x_1 + x_2 + \dots + x_n}{n}$$

 $E(\overline{x})=\mu$

$$se(\overline{x}) = \frac{s}{\sqrt{n}}$$

$$\tilde{x} = \frac{x_1 + x_2}{2}$$

$$E(\tilde{x})=\mu$$
 $se(\tilde{x})=\frac{s}{\sqrt{2}}$

Which one is better?

Estimate of σ^2 :

$$s^{2} = \frac{\sum_{i=1}^{n} (x_{i} - \overline{x})^{2}}{n-1}$$

(Unbiased)

$$s^{2} = \frac{\sum_{i=1}^{n} (x_{i} - \overline{x})^{2}}{n-1}$$

$$\tilde{\sigma}^{2} = \frac{\sum_{i=1}^{n} (x_{i} - \overline{x})^{2}}{n}$$

$$E(s^{2}) = \sigma^{2}$$

$$E(\tilde{\sigma}^{2}) = \frac{n}{n-1} \sigma^{2}$$

$$E(\tilde{\sigma}^2) = \frac{n}{n-1} \sigma^2$$

(Approximately Unbiased)

Interval Estimate

- Interval Estimate is derived by using the sample to calculate an interval $(\hat{\theta}_L, \hat{\theta}_U)$ of possible values for θ : $(\hat{\theta}_L < \theta < \hat{\theta}_U)$.
 - If $(\hat{\theta}_{L1}, \ \hat{\theta}_{U1})$ and $(\hat{\theta}_{L2}, \ \hat{\theta}_{U2})$ are two interval estimates with the same credibility for θ , the one with the shortest length is better
- Confidence Interval (CI) is an interval estimate that is used to indicate the reliability of a point estimate.
 - 95%CI for μ : $[\overline{x}$ -1.96*se (\overline{x}) , \overline{x} +1.96*se (\overline{x})] or $\overline{x} \pm 1.96 * se(\overline{x})$
 - (1- α)%CI for μ : $[\overline{x}$ $z_{\alpha/2}$ *se (\overline{x}) , \overline{x} + $z_{1-\alpha/2}$ *se (\overline{x})]
 - (1-lpha) is the confidence level, that is, how frequently the CI contains μ if the experiment was repeated many times

Which is wider 95%CI or 99%CI?

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Hypothesis Testing

- In hypothesis testing usually there is a null hypothesis and an alternative hypothesis. The **null hypothesis** typically corresponds to a default position. It is refereed to as H_0 . The **alternative hypothesis** is refereed to as H_A
- For example, let μ be the mean cholesterol for a population. A researcher wants to know if the mean cholesterol is equal (or bigger) to some value μ_0 (=130)?

 H_0 : $\mu = \mu_0$ Two sided hypothesis testing

 H_A : $\mu \neq \mu_0$

 H_0 : $\mu > \mu_0$ One sided hypothesis testing

 H_A : $\mu \leq \mu_0$

Hypothesis Testing

- Hypothesis testing is done by collecting data and then quantifying in probability terms how likely it is to have observed these data if H₀ were true
- If the collected data were unlikely to have occurred under the H_0 , then reject H_0
- Quantifying how likely it is to have observed the sample data is done via a statistical test, and based on probability distribution theory (e.g. t-test, Chi-square, F-test)

Type I Error and Type II Errors

- When testing a hypothesis the unknown parameters are estimated, which will result in an estimation error. Thus, there will be potential errors for rejecting or not rejecting the null hypothesis H_0
- Type I Error is the incorrect rejection of a true H₀
 - $Pr(\text{Reject } H_0 | H_0 \text{ is true}) = \alpha$
- Type II Error is failure to reject a false H₀
 - $Pr(Do not Reject H_0 | H_A is true) = \beta$

Type I Error and Type II Errors

- A type I error is a false positive result. For example, showing that a
 particular gene relates to some disease when that's not the case.
 Or convicting an innocent person
- A type II error is a false negative result. An example of type II error would be a failing to show that a particular gene relates to some disease when it really does
 Or failing to convict a guilty person
- All statistical hypothesis tests have a probability of making type I and type II errors. It's important to keep Type I error low

