REVIEW ARTICLE

How to calculate sample size for observational and experimental nursing research studies?

Suresh Kumar Sharma, Shiv Kumar Mudgal, Kalpana Thakur, Rakhi Gaur

Department of Nursing, College of Nursing, All India Institute of Medical Sciences, Rishikesh, Uttarakhand, India

Correspondence to: Shiv Kumar Mudgal, E-mail: peehupari05@gmail.com

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ABSTRACT

Sample size estimation for a research study is the most crucial part of the research process because it helps to produce reliable results which improve generalizability of study results. A researcher must have understanding about significance level, effect size, study’s power, and effect size; margin of error and ratio in event among population and design effect to use sample size calculation formulas efficiently. There are different formulas of sample size calculation for different types of variables measured in distinct study designs, namely descriptive, epidemiological, comparative, and interventional research studies which are covered in this article. Review authors searched online and grey literature related to sample size and read extensively. There were two authors who extracted and complied information related to topic.

KEY WORDS: Sample Size Calculation; Observational Studies; Experimental Studies; Case–Control and Cohort Studies

INTRODUCTION

Sample size estimation is the most crucial methodological part of a research study for precisely drawing inferences about population or generalization of study results. A too large sample is merely a wastage of resources and time and on the other hand, too small size sample fails to produce a conclusive and reliable results.[1] Therefore, it is essential for an investigator to estimate optimum sample size to produce reliable results, which can serve as strong foundation for evidence-based practices. It has been observed that a large number of published researches lacks clarity about the sample size estimation as well as indicate less power due to suboptimal sample size.[2-7] Sample size estimation is also essential to know the feasibility of study in terms of required cost and time. There is no magic solution for sample size estimation; however, different statistical formulas are available for sample size calculation when investigator is dealing with different types of parameters and variables in different study designs. When investigators have understanding of basic statistical conceptions for sample size calculation, they may also use available software for sample size estimation.

Review authors extensively searched, reviewed, and extracted information about basic statistical conceptions of sample size calculation and provides an easy and practical approach of sample size calculation, while investigator is dealing with different types of parameters and variables in observational studies, randomized controlled trials, and diagnostic studies.

BASIC STATISTICAL CONCEPTIONS OF SAMPLE SIZE CALCULATION

A researcher needs to have the following information in hand for the estimating sample size for a particular study: [8-10]

Null Hypothesis and Alternative Hypothesis

Experimental and correlation observational studies are used null hypothesis (indicate no observed difference), which are
set to be rejected and always used in conjunction with an alternative hypothesis (significant difference). Sometimes, it is not possible that the null hypothesis can be rejected, but it does not mean that it is true, and it just states that we are unable to produce enough evidence to reject stated null hypothesis. Thus, investigator must have clearly defined null hypothesis in hand.

Acceptable significance level

it is in continuation with the previous line such as, (it is denoted by a and acceptable level for significance means when a really true null hypotheses is rejected, in other words a = P [Type -I error]). Conventionally, 5% (α/P = 0.05) or 1% (α/P = 0.001) level of significance is considered by the biomedical researchers; which means that research accepts that there could be 5% or 1% probability that results observed are due the chance not by our intervention. The resembling confidence levels (confidence interval [CI]) for the appropriate level of significance are: (a) CI-95% for the 5% (α/P = 0.05) level of significance and (b) CI-99% for the 1% (α/P = 0.001) level of significance.

Study power

It means possibility to rule out a significant difference when it really exists. In other words, it is a probability of generalization of study findings to population at large. An increase in statistical power will decrease the possibility of Type II error (β) occurrence; means reduces risk of false-negative results. Therefore, it is denoted as 1-β. In most of clinical trial the power of 0.8 (80%) or greater is considered more appropriate to find out a statistically significant difference. Power of 80% means there are 20% chances that we may fail to identify a significant difference even though it really exists.

