

**How many study subjects are required ?
(Estimation of Sample size)**

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Objectives of this session:

Students able to

- (1) know the importance of sample size in a research project.
- (2) understand the simple mathematics & assumptions involved in the sample size calculations.
- (3) apply sample size methods appropriately in their research projects.



Why to calculate sample size?

- To show that under certain conditions, the hypothesis test has a good chance of showing a desired difference (if it exists)
- To show to the IRB committee and funding agency that the study has a reasonable chance to obtain a conclusive result
- To show that the necessary resources (human, monetary, time) will be minimized and well utilized



What do I need to know to calculate sample size?

- Most Important: sample size calculation is an **educated guess**
- It is more appropriate for studies involving **hypothesis testing**
- There is no magic involved; only statistical and mathematical logic and some algebra
- Researchers need to know something about what they are measuring and how it varies in the population of interest



■ SAMPLE SIZE:

How many subjects are needed to assure a given probability of detecting a statistically significant effect of a given magnitude if one truly exists?

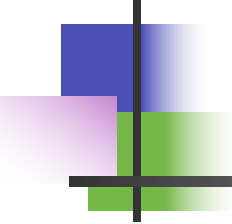
■ POWER:

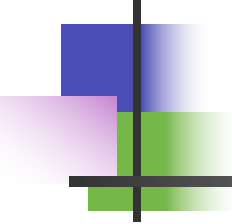
If a limited pool of subjects is available, what is the likelihood of finding a statistically significant effect of a given magnitude if one truly exists?



Before We Can Determine Sample Size We Need To Answer The Following:

1. What is the primary objective of the study?
2. What is the main outcome measure?
Is it a continuous or dichotomous outcome?
3. How will the data be analyzed to detect a group difference?
4. How small a difference is clinically important to detect?

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5. How much variability is in our target population?
 6. What is the desired α and β ?
 7. What is the anticipated drop out and non-response % ?



Where do we get this knowledge?

- Previous published studies
- Pilot studies
- If information is lacking, there is no good way to calculate the sample size



■ Type I error: Rejecting H_0 when H_0 is true

■ α : The type I error rate.

■ Type II error: Failing to reject H_0 when H_0 is false

■ β : The type II error rate

■ Power ($1 - \beta$): Probability of detecting group difference given the size of the effect (Δ) and the sample size of the trial (N)

Diagnosis and statistical reasoning

		Disease status	
		Present	Absent
Test result	+ve	True +ve (sensitivity)	False +ve
	-ve	False -ve	True -ve (Specificity)

		<u>Significance Difference is</u>	
		Present (Ho <i>not</i> true)	Absent (Ho is true)
<u>Test result</u>	Reject Ho	No error $1-\beta$	Type I err. α
	Accept Ho	Type II err. β	No error $1-\alpha$

α : significance level

$1-\beta$: power

Estimation of Sample Size by Three ways:



By using

- (1) Formulae (manual calculations)
- (2) Sample size tables or Nomogram
- (3) Softwares

Scenario 1
Precision

All studies

Scenario 2
Power

Descriptive

Hypothesis testing

Sample
surveys

Simple - 2 groups

More than 2
groups &
Complex studies



SAMPLE SIZE FOR ADEQUATE PRECISION

- In a descriptive study,
- Summary statistics (mean, proportion)
- Reliability (or) precision
- By giving “confidence interval”
- Wider the C.I – sample statistic is not reliable and it may not give an accurate estimate of the true value of the population parameter

Sample size formulae for reporting precision

For single mean : $n = Z_{\alpha}^2 S^2 / d^2$

where $S = \text{sd} (\sigma)$

For a single proportion : $n = Z_{\alpha}^2 P(1-P) / d^2$

Where , $Z_{\alpha} = 1.96$ for 95% confidence level

$Z_{\alpha} = 2.58$ for 99% confidence level



Problem 1 (Single mean)

A study is to be performed to determine a certain parameter(BMI) in a community. From a previous study a sd of 46 was obtained.

If a sample error of up to 4 is to be accepted. How many subjects should be included in this study at 99% level of confidence?

Answer

$$n = (Z_{\alpha/2})^2 \sigma^2 / d^2$$

σ : standard deviation = 46

d : the accuracy of estimate (how close to the true mean)= given sample error =4

$Z_{\alpha/2}$: A Normal deviate reflects the type I error.
For 99% the critical value =2.58

$$n = \frac{2.58^2 \times 46^2}{4^2} = 880.3 \sim 881$$

Problem 2 (Single proportion)



It was desired to estimate proportion of anemic children in a certain preparatory school. In a similar study at another school a proportion of 30 % was detected.