Type I Error and Type II Errors

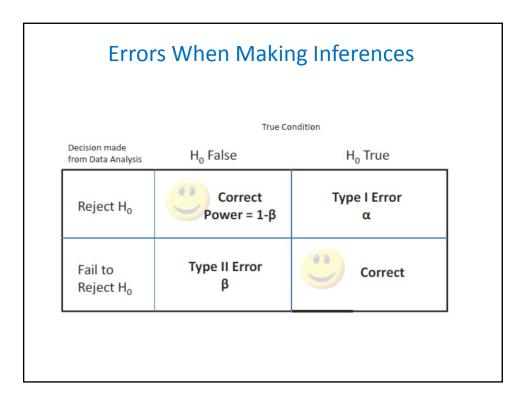
What is the Type I and Type II error for a test that <u>always</u> rejects H₀?

 $Pr(\text{Reject } H_0 | H_0 \text{ is true}) = 1$ $Pr(\text{Do not Reject } H_0 | H_A \text{ is true}) = 0$

• What is the Type I and Type II error for a test that <u>never</u> rejects H_0 ?

 $Pr(\text{Reject } H_0 | H_0 \text{ is true})=0$ $Pr(\text{Do not Reject } H_0 | H_A \text{ is true})=1$

• A good test has <u>both</u> small α and small β . α is usually set at 0.05 or 0.01; and β is usually set at 0.2 or 0.1



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Comparing Means: Parametric Test Student's t-test

- Student's t-test is commonly used for comparing means and it is based on the T-distribution. Depending on the hypothesis one of the following three tests are used
 - 1. One-sample t-test
 - 2. Paired t-test
 - 3. Two-sample t-test
- The t-test is a parametric test. It is based on the parametric assumption that the data have a normal distribution

One-Sample t-test

- Let, $x_1, x_2,, x_n$, be an independently normally distributed sample from a population with mean μ and sampling variance s^2 . We want to test whether the population mean μ is equal to some value μ_0
- Then, the null and alternative hypotheses are:

$$H_0$$
: $\mu = \mu_0$

vs.

$$H_A$$
: $\mu \neq \mu_0$

One-Sample t-test

How to build a statistical test for: H_0 : $\mu = \mu_0$ vs. H_A : $\mu \neq \mu_0$

- First, use \overline{x} to estimate μ , then compare \overline{x} to μ_0 . If the difference, $\overline{x}-\mu_0$, is large it suggests that $\mu\neq\mu_0$. So, reject H_0 : $\mu=\mu_0$ for "large" values of $\overline{x}-\mu_0$
- How large should $\overline{x} \mu_0$ be to reject H_0 ?
 - It depends on the margin of the error, $\operatorname{se}(\overline{x})$. The larger the error the larger the $\overline{x}-\mu_0$ should be

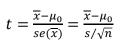
Q: Which difference $\overline{x} - \mu_0$ would be considered "larger"? (a) 10 with $se(\overline{x})=1$ or (b) 100 with $se(\overline{x})=100$

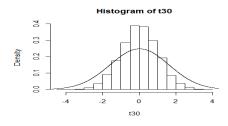
One-Sample t-test

- Calculate the difference relative to the error, $\frac{\overline{x}-\mu_0}{se(\overline{x})}$, if the ratio is large, then reject H_0 : $\mu=\mu_0$
- Next, define in probability terms, what values of $\frac{\overline{x}-\mu_0}{se(\overline{x})}$ are considered large to reject H₀

Using T-distribution for Testing One-sample t-test

• If H₀ is true, then the following test statistics has a T-distribution with *n-1 df*:





 T-distribution is symmetric around 0, and very large/small values are less likely to occur. So, if H₀ was true one would expect to see t (or t-values) that are not too far from 0

Using T-distribution for Testing One-sample t-test

• If t-value is large, $|t| \ge C$, then H_0 is rejected $(\mu \ne \mu_0)$. C is referred to as critical value and is defined such that $Pr(|T| \ge C) = \alpha$ (usually $\alpha = 0.05$, for which $C \approx 2$)

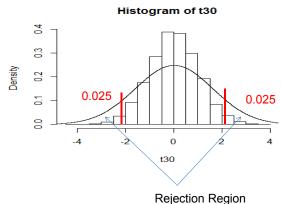
- If |t-value| > 2 reject H₀

- Alternatively, p-value is used as a criteria for rejecting H₀
 - **p-value** is defined as $Pr(|T| \ge t\text{-value})$ =p-value. For any t-value the p-value is calculated using t-distribution tables or statistical programs

