Chapter 381

Reference Intervals – Age-Specific

Introduction

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 estimates an **age-specific reference interval** for cross-sectional studies using the methodology of Altman (1993), Royston and Wright (1998), and Royston and Sauerbrei (2008). It provides formulas that may be used to produce (per)centiles as well as z-scores for new measurements not included in the original analysis.

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

Technical Details

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 √(π/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 √(π/2). Occasionally, a quadratic polynomial is required, but usually nothing more complicated than a linear polynomial is needed.

Step 4 – Calculate Z-Scores

The next step is to calculate a z-score for each observation. The z-score for the k^{th} 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

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

Fractional Polynomials

A polynomial function is of the form

$$B0 + B1\, X + B2\, X^2 + B3\, X^3 + \cdots$$

where the exponents of X are positive integers. Although popular, low order polynomials suffer from many deficiencies. First, they offer only a few model shapes which often do not fit the data well, especially near the ends of the data range. Polynomial functions do not have asymptotes, so they can't model this type of behavior.

A generalization of the polynomial function, called fractional polynomials (FP for short), was proposed by Royston and Altman (1994) and Royston and Sauerbrei (2008). FPs are of the form

$$B0 + B1\, X^{p1} + B2\, X^{p2} + \cdots$$

where p1, p2, … are selected from {-2, -1, -0.5, 0, 0.5, 1, 2, 3}. The convention is that X^0 equals LN(X). Hence the model FP(1, 0, -2) is

$$B0 + B1\, X + B2\, LN(X) + B3\, 1/X^2$$

An additional extension is with models that involve repeated powers such as (1, 1). Here, the second term is multiplied by LN(X). For example, the model FP(2, 2) is

$$B0 + B1\, X^2 + B2\, X^2\, LN(X)$$

It turns out the models that involve only two terms are usually adequate for creating reference intervals.

Ratio of Two Polynomials

Another useful extension that NCSS provides is the availability of ratios of polynomials. These models are of the form

$$Y = \frac{A0 + A1\, X}{1 + B1\, X}$$

These models approximate many different curve shapes. They offer a wide variety of curves and often provide better fitting models than polynomials and fractional polynomials. Unfortunately, the presence of the terms in the denominator causes severe problems since the denominator can become zero. When this happens, the model must be discarded.

Data Structure

The data are entered in two variables: one for Y and one for X.

Missing Values

Rows with missing values in the variables being analyzed are ignored in the calculations. If transformations are used which limit the range of X and Y (such as the logarithm), observations that cannot be transformed are treated as missing values.

Example 1 – Creating a Reference Interval Equation

This section presents an example of how to create a reference interval equation from a set of gestation data. In this dataset, the length of gestation (Gestation) and an ultrasonic measurement (Response) of 100 individuals is recorded. The program will conduct a search of 44 possible models and select the model that fits the data the best. A straight-line linear regression model appeared to fit the scaled absolute residuals. These models will be used to create the reference interval equation.

Setup

To run this example, complete the following steps:

1 Open the ReferenceInterval example dataset

- From the File menu of the NCSS Data window, select **Open Example Data**.
- Select **ReferenceInterval** and click **OK**.

2 Specify the Reference Intervals – Age-Specific procedure options

- Find and open the **Reference Intervals – Age-Specific** procedure using the menus or the Procedure Navigator.
- The settings 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.

Variables Tab

Y (Response).. **Response**
X (Covariate).. **Gestation**
Model Type.. **Find the Best Fitting Model**
x.. **Checked**

Reports Tab

Model Search: Candidates Models Reports .... **Checked**
Summary Report.. **Checked**
Iterations Reports .... **Checked**
Coefficient Estimation Reports.. **Checked**
Coefficient Estimation Reports - High-Precision .... **Checked**
Analysis of Variance Tables .... **Checked**
Coefficient Correlation Matrix.. **Checked**
Normality Test.. **Checked**
Percentile Report.. **Checked**
Residual Reports .... **Checked**

3 Run the procedure

- Click the **Run** button to perform the calculations and generate the output.

Model Search Summary Report

Model Search Summary Report

Item	Mean Model	Standard Deviation Model
Y Variable	Response	Scaled Absolute Residuals
X Variable	Gestation	Gestation
Rows Read	100	100
Rows Used	100	100
Residual Scale Factor	√(pi/2)	
Models Tried	44	1
Selected Model	y=A0+A1*x²+A2*1/x²	|Resid|=C0+C1*x
Iterations	0	0
R^2 of Selected Model	0.857448	0.125270
SE = √(MSE)	0.04496217	0.03399233

This report summarizes the fitting of the two models: the first column of the Mean and the second column of the Standard Deviation.

Variable Names

These entrees give the names of the X and Y variables.

Rows Read

The number of rows in the X and Y variables.

Rows Used

The number of rows used in the calculations. This is the number of rows with non-missing values in both X and Y.

Residual Scale Factor

During the estimation of the standard deviation model, each residual is multiplied by this value.

Models Tried

The number of models considered during the search for the best fitting model.

Select Model

The selected model in symbolic form.