Compute the minimal sample size required at a confidence limit of 95% and accepting a difference of up to 4% of the true population.

Answer

$$n = (Z_{\alpha/2})^2 p(1-p) / d^2$$

p : proportion to be estimated = 30% (0.30)

d : the accuracy of estimate (how close to the true proportion) = 4% (0.04)

$Z_{\alpha/2}$: A Normal deviate reflects the type I error

For 95% the critical value = 1.96

$$n = \frac{1.96^2 \times 0.3(1 - 0.3)}{(0.04)^2} = 504.21 \sim 505$$

Scenario 2

Three bits of information required to
determine the sample size



Type I & II
errors



Clinical
effect



Variation



Quantities related to the research question (defined by the researcher)

- ❖ α = Probability of rejecting H_0 when H_0 is true
- ❖ α is called **significance level** of the test
- ❖ β = Probability of not rejecting H_0 when H_0 is false
- ❖ $1-\beta$ is called **statistical power** of the test



- Researcher **fixes** probabilities of type I and II errors

- Prob (type I error) = Prob (reject H_0 when H_0 is true) = α
 - Smaller error \Rightarrow greater precision \Rightarrow need more information \Rightarrow need larger sample size
- Prob (type II error) = Prob (don't reject H_0 when H_0 is false) = β
- Power = $1 - \beta$
 - More power \Rightarrow smaller error \Rightarrow need larger sample size



Quantities related to the research question (defined by the researcher)

- ❖ Size of the measure of interest to be detected
 - ❖ Difference between two or more means
 - ❖ Difference between two or more proportions
 - ❖ Odds ratio, Relative risk, Correlation, Regression coefficients
 - ❖ Change in R^2 , etc

- ❖ The magnitude of these values depend on the research question and objective of the study (for example, clinical relevance)



Clinical Effect Size

“What is a meaningful difference between the groups”

- It is truly an estimate and often the most challenging aspect of sample size planning
- Large difference – small sample size
- Small differences – large sample size
- Cost/benefit

Variability

Variation

All statistical tests are based on the following ratio:

Test Statistic =
$$\frac{\text{Difference between parameters}}{v / \sqrt{n}}$$

As $n \uparrow$ $v/\sqrt{n} \downarrow$ Test statistic \uparrow

Sample size formulae for comparing

$$\text{two means : } n = 2 S^2 (Z_{\alpha} + Z_{\beta})^2 / d^2$$

where S=sd; d= difference

two proportions :

$$n = \frac{(Z_{\alpha} + Z_{\beta})^2 ((p_1 q_1) + (p_2 q_2))}{(p_1 - p_2)^2}, \text{ where } q_1 = (1 - p_1), q_2 = (1 - p_2)$$

$Z_{\alpha} = 1.96$ for 95% confidence level

$Z_{\alpha} = 2.58$ for 99% confidence level ;

$Z_{\beta} = 0.842$ for 80% power

$Z_{\beta} = 1.282$ for 90% power

Example 1: *Does the consumption of large doses of vitamin A in tablet form prevent breast cancer?*

- Suppose we know from our tumor-registry data that incidence rate of breast cancer over a 1-year period for women aged 45 – 49 is 150 cases per 100,000
- Women randomized to Vitamin A vs. placebo

Example 1 continued

- Group 1: Control group given placebo pills.
Expected to have same disease rate as registry
(**150 cases per 100,000**)
- Group 2: Intervention group given vitamin A tablets.
Expected to have 20% reduction in risk (**120 cases per 100,000**)
- Want to compare incidence of breast cancer over 1-year
- *Planned statistical analysis*: **Chi-square test to compare two proportions from independent samples**

$$H_0: p_1 = p_2 \quad \text{vs.} \quad H_A: p_1 \neq p_2$$

Example 1: Does ingestion of large doses of vitamin A prevent breast cancer?