- If p-value < 0.05 reject H_0

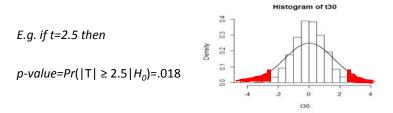
T-distribution and the Rejection Region

• Let $t=\frac{\overline{x}-\mu_0}{se(\overline{x})}$. The rejection region, is the set of t-values for which H_0 is rejected. The area under the curve for the values in the rejection region is the Type I error and equals to 0.05



Meaning of the p-value

- A p-value is not the probability that H₀ is true. On the contrary, the p-value is based on the assumption that H₀ is true
- A p-value is an estimate of the probability that the observed result, or a more extreme result, could have occurred by chance, if H₀ were true



The p-value is a measure of the credibility of the null hypothesis. A large p-value (> 0.05) means that there is no compelling evidence to reject H₀

Example of One-Sample t-test

(TROPHY Data)

• H_0 : Mean of LDL = 130 vs. H_A : Mean of LDL \neq 130

$$t = \frac{\overline{x} - 130}{se(\overline{x})} = \frac{\overline{x} - 130}{\sqrt{s} / \sqrt{n}}$$

- mean(LDL)=123.06
- sd(LDL)=32.1/
- n=length(LDL)=255

 $t=(123.06-130)/(32.1/\sqrt{255})=-3.453$ (Reject H_0)

Example of One-Sample t-test in R (TROPHY Data)

- H_0 : Mean of LDL = 130 vs. H_A : Mean of LDL \neq 130
- Code in R: t.test(LDL, m=130)
- The output for this t.test is:

One Sample t-test data: LDL

t = -3.4529, df = 254, p-value = 0.0006494 \rightarrow p < .05, Reject H_0 alternative hypothesis: true mean is not equal to 130 percent confidence interval: 119.1000 127.0176 \rightarrow Does not include 130, hence Reject H_0

Paired t-test

- Let, (x₁,y₁),(x₂,y₂),....,(x_n,y_n) be a sample of pairs independently normally distributed with mean (μ₁,μ₂) and sampling standard deviation (s₁,s₂). Here x_i and y_i are data on the same subject, for example, x_i is the BP measured before taking some medication and y_i is the BP 2 years after.
- These type of data are used to test whether there is a treatment effect by comparing the before (μ_1) and after treatment (μ_2) BP values.
- The null and the alternative hypotheses are:

$$H_0$$
: $\mu_1 = \mu_2$

VS.

$$H_A$$
: $\mu_1 \neq \mu_2$

Paired t-test

- Paired t-test is derived using one-sample t-test on d_i = y_i x_i
- Calculate $d_i = y_i x_i$, then $d_1, d_2, ..., d_n$, are independently normally distributed, with mean $\mu_d = \mu_2 \mu_1$. If there were no treatment effect μ_d should be zero
- · Then testing

$$H_0: \mu_1 = \mu_2$$

$$H_A$$
: $\mu_1 \neq \mu_2$

is the same as testing:

$$H_0$$
: $\mu_d = 0$

$$H_A$$
: $\mu_d \neq 0$

Use the one-sample t-test on d₁,d₂,...,d_n

Example of Paired t-test in R

(TROPHY Data)

- Let μ_1 and μ_2 be the mean of DBP for patients in treatment group at baseline (before treatment) and 2 years later (after treatment):
- $H_0: \mu_2 \mu_1 = 0$ vs. $H_A: \mu_2 \mu_1 \neq 0$
- t.test(DBP24,DBP0,paired=T)

Paired t-test data: DBP0 and DBP24

t = -6.712, df = 126, p-value = 5.862e-10 \rightarrow p<.05, Reject H_0 alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -4.686208 -2.552063

sample estimates: mean of the differences -3.619135

Does not contain 0, hence Reject H_0

Two-Sample t-test

• Let, $x_1, x_2,, x_{n1}$, and $y_1, y_2,, y_{n2}$ be two samples independently normally distributed with mean μ_1 , μ_2 and sampling variance s_1, s_2 . For example x is the sample for a treated group and y is the sample for a control (placebo) group. We want to test whether the means between two groups are equal or not. The null and alternative hypotheses are:

$$H_0: \mu_1 = \mu_2$$

Vs.

