Chapter 821

Reference Intervals for Clinical and Lab Medicine

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 with a covariate (such as age).

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. In the discussion to follow, we will assume *age* is the covariate, but the methodology works for any continuous covariate.

This procedure calculates sample sizes for an **age-specific reference interval** for cross-sectional studies using the methodology given in Bellera and Hanley (2007). They provide sample size results for the reference interval methodology of Altman (1993), Royston and Wright (1998), and Royston and Sauerbrei (2008).

This methodology gives results that are similar to those obtained by quantile regression.

Technical Details

Background

There are many methods that have been proposed for estimating reference intervals (RIs). The article by Bellera and Hanley (2007) provides a method for computing necessary sample sizes for a specific method developed in a series of articles by Altman and Royston. This section will describe this method.

Data Collection

Data should be collected specifically to calculate an RI, with only one measurement per subject. The subjects selected should form a representative group without prior selection to avoid biasing the results. It is desirable to have approximately equal numbers of individuals at each age.

Models of the Mean and Standard Deviation (SD)

The fundamental assumption of this method is that at each age, the measurement of interest is normally distributed with a given mean and standard deviation. Furthermore, the means and standard deviations are smooth functions across age. Various types of models are available to model the mean and SD functions, including polynomial, fractional polynomial, and ratios of polynomials.

The reference interval equation takes the form

$$Y = M(X) + z_\alpha \, SD(X), \quad 0 < X < \infty$$

where X is age, M(X) is an estimate of the mean of Y at X, SD(X) is an estimate of the standard deviation of Y at X, and z_α is the appropriate percentile of the standard normal distribution. M(X) is estimated using nonlinear least squares. SD(X) is estimated using a separate (possibly nonlinear) least squares regression in which Y is replaced by the scaled absolute residuals. The scaling of the residuals (Y - M(X)) is made by multiplying them by $\sqrt{(\pi/2)}$ (which is approximately equal to 1.2533).

The Six Step Estimation Process

The following six step procedure was suggested by Altman and Chitty (1994).

Step 1 – Fit the Mean Function

The first step is to fit the mean function with a reasonable, well-fitting model. This is usually accomplished by fitting a polynomial, a fractional polynomial, or the ratio of two polynomials. Also, the possibility of transforming Y using the logarithm, square root, or some other power transformation function is considered.

During this step, various models are investigated by considering the goodness-of-fit (R^2), the Y-X scatter plot, and the residual versus X plot.

Step 2 – Study the Residuals from the Mean Fit

During this step, the residuals (differences) between the data and the fitted line are examined more closely. Usually, the vertical spread of the residuals will change with X. This will be treated in the next step. But another feature that should be considered is whether the residuals are symmetric or skewed about zero across X. Skewing is not modelled during the next step, so it must be fixed here. Skewing is usually corrected by using the logarithm of Y instead of Y itself.

Step 3 – Fit a Standard Deviation Function

The next step is to estimate the SD function. This is usually accomplished by fitting a linear polynomial to the scaled absolute residuals (SAR). The scaling factor is $\sqrt{(\pi/2)}$. Occasionally, a quadratic polynomial is required, but usually nothing more complicated is needed.

Step 4 – Calculate Z-Scores

The next step is to calculate a z-score for each observation. The z-score for the kth observation is calculated using

$$Z_k = \frac{Y_k - M(X_k)}{SD(X_k)}$$

Step 5 – Check the Goodness-of-Fit of the Models

The first item to consider is the value of R^2. This value should be as high as possible, although a high R^2 is not the only consideration. But it is a starting point. The plot of the fit of the mean overlaid on the X-Y plot allows you determine whether the model is appropriate.

The z-scores should also be checked to determine that they are approximately normally distributed. This can be done by looking at a normal probability plot of the z-scores and by considering the results of a normality test such as the Shapiro-Wilk test.

Step 6 – Calculate the Percentiles

The final step is to calculate the percentiles. The formula for a percentile is reference interval equation takes the form

$$Y_{(X,\alpha)} = M(X) + z_\alpha \, SD(X)$$

Sample Size Formulas

Bellera and Hanley (2007) derive the following formula relating the sample size to the precision of a confidence interval for a reference limit (note that a reference interval is composed of a high, and a low, reference limit). The formula is

$$n \geq \frac{z_{1-\frac{\alpha}{2}}^2 \left(D + \frac{z_p^2}{2} \right)}{z_{1-\frac{\beta}{2}}^2 \left(\frac{\Delta}{100} \right)^2}$$

The parameter p is the quantile of the $100p\%$ reference limit of interest and corresponds to the standard normal deviate, z_p.