Iterations

The number of iterations required. A '0' here indicates that convergence occurred before iteration began. . If the number of iterations is equal to the Maximum Iterations that you set, the algorithm did not converge, but was aborted.

R^2

This value is computed in the usual way for models that do not include a denominator polynomial. When a denominator is included, this value is only approximately correct.

R^2 varies between 0 and 1, with 0 indicating a poor fit and 1 indicating a perfect fit. Note that the R^2 of the standard deviation model will usually be close to zero. That is okay.

The R^2 value allows you to compare various models. This value, combined with the plots, is used to determine the best fitting model.

SE

An estimate of the standard error.

Model Search: Candidate Models Sorted by R^2

Model Search: Candidate Models Sorted by R^2

Rank	Mean Model	Mean Model R^2	R^2 minus Best R^2	SD Model	SD Model R^2	Prob Level of Normality Test Z-Scores
1	**$x^2 + 1/x^2$**	**0.857448**	**0.000000**	**x**	**0.125270**	**0.6878**
2	$x + 1/x^2$	0.857414	-0.000034	x	0.124475	0.6539
3	$1/x^2 + x^3$	0.857375	-0.000074	x	0.126365	0.7199
4	$\sqrt{x} + 1/x^2$	0.857353	-0.000095	x	0.123920	0.6360
5	$1/x + x^3$	0.857294	-0.000154	x	0.122213	0.5748
6	$LN(x) + 1/x^2$	0.857262	-0.000187	x	0.122963	0.6127
7	$1/x + x^2$	0.857208	-0.000240	x	0.121813	0.5804
8	$1/\sqrt{x} + 1/x^2$	0.857139	-0.000309	x	0.121890	0.6070
9	$x + 1/x$	0.857133	-0.000316	x	0.121470	0.5902
10	$\sqrt{x} + 1/x$	0.857100	-0.000348	x	0.121320	0.5954
.	.	.	.	.	.	.
.	.	.	.	.	.	.
.	.	.	.	.	.	.

Rank

The rank number after sorting the models by R^2.

Mean Model

The generic model of the mean being reported on in this row.

Mean Model R^2

The R^2 value of this model.

R^2 minus Best R^2

The difference between the R^2 value of this model and the R^2 value of the best model encountered.

SD Model

The generic model of the standard deviation being reported on in this row.

SD Model R^2

The R^2 value of this model.

Prob Level of Normality Test of Z-Scores

The p-value of the Shapiro-Wilk normality test of the z-scores. If this value is greater than 0.05, there is not enough evidence to conclude that the data are not normally distributed.

Individual Model Summary Report

Individual Model Summary Report

Item	Mean Model	Standard Deviation Model
Y Variable	Response	Scaled Absolute Residuals
X Variable	Gestation	Gestation
Rows Read	100	100
Rows Used	100	100
Residual Scale Factor	√(pi/2)	
Model	y=A0+A1*x²+A2*1/x²	|Resid|=C0+C1*x
Iterations	0	0
R²	0.857448	0.125270
SE = √(MSE)	0.04496217	0.03399233

This report summarizes the fitting of the two models: the first column of the Mean and the second column of the Standard Deviation.

Variable Names

These entrees give the names of the X and Y variables.

Rows Read

The number of rows in the X and Y variables.

Rows Used

The number of rows used in the calculations. This is the number of rows with non-missing values in both X and Y.

Residual Scale Factor

During the estimation of the standard deviation model, each residual is multiplied by this value.

Model

The models in symbolic form.

Iterations

The number of iterations required. A '0' here indicates that convergence occurred before iteration began. . If the number of iterations is equal to the Maximum Iterations that you set, the algorithm did not converge, but was aborted.

R^2

This value is computed in the usual way for models that do not include a denominator polynomial. When a denominator is included, this value is only approximately correct.

R^2 varies between 0 and 1, with 0 indicating a poor fit and 1 indicating a perfect fit. Note that the R^2 of the standard deviation model will usually be close to zero. That is okay.

The R^2 value allows you to compare various models. This value, combined with the plots, is used to determine the best fitting model.

SE

An estimate of the standard error.

Iterations Reports

Mean Function Estimation Iterations Report

Iteration Number	Error Sum Lambda	Lambda	A0	A1	A2
0	0.1960949	4E-05	10.31614	-8.090378E-05	75.65155

Convergence criterion met.

Standard Deviation Estimation Iterations Report

Iteration Number	Error Sum Lambda	Lambda	C0	C1
0	0.1132369	4E-05	-0.00397401	0.001716751

Convergence criterion met.

This report displays the error (residual) sum of squares, lambda, and parameter estimates for each iteration of each model. They allow you to observe the progress of the estimation algorithms.