Expected effect size

It refers to the magnitude of the relationship between two variables as it occurs in population. For example, average hemoglobin rise with one type of diet is 5 g/dl and another is 2 g/dl; then, the absolute effect size would be 5–2 = 3 g/dl. Thus, effect size is considered as the calculated difference between the measured effects of interventional and control group. Pilot studies and previously reported data can be used to estimate the effect size. Cohen guide for effect size which is preferred by many social scientist states that it is considered to be small effect if effect size is <0.1, effect size between 0.3 and 0.5 is assumed to be medium effect and more than 0.5 is called as large difference effect. Hence, effect size of 0.5 is commonly used as it comes in reflects moderate to large difference. Nevertheless, it is important to mention that effect size and sample size are inversely proportional to each other; when effect size is large research needs smaller sample size and vice versa.

Underlying event rate in the population

It is very essential to consider a prevalence rate or bottom line event rate of the condition under study population while calculating for sample size. It is usually estimated from previous literature, including observational cohorts. For example, studying the association of alcohol and liver disease, the prevalence rate for liver disease in studying population should be known before the study.

Margin of error

It is a random sampling error, which is a likelihood of sample results variation from the population. For example, suppose there is 40% prevalence of anemia study sample and we set margin of error as 5%; it means that range of anemia in population would be between 40 ± 5, i.e., 35% and 45% prevalence of anemia.

Standard deviation (SD) in the population

A researcher must anticipate the population variance of given outcome variable that may be calculated by mean that of SD. For homogenous population, smaller sample size will be needed as variance or SD will be less in this population. For example, for studying the effect of exercise regimen on blood glucose, we include a population with blood glucose ranging from 150 to 350 mg/dl. Now, it is simple to understand that we might require more number of samples to find out differences among interventions because SD in this group will be more. Although if we consider a sample from population with blood sugar reading in between 150 and 250 mg/dl, then researcher may receive a more similar group representing homogeneity, therefore, decreases SD and number of samples for study.

One tail and two tail inferential statistical test

The choice of one-tail or two-tail test depends on the objective of the study. Research has a hypothesis that a new drug is more effective in reducing the blood pressure; then the one-tail test could be sufficient to test the hypothesis, but if not sure that the new drug may be more or less effective in lowering BP as compared to existing drug then it is always better to use two-tail test. Inputs for the one-tail and tow tail tests are same except the critical ratio (Z value); which is different in one-tail test ($Z_{1-α}$) and two-tail test ($Z_{1-α/2}$) as depicted in Table 1.

Design effect (DEFF)

The sample size calculation formulas provided in this article helps to estimate an adequate sample size when simple random

<table>
<thead>
<tr>
<th><strong>Table 1:</strong> Z values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level of confidence (%)</td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>0.05 (95)</td>
</tr>
<tr>
<td>0.01 (99)</td>
</tr>
<tr>
<td>0.001 (99.9)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Power of the test</th>
<th>Z value ($Z_{1-α}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.80</td>
<td>0.84</td>
</tr>
<tr>
<td>0.90</td>
<td>1.28</td>
</tr>
<tr>
<td>0.95</td>
<td>1.65</td>
</tr>
<tr>
<td>0.99</td>
<td>2.33</td>
</tr>
</tbody>
</table>
sampling technique is assumed to be used in the study. However, whenever simple random sampling is not possible to use, then calculated sample may not be adequate and to overcome this problem, calculated sample size has to be adjusted in terms of DEFF. This is equal to the ratio of expected variance in cluster random sampling with expected variance in simple random sampling. The DEFF is generally \( \geq 1 \). Therefore, in cluster design, we assume \( DEFF = 2 \).

\[
DEFF = 1 + \delta(n-1) \quad (\text{Hers, } \delta = \text{interclass correlation}; \quad n = \text{common size of the cluster})
\]

**EXAMPLES FOR SAMPLE SIZE ESTIMATION FOR OBSERVATIONAL AND EXPERIMENTAL STUDIES**[11-23]

**Estimation of Sample Size for Cross-sectional or Descriptive Research Studies**

These studies or surveys are generally conducted to find out, observe, describe, and document aspects of a situation as it naturally occurs. It is not used to identify the causation of something, such as a reason of any epidemic. Researchers do not manipulate variables. A researcher might collect cross-sectional data on past alcohol habits and current diagnoses of liver disease, for example.