The parameter α gives the confidence level of the $100\alpha\%$ confidence interval for a reference limit and corresponds to the standard normal deviate, $z_{1-\alpha/2}$.

The parameter β gives the quantile of the $100\beta\%$ reference interval and corresponds to the standard normal deviate, $z_{1-\beta/2}$. This reference interval is constructed to include the middle $100\beta\%$ of the population. Usually, β is set equal to α.

The parameter Δ is the relative margin of error. It is the percentage ratio of the width of the confidence interval for the reference limit to the width of the reference limit. The value of Δ is usually set to a small value such as 10% so that the width of the confidence interval is small compared to the width of the reference interval.

The parameter D is a constant that depends on the assumed distribution of the covariate. If the covariate is uniformly distributed, D is 4. If the covariate is normally distributed, D is 5. If the covariate is grouped into three groups (low, medium, and high), D is 5/2. The last three options work for any value of the covariate. If, however, you are only interested in the reference limit at the average value of the covariate, D is 1.

Unequal Variance

The above sample size formula assumes that the variance of the measurement is the same across all values of the covariate. This is often not the case. In fact, the method anticipates that variance will change and attempts to model the change. In this case, Bellera and Hanley on page 613 of their article advise that the above formula can still be used as a rough guide for sample size planning.

Example 1 – Calculating Sample Size

Suppose a study is being planned to obtain 80%, 90%, or 95% confidence interval for the 95% reference limit of blood pressure (BP) for different ages. Sample sizes are desired for relative margin of errors from 6% to 10% compared with a 95% reference interval. It is assumed that the age distribution of the sample is uniform.

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 ....**Sample Size**
Design Type...**Uniform Distribution**
Reference Limit Percentage **95**
R.I. Percentage Difference..............................**95**
Confidence Level Percentage.........................**80 90 95**
Percentage Margin of Error............................**6 7 8 9 10**

Output

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

Numeric Reports

Numeric Results for Reference Intervals

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Solve For: Sample Size
Design Type: Uniform Distribution

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Sample Size N	Percentage Margin of Error	Reference Limit Percentage	Reference Interval Percentage Difference	Confidence Level Percentage
636	6	95	95	80
468	7	95	95	80
358	8	95	95	80
283	9	95	95	80
229	10	95	95	80
1048	6	95	95	90
770	7	95	95	90
590	8	95	95	90
466	9	95	95	90
377	10	95	95	90
1487	6	95	95	95
1093	7	95	95	95
837	8	95	95	95
661	9	95	95	95
536	10	95	95	95

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N	The total sample size of the study.
Percentage Margin of Error	The measure of precision. It is the percentage that the width of the confidence interval of the reference limit is of the width of the reference interval.
Reference Limit Percentage	The percentage at which the reference limit is calculated. Assuming the data are normally distributed, it gives the percentage of the data values that are below the specified limit.
Reference Interval Percentage Difference	The difference between the two reference interval percentile percentage values.
Confidence Level Percentage	The percentage of such confidence intervals that cover the true reference limit.

Summary Statements

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A single-group age-specific reference interval design (cross-sectional study) will be used to obtain a two-sided reference interval. It is assumed that the distribution of the covariate is Uniform and the reference limits may be computed at any covariate value. To obtain a 95% reference interval with a 80% confidence interval of a 95% reference limit, and with a target percentage margin of error of 6%, 636 subjects will be needed. The percentage margin of error is the width of the confidence interval of the limit as a percentage of the width of the reference interval.

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Dropout-Inflated Sample Size

Dropout Rate	Sample Size N	Dropout-Inflated Enrollment Sample Size N'	Expected Number of Dropouts D
20%	636	795	159
20%	468	585	117
20%	358	448	90
20%	283	354	71
20%	229	287	58
20%	1048	1310	262
20%	770	963	193
20%	590	738	148
20%	466	583	117
20%	377	472	95
20%	1487	1859	372
20%	1093	1367	274
20%	837	1047	210
20%	661	827	166
20%	536	670	134

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, 795 subjects should be enrolled to obtain a final sample size of 636 subjects.

References

Bellera, C. A. and Hanley, J. A. 2007. 'A method is presented to plan the required sample size when estimating regression-based reference limits.' Journal of Clinical Epidemiology. Vol. 60, pages 610-615

Royston, P. 1991. 'Constructing time-specific reference ranges.' Statistics in Medicine. Vol. 10, pages 675-690.