$$H_A$$
: $\mu_1 \neq \mu_2$

Two-Sample t-test

If H₀ is true, then the following test statistics has a T-distribution:

$$t-value = \frac{\overline{x} - \overline{y}}{\sqrt{\frac{s_1^2 + s_2^2}{n_1 + n_2}}}$$

where, $\overline{x} = \frac{\sum x_i}{n_1}$, $\overline{y} = \frac{\sum y_i}{n_2}$, $s_1^2 = \frac{\sum (x_i - \overline{x})^2}{n_1 - 1}$, and $s_2^2 = \frac{\sum (y_i - \overline{y})^2}{n_2 - 1}$. The same argument described for the one-sample t-test applies here:

- If |t-value| > 2 reject H₀
- If p-value <.05 reject Ho

Note: If s_1 and s_2 are the same the t-test with equal varience is used.

Example of Two-Sample t-test in R (TROPHY Data)

- Let μ_1 and μ_2 be the mean of LDL for patients in treatment group and placebo group respectively
- H_0 : $\mu_1 = \mu_2$ vs. H_A : $\mu_1 \neq \mu_2$
- Code in R: t.test(LDL~Trt)

Welch Two Sample t-test data: LDL by Trt

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Nonparametric Test for Comparing Means

- The Student's t-test is a parametric test, it assumes that data are normally distributed, which may or may not be true
- Nonparametric test is an alternative to the t-test for comparing means. Nonparametric test does not assume normality for the data
- If the data are not normally distributed and have large outliers, a nonparametric test is preferred

Nonparametric Test for Comparing Means Wilcoxon test

 Wilcoxon test is the most common used among nonparametric tests. As with t-test, there are three Wilcoxon tests related to a specific H₀

One-sample test: Wilcoxon sign rank test
 Paired test: Wilcoxon sign rank test

3. Two-sample test: Wilcoxon sum rank test (aka Mann-Whitney test)

Wilcoxon Test for Comparing Means From Two Samples

Let's consider the following data on two samples:

X sample: 2,5,3,6Y sample: 3,7,99,8,9

- The H_0 states that the data for the X and Y samples come from the same distribution with the same mean ($\mu_{\chi}=\mu_{\gamma}$)
- If H_0 is true then "X > Y" or "Y > X" are equally likely to occur:

$$Pr(X > Y) = Pr(Y > X)$$

That is, smaller (or larger) values are equally likely to be from either X or Y sample