Example I: Sample size in case data is on nominal/ordinal scale and proportion is one of the parameters

\[
\text{Sample size } n = \frac{(Z_{1-\alpha/2})^2 \cdot p \cdot q}{d^2}
\]

\( n \) = Desired sample size
\( Z_{1-\alpha/2} \) = Critical value and a standard value for the corresponding level of confidence.
(At 95% CI or 5% level of significance (type-I error) it is 1.96 and at 99% CI it is 2.58)
\( p \) = Expected prevalence or based on previous research
\( q = 1-p \)
\( d \) = Margin of error or precision

A researcher wants to carry out a descriptive study to understand the prevalence or proportion of diabetes mellitus among adults in a city. A previous study stated that diabetes in the adult population was 40%. At 95% CI and 5% margin of error, calculate the sample required to conduct other new research?

On applying:

\[
(n) = \frac{(1.96)^2 \cdot (0.4)(0.6)(0.05)}{(0.05)^2}
\]

\( n \) = 368.79

\( n \) = 369+37 (considering 10% dropout of study participants)

Sample size \( n \) = 406

\( Z_{1-\alpha/2} = 1.96 \)

\( p = 40\% = 0.4 \)

\( q = 1-0.4 = 0.6 \)

\( d = 5\% = 0.05 \)

Hence, for conducting a new cross-sectional study to identify the prevalence or proportion of DM among patients, minimum 406 subjects will be required.

Example II: Sample size, when mean is of the study or data are on interval/ratio scale

\[
\text{Sample size } n = \frac{(Z_{1-\alpha/2})^2 \cdot (\sigma)^2}{d^2}
\]

\( n \) = Desired number of samples
\( Z_{1-\alpha/2} \) = Standardized value for the corresponding level of confidence.
(At 95% CI, it is 1.96 and at 99% CI or 1% type I error it is 2.58)
\( d \) = Margin of error or rate of precision
\( \sigma \) = SD which is based on previous study or pilot study

Suppose a researcher wants to know the average hemoglobin level among adults in the city at 95% CI and the margin of error is 2 g/dl. From a previous study, the SD of hemoglobin level among adults was found to be 4.5 g/dl. How many study subjects will be required to conduct a new study?

On applying:

\[
(n) = \frac{(1.96)^2 \cdot (4.5)^2}{(2)^2}
\]

\( n \) = 19.44

\( n \) = 19+2 (considering 10% dropout of study participants)

Sample size \( n \) = 21

\( Z_{1-\alpha/2} = 1.96 \)

\( \sigma = 4.6 \text{ g/dl} \)

\( d = 2 \text{ g/dl} \)

Hence, for conducting a new cross-sectional study to estimate the average hemoglobin level among adults, minimum 21 subjects will be required.

Example III: Sample size for finite population

\[
\text{Sample required } n = \frac{N}{1+N*d^2}
\]

\( N \) = Total population
\( d \) = Margin of error or precision

Suppose a researcher wants to conduct a survey to assess the prevalence of regular foot care among adults with diabetes
in a town. Now, if the population of adults suffering from diabetes is 2500 and researcher wants to work at 95% CI, where margin of error or precision rate decided by researcher is 5%. Find out the sample size for the upcoming survey?

On applying: Above formula

\[ n = \frac{2500}{1 + 2500 \times 0.05^2} \]

\[ n = 344.82 \]

\[ n = 345 + 36 \text{ (considering 10\% dropout of study participants)} \]

Sample size (n) = 379

Hence, for conducting a new cross-sectional study to assess the prevalence of regular foot care among adults with diabetes, minimum (379 subjects) will be required.

Sample Estimation for Case–control Studies

It is a study that determines the cause and effect to see whether exposure is correlated with an outcome or not. By way of explanation, it determines whether an exposure is correlated with an outcome (i.e., disease or condition of interest). It is a type of observational study in which commonly assumed causation is studied among two groups differing in outcome. For example, a case–control research to find out the relationship between alcohol and liver disease.

Example I: Sample size, when proportion is parameter of the study or data are on nominal/ordinal scale:

\[ n = \frac{(r + 1) \times p(1 - p) \left( Z_{1 - \beta} + Z_{1 - \alpha/2} \right)^2}{\left( p_1 - p_2 \right)^2} \]

n = Desired number of samples
r = Control to cases ratio (1 if same numbers of subject in both groups)
p = Proportion of population = (P_1 + P_2)/2
\( Z_{1 - \beta} \) = It is the desired power (0.84 for 80% power and 1.28 for 90% power)
\( Z_{1 - \alpha/2} \) = Critical value and a standard value for the corresponding level of confidence.
(At 95% CI it is 1.96 and at 99% CI or 1% type I error it is 2.58)
P_1 = Proportion in cases
P_2 = Proportion in controls

If a researcher wants to conduct a case–control design to identify the link between deep vein thrombosis and pulmonary embolism. He decides to work at 95% CI and 80% power of the study. He assumes expected proportion in case is 40% and control group is 30% and decides to have a same number of cases in both groups. Find out the optimum sample size for each group in study.

