Chapter 814

Reference Intervals for Normal Data

Introduction

This routine calculates the sample size needed to obtain a specified relative width of a confidence interval about the limits of a reference interval calculated from normally distributed data.

Consider a measurement made on a population of individuals (usually healthy patients). A **reference interval** (RI) of this measurement gives the boundaries between which a typical measurement is expected to fall. When a measurement occurs that is outside these reference interval boundaries, there is cause for concern. That is, the measurement is unusually high or low. The reference interval is often presented as percentiles of a reference population, such as the 2.5th percentile and the 97.5th percentile. Of course, the choice of the reference population is important, and you would expect that there is often differences according to age, size, and so on. Hence, the population must be partitioned into homogeneous subpopulations and individual reference intervals determined for each.

This procedure calculates sample sizes based on the methods shown in Harris and Boyd (1995), Horn and Pesce (2005), and Machin, Campbell, Tan, and Tan (2018).

Technical Details

Reference Interval

Assume that a sample of N observations of a normally distributed random value Y are summarized by their mean and standard deviation. The $100(1-\alpha)\%$ *reference interval* is given by the *reference limits* R_L and R_U where $R_L = \bar{y} - cs\sqrt{(N+1)/N}$ and $R_U = \bar{y} + cs\sqrt{(N+1)/N}$.

The *critical value* c is obtained from the t distribution or the normal distribution as $c = t_{N-1,1-\frac{\alpha}{2}}$ or $c = z_{1-\frac{\alpha}{2}}$, respectively.

The width of this interval is

$$W_{RI} = 2cs\sqrt{(N+1)/N}.$$

Confidence Intervals of Reference Limits

Each of the reference limits are data estimates of population parameters, so they can be estimated by confidence intervals. These $100(1-\gamma)\%$ CIs are

$$R_L - z_{1-\frac{\gamma}{2}} SE(R_L) \quad \text{and} \quad R_L + z_{1-\frac{\gamma}{2}} SE(R_L)$$

$$R_U - z_{1-\frac{\gamma}{2}} SE(R_U) \quad \text{and} \quad R_U + z_{1-\frac{\gamma}{2}} SE(R_U)$$

where

$$SE(R_L) = SE(R_U) = s\sqrt{(2+c^2)/2N}$$

The width of these intervals is

$$W_{CI} = 2z_{1-\frac{\gamma}{2}} s\sqrt{(2+c^2)/2N}.$$

Relative Margin of Error

The ratio of the widths of these intervals is used as a basis for determining the sample size. This ratio is called the relative percentage margin of error and is calculated as

$$ME = 100\, W_{CI}/W_{RI}.$$

This is called the relative margin of error because the *s* cancels out of the ratio.

Hence, the sample size is selected to obtain a specified value of *ME*.

Example 1 – Calculating Sample Size

Suppose a study is being planned to obtain 90%, confidence intervals of the 95% reference limits of blood pressure (BP) for a certain gender and age range. Sample sizes are need for relative margin of errors from 10% to 30%. The goal is to determine the necessary sample size for each value of the relative margin of error.

Setup

If the procedure window is not already open, use the PASS Home window to open it. The parameters for this example are listed below and are stored in the **Example 1** settings file. To load these settings to the procedure window, click **Open Example Settings File** in the Help Center or File menu.

Design Tab

Solve For	**N (Sample Size)**
Reference Interval Distribution	**Student's t (Exact)**
RI (Reference Interval Percentage)	**95**
CL (Confidence Level Percentage)	**90**
ME (Percentage Margin of Error)	**10 20 30**

Output

Click the Calculate button to perform the calculations and generate the following output.

Numeric Reports

Numeric Results

Solve For: N (Sample Size)
Reference Interval Distribution: Student's t (Exact)

Sample Size N	Relative Margin of Error ME	Percentage of the Population in the Reference Interval RI	Confidence Level of the Confidence Intervals for the Reference Limits CL	Width of the Confidence Intervals for the Reference Limits W_{CL}*	Width of the Reference Interval W_{RI}*
203	10.0	95	90	0.395	3.953
48	20.0	95	90	0.811	4.065
19	29.9	95	90	1.290	4.311

* The standard deviation was assumed to be 1.0.

N The total sample size of the study.
ME The margin of error, which is a measure of imprecision. It is the percentage that the width of the confidence interval of the reference limits is of the width of the reference interval. ME = 100 W_{CL} / W_{RI}.
RI The reference interval percentage. It is the percentage of data values that are anticipated to be between the reference limits assuming a normal distribution.
CL The confidence level of the confidence intervals made for each reference limit. It is a percentage.
W_{CL} The width of the confidence interval made for each reference limit. The standard deviation was assumed to be 1.0.
W_{RI} The width of the reference interval. The standard deviation was assumed to be 1.0.

Summary Statements

A single-group design will be used to obtain a two-sided reference interval based on the Student's t distribution. To obtain a 95% reference interval with 90% confidence intervals of the limits, and with a target relative margin of error of 10, 203 subjects will be needed. The relative margin of error (ME) is the width of the confidence intervals of the limits (WCL) as a percentage of the width of the reference interval (WRI) (ME = 100 * WCL / WRI). For this scenario, if the standard deviation is assumed to be 1, the width of the confidence intervals of the reference limits is 0.395, and the width of the reference interval is 3.953.