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

Plots Section

Plots
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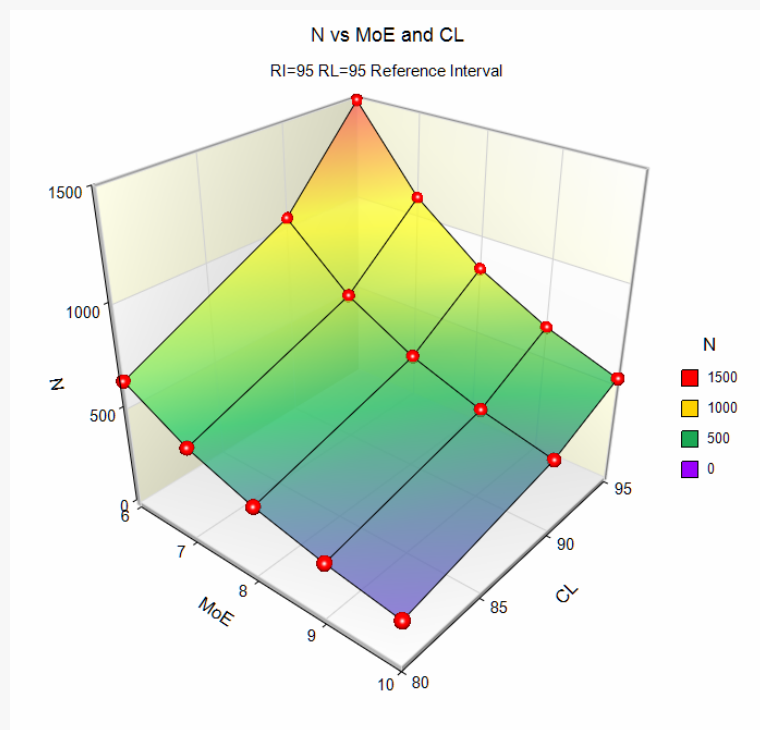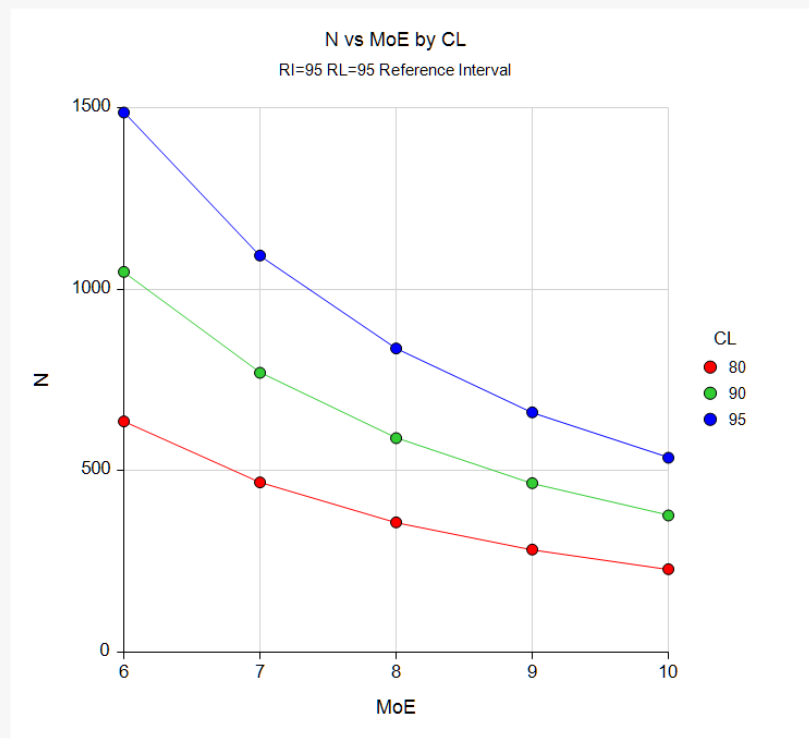
These plots show the sample size versus the margin of error (MoE) for the three confidence levels.

Example 2 – Validation using Bellera and Hanley (2007)

Bellera and Hanley (2007), page 612, give an example of a study is planned to obtain 95% confidence interval for the 95% reference limit of blood pressure (BP) for different ages. They calculate the sample size for a relative margin of error of 10% compared with a 95% reference interval, assuming the age distribution is uniform. They obtain a sample size value of 536.

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 ....**Sample Size**
Design Type..**Uniform Distribution**
Reference Limit Percentage **95**
R.I. Percentage Difference.............................**95**
Confidence Level Percentage.........................**95**
Percentage Margin of Error............................**10**

Output

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

Numeric Results for Reference Intervals

Solve For: Sample Size
Design Type: Uniform Distribution

Sample Size N	Percentage Margin of Error	Reference Limit Percentage	Reference Interval Percentage Difference	Confidence Level Percentage
536	10	95	95	95

PASS also calculates the sample size to be 536.