On applying:

\[ n = \frac{(r + 1) \times p(1 - p) \left( Z_{1 - \beta} + Z_{1 - \alpha/2} \right)^2}{\left( p_1 - p_2 \right)^2} \]

\[ n = \frac{2 \times 178.36}{1} \]

\[ n = 356.72 \]

\[ n = 357 + 36 \text{ (considering 10\% dropout of study participants)} \]

Sample size (n) = 393

p_1 = 40\% = 0.4
p_2 = 30\% = 0.3
r = 1
\( Z_{1 - \beta} = 0.84 \)
\( Z_{1 - \alpha/2} = 1.96 \)
p = 0.4 + 0.3/2 = 0.35

Therefore, a researcher is supposed to take a minimum of 393 subjects in case as well as in the control group.

Example II: Sample size in case data is on interval/ratio (quantitative) scale and mean as a parameter of the study

\[ n = \frac{(r + 1) \times \sigma^2 \left( Z_{1 - \beta} + Z_{1 - \alpha/2} \right)^2}{d^2} \]

n = Number of samples which we need to find out
r = Control to cases ratio
p = Proportion of population = P_1 + P_2/2
\( Z_{1 - \beta} \) = It is the desired power (0.84 for 80% power and 1.28 for 90% power)
\( Z_{1 - \alpha/2} \) = Critical value and a standard value for the corresponding level of confidence.
(At 95% CI it is 1.96 and at 99% CI or 1% type I error it is 2.58)
\( \sigma \) = SD which is based on a previous study or pilot study
d = Effect size (difference in the means from previous studies or pilot study)

Suppose a researcher wants to conduct a study to identify the association between the amount or quantity of alcohol consumption and liver cirrhosis. From the previous study, he finds that the mean difference in alcohol consumption between the case and control groups was 10 ml/day and SD was 18 ml/day. He decides to conduct the study at 95% CI and fix power of the study at 80%. Find the sample size to have a equal number in case and control group for study.

On applying:

\[ n = \frac{(r + 1) \times \sigma^2 \left( Z_{1 - \beta} + Z_{1 - \alpha/2} \right)^2}{d^2} \]

\[ n = \frac{(1 + 1) \times (18)^2 \left( 0.84 + 1.96 \right)^2}{10} \]

\[ n = 352.4 \]

\[ n = 353 + 36 \text{ (considering 10\% dropout of study participants)} \]

Sample size (n) = 389
Sample size estimation for cohort studies

It is defined as a longitudinal research study that includes a category of people who share the same characteristic, typically those who experienced a common event in a selected period, such as disease or education. There is no control group, and no intervention or treatment is given to patient that is why it is different from randomized control trials. For example, researcher asks study subject to record their eating practices over the period of time and then correlates between eating practices and their sleep pattern.

Example I: Sample size estimation for independent cohort studies

\[
\begin{align*}
Z_{1-\beta} & = 0.84 \\
Z_{1-\alpha/2} & = 1.96 \\
p_0 & = 0.2 \\
p_1 & = 0.3 \\
m & = 1 \\
p & = \frac{p_1 m + p_0}{m + 1}
\end{align*}
\]

A researcher wants to identify the impact of smoking on lung cancer. A previous study stated that proportion of lung cancer in the case group is 30% and in the control group is 20%. Calculate the sample size, if a researcher wishes to conduct the study at 95% CI and 80% power of the study with the equal number of case and control subjects.

On applying:

\[
\begin{align*}
n = \frac{\left[1.96 \sqrt{\left\{(1 + 1/m) p_1 (1 - p_1)\right\}} + Z_{1-\beta}\right]}{\left[\left(p_0 * (1 - p_0 / m) \right.\right. (1 - p_1)]^2 \left(p_0 - p_1\right)^2}}
\end{align*}
\]

\[n = 183.17\]

Sample size (n) = 183+18 = 201 (considering 10% dropout of study participants)

Therefore, a researcher will require a minimum (201 subjects) for the study.