- Test $H_0: p_1 = p_2$ vs. $H_A: p_1 \neq p_2$
- Assume 2-sided test with $\alpha=0.05$ and 80% power

$$n = \frac{(Z_{\alpha/2} + Z_{\beta})^2 ((p_1q_1) + (p_2q_2))}{(p_1 - p_2)^2}, \text{ where } q_1 = (1 - p_1), q_2 = (1 - p_2)$$

- $p_1 = 150$ per 100,000 = .0015
- $p_2 = 120$ per 100,000 = .0012 (20% rate reduction)
- $\Delta = p_1 - p_2 = .0003$
- $z_{1-\alpha/2} = 1.96$ $z_{1-\beta} = .84$
- n per group = 234,882
- **Too many to recruit in one year!**

Example 2: *Does a special diet help to reduce cholesterol levels?*

- Suppose an investigator wishes to determine sample size to detect a 10 mg/dl difference in cholesterol level in a diet intervention group compared to a control (no diet) group
- Subjects with baseline total cholesterol of at least 300 mg/dl randomized

Example 2 continued

- Group 1: A six week diet intervention
- Group 2: No changes in diet
- Investigator wants to compare total cholesterol at the end of the six week study
- *Planned statistical analysis: two sample t-test (for independent samples)(comparison of two means)*

$$H_0: \mu_1 = \mu_2 \quad \text{vs.} \quad H_A: \mu_1 \neq \mu_2$$

Sample Size Formula

To Compare Two Means From Independent Samples: $H_0: \mu_1 = \mu_2$

1. α level
2. β level (1 – power)
3. Expected population difference ($\Delta = |\mu_1 - \mu_2|$)
4. Expected population standard deviation (σ_1 , σ_2)

Continuous Outcome

(2 Independent Samples)

- Test $H_0: \mu_1 = \mu_2$ vs. $H_A: \mu_1 \neq \mu_2$
- Two-sided alternative
- Assume outcome normally distributed with:

$$n_{per\ group} = \frac{2S^2 (z_{\alpha} + z_{\beta})^2}{d^2}$$

S= standard deviation; d=difference between two means;
 $Z_{\alpha} = 1.96$ for 95% confidence level; $Z_{\beta} = 1.28$ for 90% power

Example 2: *Does a special diet help to reduce cholesterol levels?*

- Test $H_0: \mu_1 = \mu_2$ vs. $H_A: \mu_1 \neq \mu_2$
- Assume 2-sided test with $\alpha = 0.05$ and 90% power
- $d = \mu_1 - \mu_2 = 10$ mg/dl
- $\sigma_1 = \sigma_2 = (50$ mg/dl)
- $z_\alpha = 1.96$ $z_\beta = 1.28$
- n per group = 525
- Suppose 10% loss to follow-up expected, adjust $n = 525 / 0.9 = 584$ per group

Problem (comparison of two means)



- A study is to be done to determine effect of 2 drugs (A and B) on blood glucose level. From previous studies using those drugs, Sd of BGL of 8 and 12 g/dl were obtained respectively.
- A significant level of 95% and a power of 90% is required to detect a mean difference between the two groups of 3 g/dl. How many subjects should be include in each group?

Answer

$$n = \frac{(SD1 + SD2)^2}{\Delta^2} * f(\alpha, \beta)$$

$$n = \frac{(8^2 + 12^2) \times 10.5}{3^2} = 242.6 \sim 243$$

in each group

Comparison of two means



Objective:

To observe whether feeding milk to 5 year old children enhances growth.

Groups:

Extra milk diet

Normal milk diet

Outcome:

Height (in cms.)

Assumptions or specifications:

Type-I error (α) = 0.05

Type-II error (β) = 0.20

i.e., Power($1-\beta$) = 0.80


Clinically significant difference (Δ) = 0.5 cm.,
Measure of variation (SD.,) = 2.0 cm.,
(from literature or “Guesstimate”)

Using the appropriate formula:

$$N = \frac{2(\text{SD})^2}{\Delta^2} f(\alpha, \beta)$$

$$\begin{aligned} &= \frac{2(2)^2}{(0.5)^2} 7.9 \\ &= 252.8 \text{ (in each group)} \end{aligned}$$

The following steps constitute a pragmatic approach to decision taking on Sample size:

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- (1) Remember that there is no stock answer.
 - (2) Initiate early discussion among research team members.
 - (3) Use correct assumptions – consider various possibilities.
 - (4) Consider other factors also– eg., availability of cases, cost, time.
 - (5) Make a balanced choice
 - (6) Ask if this number gives you a reasonable prospect of coming to useful conclusion.
 - (7) If yes, proceed if no, reformulate your problem for study.



Summary

- Define research question well
- Consider study design, type of response variable, and type of data analysis
- Decide on the type of difference or change you want to detect (make sure it answers your research question)
- Choose α and β
- Use appropriate equation for sample size calculation or sample size tables/ nomogram or software.

Any Q's