Dropout-Inflated Sample Size

Dropout Rate	Sample Size N	Dropout-Inflated Enrollment Sample Size N'	Expected Number of Dropouts D
20%	203	254	51
20%	48	60	12
20%	19	24	5

Dropout Rate	The percentage of subjects (or items) that are expected to be lost at random during the course of the study and for whom no response data will be collected (i.e., will be treated as "missing"). Abbreviated as DR.
N	The evaluable sample size at which the reference interval is computed. If N subjects are evaluated out of the N' subjects that are enrolled in the study, the design will achieve the stated reference interval.
N'	The total number of subjects that should be enrolled in the study in order to obtain N evaluable subjects, based on the assumed dropout rate. After solving for N, N' is calculated by inflating N using the formula N' = N / (1 - DR), with N' always rounded up. (See Julious, S.A. (2010) pages 52-53, or Chow, S.C., Shao, J., Wang, H., and Lokhnygina, Y. (2018) pages 32-33.)
D	The expected number of dropouts. D = N' - N.

Dropout Summary Statements

Anticipating a 20% dropout rate, 254 subjects should be enrolled to obtain a final sample size of 203 subjects.

References

Harris, E.K. and Boyd, J.C. 1995. 'Statistical Bases of Reference Values in Laboratory Medicine'. Marcel Dekker. New York, New York.

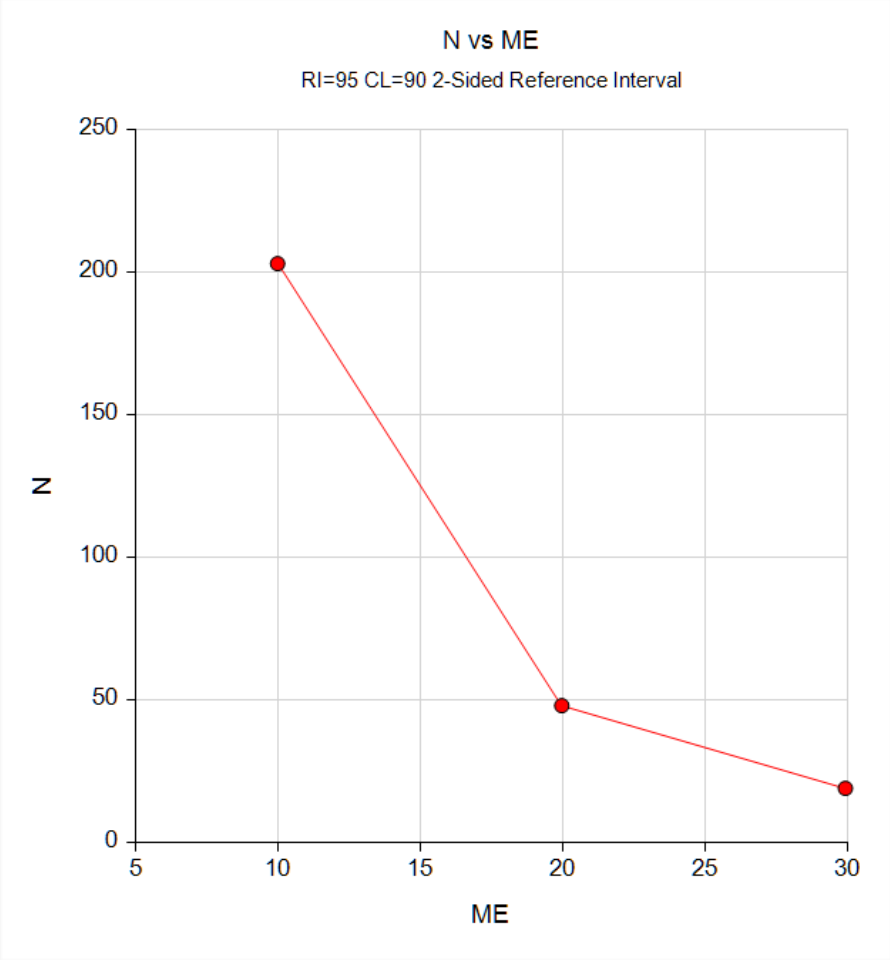
Horn, P.S. and Pesce, A.J. 2005. 'Reference Intervals - A User's Guide'. AACC Press. Washington D.C.

Machin, D, Campbell, M.J., Tan, S.B, Tan, S.H. 2018. 'Sample Sizes for Clinical, Laboratory and Epidemiology Studies, Fourth Edition'. John Wiley and Sons. Hoboken, New Jersey.

This report shows the calculated sample size for each of the scenarios.

Plots Section

Plots



This plot shows the sample size versus the margin of error (ME).

Example 2 – Validation using Harris and Boyd (1995)

Harris and Boyd (1995) page 69 give an example of a study planned to obtain 90% confidence intervals for the 95% reference limits. They calculate the sample size for a relative margin of error of 20% assuming the reference interval uses the normal distribution. They obtain a sample size value of 51.4.

Setup

If the procedure window is not already open, use the PASS Home window to open it. The parameters for this example are listed below and are stored in the **Example 2** settings file. To load these settings to the procedure window, click **Open Example Settings File** in the Help Center or File menu.

Design Tab	
Solve For	**N (Sample Size)**
Reference Interval Distribution	**Normal (Approximate)**
RI (Reference Interval Percentage)	**95**
CL (Confidence Level Percentage)	**90**
ME (Percentage Margin of Error)	**20**

Output

Click the Calculate button to perform the calculations and generate the following output.

Numeric Results

Solve For: N (Sample Size)
Reference Interval Distribution: Normal (Approximate)

Sample Size N	Relative Margin of Error ME	Percentage of the Population in the Reference Interval RI	Confidence Level of the Confidence Intervals for the Reference Limits CL	Width of the Confidence Intervals for the Reference Limits W_{CL}*	Width of the Reference Interval W_{RI}*
51	19.9	95	90	0.787	3.958

* The standard deviation was assumed to be 1.0.

PASS also calculates the sample size to be 51 which is within rounding of the 51.4 calculated by Harris and Boyd (1995).