Wilcoxon Test for Comparing Means From Two Samples

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X sample: 2,5,3,6Y sample: 3,7,99,8,9
```

- · Rank all the data from smallest to largest:
 - Rank all data: 2 3 3 5 6 7 8 9 99
 Assign ranks: 1 2.5 2.5 4 5 6 7 8 9
 (In case of a tie, assign an average rank to each data point)
- Calculate R₁ = 12.5 sum of ranks from X sample. R₂ = 32.5 sum of ranks from Y sample
- If the low ranking is dominated by one sample (e.g. $R_1 < R_2$), that is evidence against H_0 , which is rejected using the p-value < 0.05 criteria

Example of Wilcoxon two-sample test in R (TROPHY Data)

- Let μ_1 and μ_2 be the mean of HDL for patients in the treatment group and the placebo group respectively
- H_0 : $\mu_1 = \mu_2$ vs. H_A : $\mu_1 \neq \mu_2$
- Code in R: wilcox.test(HDL~Trt)

Wilcoxon rank sum test with continuity correction

HDL by Trt W = 7574, **p-value = 0.3471** alternative hypothesis: true location shift is not equal to 0

Compare t-test vs. Wilcoxon test

X sample: 2,5,3,6Y sample: 3,7,99,8,9

wilcox.test(X,Y) Wilcoxon rank sum test with continuity correction

data: x and y W = 2.5, p-value = 0.0851

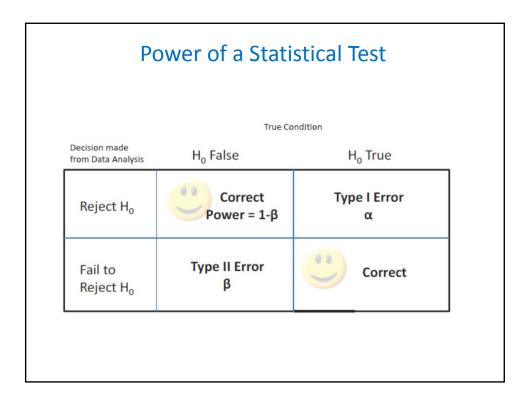
• t.test(X,Y) Welch Two Sample t-test

data: x and y t = -1.1459, df = 4.02, p-value = 0.3154

• Usually Wilcoxon is better to use if n is small and there are outliers. If data are normally distributed, the t-test is better

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Power of a Statistical Test

- **Power** is the probability of detecting a difference, when it exists, for a given Type I error, say α =.05
 - Power= $Pr(Reject H_0 | H_A is true)$

or

- Power=1-Pr(Do not Reject $H_0 | H_A$ is true)=1- β
- A good test should have sufficient Power, usually 80% or 90%

What Impacts Power?

(e.g. two sample t-test)

• The two-sample t-test which tests H_0 : μ_1 = μ_2 is

$$t = \frac{\overline{x}_1 - \overline{x}_2}{se(\overline{x}_1 - \overline{x}_2)} = \frac{\overline{x}_1 - \overline{x}_2}{\left|\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right|} \rightarrow \text{smal}$$

- Power= $Pr(\text{Reject }H_0/H_A \text{ is true})=Pr(|t|>C_{\alpha})$. Thus the larger the value of t the bigger the Power.
- t is large when:
 - $-\overline{x}_1-\overline{x}_2$ (or $\mu_1{-}\mu_2$) is large.
 - $-se(\overline{x}_1 \overline{x}_2)$ is small (more precision)
 - s₁ and s₂ are small
 - n₁ and n₂ are large (large sample)

Examples: Which Experiment Will Have the Larger t-value?

	\overline{x}_1	\overline{x}_2	s_1	s_2	n_1	n_2	t-value
Exp 1a	40	50	30	30	100	100	?
Exp 1b	40	50	30	30	200	200	
Exp 1c	40	50	30	30	300	300	?
Exp 2a	40	47	30	30	100	100	
Exp 2b	40	47	30	30	200	200	
Exp 2c	40	47	30	30	300	300	
Ехр За	40	50	20	20	100	100	
Exp 3b	40	50	20	20	200	200	
Exp 3c	40	50	20	20	300	300	

$$H_0\colon \mu_1 = \mu_2 \qquad \qquad \mathsf{t} = \frac{\overline{x}_1 - \overline{x}_2}{se(\overline{x}_1 - \overline{x}_2)} = \frac{\overline{x}_1 - \overline{x}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

Examples: Which Experiment Will Have the Larger t-value?

	\overline{x}_1	\overline{x}_2	s_1	s_2	n_1	n_2	t-value
Exp 1a	40	50	30	30	100	100	-1.67
Exp 1b	40	50	30	30	200	200	-2.36
Exp 1c	40	50	30	30	300	300	-3.33
Exp 2a	40	47	30	30	100	100	-1.17
Exp 2b	40	47	30	30	200	200	-1.65
Exp 2c	40	47	30	30	300	300	-2.33
Ехр За	40	50	20	20	100	100	-2.5
Exp 3b	40	50	20	20	200	200	-3.54
Ехр 3с	40	50	20	20	300	300	-5

$$H_0\colon \mu_1 = \mu_2 \qquad \qquad \mathsf{t} = \frac{\overline{x}_1 - \overline{x}_2}{se(\overline{x}_1 - \overline{x}_2)} = \frac{\overline{x}_1 - \overline{x}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

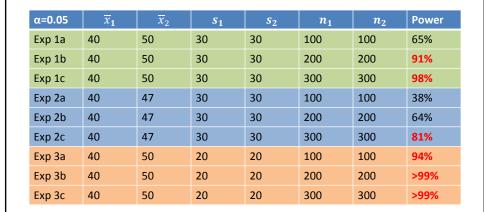
Power Calculations

