Research Technical Note

How to Establish Sample Size Before Starting a Scientific Experiment

One important factor for consideration prior to beginning a new experiment, especially when dealing with animal models, is sample size. While it is always possible to repeat experiments at a later time to gather the necessary sample size, unplanned experiments can add significant time delays and cost. Sample size is important for showing statistical significance. Sample size and power selection prior to the experiment helps to decrease resources, space and time to obtain valuable results.

Factors to consider for determining minimum sample size:

- Method of statistical analysis. This is often driven by the set-up of the experiment and the number/type of variables being measured.
- Intensity of condition being analyzed and the overall variability between conditions. If the two conditions are inherently very similar, then it is harder to detect differences in data. Analyzing mild or subtle conditions is far more difficult than looking at strong variations and will often require larger sample sizes.
- Predicted success rate of treatment/procedure. Once the conditions for the experiment are established, the actual testing may begin. The nature of the procedure and its subsequent likelihood for success helps determine the number of subjects needed; where a lower success rate requires more subjects.
- Precision of results. Consider the ability of a sample to predict the true mean of a population. The precision of the results depends on the variability of the parameter of interest and the accuracy of the method of measurement.
- Confidence intervals and related parameters. Statistics lets the user decide what constitutes statistical significances (95% confidence interval is a common choice). Using a higher standard of significances requires larger sample sizes.
- Type I error and Type II error. Another factor is the ability to correctly determine a condition which is limited by the measurement/detection method used. In most cases it is not possible to determine with 100% accuracy whether a treatment failed or succeeded. Both false positives (Type I error) and false negatives (Type II error) are detrimental to accurate results. The higher the possibility of either type of error the larger the sample size must be to achieve significant results.

BEFORE CONSIDERING STATISTICS...

Every effort should be made to choose a protocol which limits the number of subjects needed in each group. For this reason it is important to limit inconsistencies in group conditions and use appropriate, accurate data collection methods. A well planned experimental protocol can help control costs, reduce time, minimize space requirements and maximize resources.

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Example of How to Establish Sample Size before an Experiment

MYOCARDIAL INFARCTION MODEL

To illustrate the process of sample size determination, consider the following example:

What is the minimum sample size needed to show that the treatment of myocardial infarction is statistically significant? For this example we will use the 2-tailed Student's T-test for statistical analysis.

$$N=2\left[\frac{(z_\alpha + z_\beta) * SD}{\Delta}\right]^2$$

Consider the extent of damage caused by the myocardial infarction prior to treatment. What size of infarct (scar area/total area of left ventricle) is necessary to claim and show the statistical significance of the treatment? In clinical research, this is a question of what segment of the disease population the treatment should address. For the sake of illustration let us assume that a scar size of 45%, as created by coronary artery ligation, is an appropriate starting point for treatment. Let us further assume that the standard deviation (SD) of the MI size as measured by planimetry is 5%**[1]**.

Next, we must consider the efficacy of the treatment. What would be the number of scar size area improvements post-coronary artery occlusion that the scientific community would consider to be successful? In this example 7-10% might be a success, so let us assume 8.5%.

Based on the previous assumptions the combined variation of the experiment (Δ) is 8.5%*45% = 3.825%

Next, we choose the assumed values for Type I (alpha) and Type II (beta) error.

- Assuming an alpha of 0.05 the z-value (z_α) will be 1.96.
- Assuming a beta of 0.1 the z-value (z_β) will be 1.28.

Using all of the values above, we can use the sample size formula to estimate the number of subjects needed for the 2-tailed T-test.

$$N=2\left[\frac{(z_\alpha + z_\beta) * SD}{\Delta}\right]^2 = 2\left[\frac{(1.96 + 1.28) * 5}{3.825}\right]^2 = 36$$

Thus, a minimum of 36 subjects per group would be sufficient to show the treatment made a significant difference when analyzed by the Student's 2 tail t-test (α=0.05 and β=0.1).

REFERENCE

[1] Takagawa J, Zhang Y, Wong ML, Sievers RE, Kapasi NK, Wang Y, Yeghiazarians Y, Lee RJ, Grossman W, Springer ML. "Myocardial infarct size measurement in the mouse chronic infarction model: comparison of area- and length-based approaches." J Appl Physiol. 2007 Jun; 102(6): 2104-11

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