Sample size estimation for comparative studies

It is the study design in which comparison is done between two or more groups on the basis of selected attributes such as knowledge, perception, and attitude. A multidisciplinary approach is best used for this type of researches. In case of comparative studies, quantitative data of secondary analysis are outspread.

Example I: Sample size in case data is on nominal/ordinal scale and proportion is parameter of the study

\[
\begin{align*}
n = \text{Sample size for one group that we need to find out} \\
p_1 \text{ and } p_2 \text{ = Proportion of two groups} \\
c \text{ = Standard value for the corresponding level of } \alpha \text{ and } \beta \text{ selected for the study. It is as follows:}
\end{align*}
\]

\[
\begin{align*}
Z_{1-\alpha/2} & = 0.8 \\
Z_{1-\alpha/2} & = 1.96 \\
p_0 & = 0.2 \\
p_1 & = 0.3 \\
m & = 1
\end{align*}
\]

A researcher wants to compare the knowledge among Group A and Group B. From the previous study, proportion of the two groups is taken, i.e., 40% and 20%, respectively. A researcher wants 95% CI and 80% power for the study. Calculate the total subjects required for a new study.

On applying:

\[
\begin{align*}
n = \frac{p_1 (1 - p_1) + p_2 (1 - p_2) \cdot C}{\left(p_1 - p_2\right)^2}
\end{align*}
\]

A researcher wants to compare the knowledge among Group A and Group B. From the previous study, proportion of the two groups is taken, i.e., 40% and 20%, respectively. A researcher wants 95% CI and 80% power for the study. Calculate the total subjects required for a new study.

On applying:

\[
\begin{align*}
n = \frac{p_1 (1 - p_1) + p_2 (1 - p_2) \cdot C}{\left(p_1 - p_2\right)^2}
\end{align*}
\]
Sharma et al. Calculation of sample size in nursing research

\[
(n) = \frac{0.4 (1 - 0.4) + 0.2 (1 - 0.2) \times 7.85}{(0.4 - 0.2)^2}
\]

\[
(n) = 10 \times 7.85
(n) = 78.5 + 7 \text{ (considering 10% dropout of study participants)}
\]

Sample size \( n \) = 86

\[ p_1 = 40\% = 0.4 \]
\[ p_2 = 20\% = 0.2 \]
\[ C = 7.85 \text{ (at 95\% CI and 80\% power)} \]

Therefore, a researcher will require minimum (86 subjects) for the study in each group.

Example II: Sample size in case data is on interval/ratio scale and mean is parameter of the study

\[
\text{Sample size } n = \left(\sigma_1^2 + \sigma_2^2\right) \left(Z_{1-\beta} + Z_{1-\alpha/2}\right)^2
\]

\[
d = \text{difference in means of two group (effect size)}
\]
\[
\sigma_1 = \text{SD of Group 1}
\]
\[
\sigma_2 = \text{SD of Group 2}
\]
\[
Z_{1-\beta} = \text{Critical value and a standard value for the corresponding level of confidence.}
\]
\[
\text{(At 95\% CI it is } 1.96 \text{ and at 99\% CI, or 1\% type I error it is } 2.58)\]

Assuming from the previous study that the mean (SD) of HbA1c level in Group A and Group B was 5.5 (2.2) and 7.5 (2.0), respectively. At 95\% CI and 80\% power of the study, find out the sample size for the new study.

On applying:

\[
(n) = \left(2.2^2 + 2.0^2\right) \left(0.84 + 1.96\right)^2
\]

\[
(n) = \left(8.84\right)^2 \left(2.8\right)^2
\]

\[
(n) = 153.15 + 15 \text{ (considering 10\% dropout)}
\]

Sample size \( n \) = 168

Therefore, a researcher will require a minimum (168 subjects) for the study in each group that means a total of 336 study subjects are required.