- To calculate Power (using R or other programs) for comparing the means between two groups the following input is required:
 - Number of samples in each group: (n₁,n₂)
 - Difference in the mean you want to detect: $\delta = \mu_1 \mu_2$ or $(\overline{x}_1 \overline{x}_2)$
 - Standard deviation of each group: (s₁,s₂)
 - Type I error: $(\alpha = 0.05)$

Power Calculations

α=0.05	\overline{x}_1	\overline{x}_2	s_1	s_2	n_1	n_2	Power
Exp 1a	40	50	30	30	100	100	?
Exp 1b	40	50	30	30	200	200	
Exp 1c	40	50	30	30	300	300	
Exp 2a	40	47	30	30	100	100	
Exp 2b	40	47	30	30	200	200	
Exp 2c	40	47	30	30	300	300	
Ехр За	40	50	20	20	100	100	
Exp 3b	40	50	20	20	200	200	
Exp 3c	40	50	20	20	300	300	

Power Calculations



How to Increase Power?

- Compare groups with larger difference in their means
- Choose conditions with less variability (small s₁,s₂)
- Use larger sample sizes (large n₁,n₂)
 - The larger the n the larger the Power, still n is constrained by the budget.
 - How large should n be?
- Sample size calculation is used to calculate how large n should be for a study

Sample Size Calculations

- The sample size calculations can be seen as the reverse of Power calculations
- In Power calculations

The sample size is fixed, say n=100, then one calculates the Power

In sample size calculations

The Power is fixed, say 80%, then one calculates the n needed to achieve 80% Power

- Sample size calculation is important when designing new studies
 - If n is too small, it will result in not enough Power to detect differences
 - If n is too large, it will result in more than sufficient Power; hence a waste of resources

Sample Size Calculations

α=0.05	\overline{x}_1	\overline{x}_2	s_1	s_2	n_1	n_2	Power
Exp 1a	40	50	30	30	?	?	80%
Exp 1b	40	50	30	30			90%
Exp 2a	40	47	30	30			80%
Exp 2b	40	47	30	30			90%
Exp 3a	40	50	20	20			80%
Exp 3b	40	50	20	20			90%

To calculate sample size

Need: delta= $\overline{x}_1 - \overline{x}_2$

sd= alpha= Power=

Sample Size Calculations

α=0.05	\overline{x}_1	\overline{x}_2	s_1	s_2	n_1	n_2	Power
Exp 1a	40	50	30	30	142	142	80%
Exp 1b	40	50	30	30	190	190	90%
Exp 2a	40	47	30	30	289	289	80%
Exp 2b	40	47	30	30	387	387	90%
Ехр За	40	50	20	20	64	64	80%
Exp 3b	40	50	20	20	85	85	90%

To calculate sample size

Power.t.test(delta=10,sd=30,power=.8)

Need: delta= $\overline{x}_1 - \overline{x}_2$

sd= alpha=

Power=

n=142.24

Summary Points

 $E(\hat{\theta}) = \theta$ Point Estimate for θ : Unbiased

se $(\hat{\theta})$ is small. Minimum Variance (error)

95%CI for μ : $[\overline{x}$ -1.96*se (\overline{x}) , \overline{x} +1.96*se (\overline{x})]

Hypothesis testing: H_0 : (Default status) $\mu_1 = \mu_2$ vs. H_A : $\mu_1 \neq \mu_2$

> Type I Error: Incorrect rejection of a true H₀ $Pr(\text{Reject } H_0|H_0 \text{ is true})=\alpha$

> Type II Error: Failure to reject a false H₀ $Pr(Do not Reject H_0|H_A is true) = \beta$

p-value: Measures the credibility of the null hypothesis. A small *p-value* (e.g., p < 0.05) results in rejection of H_0

Summary Points

Test for Mean Comparison

Hypothesis Parametric test Nonparametric test

 H_0 : $\mu = \mu_0$ One-sample t-test Wilcoxon sign rank test

(One sample)

 H_0 : $\mu_{before} = \mu_{after}$ (Matched pairs) Paired test based on one-sample

 $H_0: \mu_1 = \mu_2$ Two-sample *t*-test Wilcoxon sum rank test (Two independent samples)

(Mann-Whitney test)

Summary Points

Power Analysis

Calculates what is the statistical power (*i.e.* probability) to reject H_0 in favor of a given H_A , for a given sample size n and Type I error. A statistical test is considered to have good Power if:

Power= $Pr(Reject H_0 | H_A is true) \ge 80\%$

Sample Size Calculation

Calculate the required sample size for a study, to be able to reject the H_0 in favor of a given H_A , with a given Power (80%) and for a given Type I error alpha=0.05.