Example III: Sample size for comparison between two groups and for continuous variables

\[
\text{Sample size } n = 1 + 2C \left(\text{SD/d}\right)^2
\]

\[
(n) = 1 + 2 \times 7.85 \left(30/15\right)^2
\]

\[
(n) = 1 + 62.8
\]

\[
(n) = 63.8 + 6 \text{ (considering 10\% dropout)}
\]

Sample size \( n \) = 70

\[ d = 15 \text{ mg/dl} \]
\[ \text{SD} = 30 \text{ mg/dl} \]
\[ C = 7.85 \text{ (at 95\% CI and 80\% power)} \]

Therefore, researcher will require minimum of 70 subjects for the study in each group that means a total of 140 study subjects are required.

Sample Size Estimation for Experimental Studies

Experimental studies or randomized controlled trials are the studies in which researcher artificially manipulates variables under the study. Randomization and control group are an important aspect in these types of studies. In this investigator provides intervention and study its effect and compare in experiential and control group. There are following types of comparison:

- Superiority trial: The aim of this type of trial is to view that a novice drug/treatment/intervention is superior to a control treatment.
- Equivalence trial: The aim of this type of trial is to view that novice treatment/intervention is equally effective to control treatment.
- Non-inferiority trial: The aim of this type trial is to view that novice treatment/intervention is effective but must not superior than the control treatment.

Example I: Sample size to rule out the difference (effect size) among two groups (on the basis of difference in proportion or for dichotomous nominal/ordinal variables)

\[
\text{Sample size } n = 1 + 2C \left(\text{SD/d}\right)^2
\]

\[
(n) = 1 + 2 \times 7.85 \left(30/15\right)^2
\]

\[
(n) = 1 + 62.8
\]

\[
(n) = 63.8 + 6 \text{ (considering 10\% dropout)}
\]

Sample size \( n \) = 70

\[ d = 15 \text{ mg/dl} \]
\[ \text{SD} = 30 \text{ mg/dl} \]
\[ C = 7.85 \text{ (at 95\% CI and 80\% power)} \]

A researcher wants to know the effect of a drug A and compare the drug A with placebo. He thinks that if drug A decreases the blood sugar level by 15 mg/dl as compared to placebo, then it should be considered as clinically significant. Suppose a previous study stated that the SD was 30 mg/dl. He decided to conduct the study at 95\% CI with 80\% power of study.

On applying:

\[
\text{Sample size } n = 1 + 2C \left(\text{SD/d}\right)^2
\]

\[
(n) = 1 + 2 \times 7.85 \left(30/15\right)^2
\]

\[
(n) = 1 + 62.8
\]

\[
(n) = 63.8 + 6 \text{ (considering 10\% dropout)}
\]

Therefore, researcher will require minimum of 70 subjects for the study in each group that means a total of 140 study subjects are required.
Sharma et al. Calculation of sample size in nursing research

S = Pooled SD (both comparison groups)
p = Response rate of standard intervention
p = Response rate of new intervention

A researcher wants to assess the difference in the effectiveness of treatment A (new intervention) and treatment B (standard intervention) for the treatment of stroke in two-month protocol.

Researcher assumes all parameters as:

p = 0.30; p = 0.45; α=0.05 (95% CI); β=0.20 (80% power);

a. Non-inferiority trial:

\[ n = 2 \left( \frac{0.84 + 1.645^2 \times 0.30}{0.10} \right) \times (1 - 0.3) \]

n = 260.40+26 (considering 10% dropout)

Sample size (n) = 286

b. Equivalence trial

\[ n = 2 \left( \frac{0.84 + 1.645^2}{0.1} \right) \times (1 - 0.3) \]

n = 330.45+33 (considering 10% dropout)

Sample size (n) = 363

c. Clinical superiority trial

\[ n = 2 \left( \frac{0.84 + 1.645^2}{0.21 - 0.1} \right) \times 0.3 \times (1 - 0.3) \]

(n) = 2510.34*0.30*0.7

(n) = 214+21 (considering 10% dropout)

Sample size (n) = 235

Electronic Resources for Sample Size Calculation

Today, there are many sample size calculation software or internet URL links available. Some of the commonly used sample size calculation electronic resources are given below:
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CONCLUSION

This article has provided a simple and comprehensive discussion about sample size calculation for the observational and experimental nursing studies, which will enable nurse researcher to identify the appropriate sample size for their studies to produce more reliable results to enhance the generalization of study findings.

REFERENCES


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