Coefficient Estimation Reports

Mean Equation - Coefficient Estimation Report

Model: y = A0+A1*x²+A2*1/x²

Coefficient and Term	Coefficient Estimate	Standard Error of Estimate	Lower 95.0% Confidence Limit	Upper 95.0% Confidence Limit	T Value	Prob Level
A0	10.31614	0.03384301	10.24897	10.38331	304.82	0.0000
A1*x²	-8.090378E-05	2.342309E-05	-0.0001273921	-3.441544E-05	-3.45	0.0008
A2*1/x²	75.65155	9.254083	57.28476	94.01834	8.17	0.0000

Estimated Model of Response

(10.31614-(8.090378E-05)*Gestation^2+(75.65155)*1/(Gestation*Gestation))

Standard Deviation Equation - Coefficient Estimation Report

Model: SD = C0+C1*x

Coefficient and Term	Coefficient Estimate	Standard Error of Estimate	Lower 95.0% Confidence Limit	Upper 95.0% Confidence Limit	T Value	Prob Level
C0	-0.00397401	0.01280035	-0.02937588	0.02142786	-0.31	0.7569
C1*x	0.001716751	0.0004582565	0.0008073563	0.002626146	3.75	0.0003

Estimated Model of SD of Response

(-0.00397401+(0.001716751)*Gestation)

Estimated Z-Score Model

Z = (Response - (10.31614-(8.090378E-05)*Gestation^2+(75.65155)*1/(Gestation*Gestation))) /
(-0.00397401+(0.001716751)*Gestation)

Coefficient and Term

The name of the coefficient and term whose results are shown on this line.

Coefficient Estimate

The estimated value of this coefficient.

Standard Error of Estimate

An estimate of the standard error of the coefficient.

Lower 95% Confidence Limit

The lower value of a 95% confidence interval for this coefficient.

Upper 95% Confidence Limit

The upper value of a 95% confidence interval for this coefficient.

T Value

The value of the t-statistic used to test whether this term is statistically significant.

Prob Level

The significance level or p-value of the test statistic. If this value is 0.05 or less, the t-test is statistically significant.

Estimated Model

This is the estimated model written out so that it can be copied and pasted into another program such as Excel.

Estimated Z-Score Model

This is the estimated z-score model written out so that it can be copied and pasted into another program such as Excel.

Coefficient Estimation Reports in High Precision

Mean Equation - Coefficient Report in High-Precision

Coefficient and Term	Coefficient Estimate	Standard Error	Lower 95.0% Confidence Limit	Upper 95.0% Confidence Limit
A0	10.3161380531194	0.03384301	10.2489690552149	10.3833070510239
A1*x²	-8.09037797269359E-05	2.342309E-05	-0.000127392125124008	-3.44154343298636E-05
A2*1/x²	75.6515482561129	9.254083	57.2847554176779	94.0183410945479

Estimated Model of Response

(1-(8.09037797269359E-05)*Gestation^2+(75.6515482561129)*1/(Gestation*Gestation))

Standard Deviation Equation - Coefficient Report in High-Precision

Coefficient and Term	Coefficient Estimate	Standard Error	Lower 95.0% Confidence Limit	Upper 95.0% Confidence Limit
C0	-0.00397401029375437	0.01280035	-0.0293758806868975	0.0214278600993888
C1*x	0.00171675136127743	0.0004582565	0.000807356301336725	0.00262614642121814

Estimated Model of SD of Response

(1+(0.00171675136127743)*Gestation)

Estimated Z-Score Model

Z = (Response - (1-(8.09037797269359E-05)*Gestation^2+(75.6515482561129)*1/(Gestation*Gestation))) / (1+(0.00171675136127743)*Gestation)

This is a version of the coefficient report in which the coefficients are displayed in high-precision. In some cases, it is important to use all digits when using the estimates.

Shapiro-Wilk Normality Test of Z-Scores

Shapiro-Wilk Normality Test of Z-Scores

Test Name	Test Statistic	Prob Level	Reject Normality at 5% Level?
Shapiro-Wilk	0.99	0.6878	No

This report shows the result of a test of the normality of the z-scores. If normality is rejected, a different model should be used, possibly one that uses LN(y).

Percentile Report

Percentile Report

Model: $y = A0+A1*x^2+A2*1/x^2 + Z\alpha * (C0+C1*x)$

	Percentiles of Response						
Gestation	2.5	10.0	25.0	50.0	75.0	90.0	97.5
8.000	11.474	11.481	11.486	11.493	11.500	11.506	11.512
16.000	10.545	10.561	10.575	10.591	10.607	10.621	10.637
24.000	10.328	10.353	10.376	10.401	10.426	10.449	10.474
32.000	10.207	10.242	10.273	10.307	10.342	10.372	10.407
40.000	10.107	10.151	10.190	10.234	10.278	10.317	10.361

This report shows the estimated percentiles at the Gestation values and Percentile values that were selected. Note that 'Z' stands for standard normal deviate corresponding to the indicated percentile.

Analysis of Variance Tables

Mean Equation - Analysis of Variance Table

Model Term(s)	DF	Sum of Squares	Mean Square
Mean	1	10787.3	10787.3
Model	3	10788.48	10788.61
Model (Adjusted)	2	1.179512	0.5897558
Error	97	0.1960949	0.002021597
Total (Adjusted)	99	1.375606	
Total	100	10788.67	

Standard Deviation Equation - Analysis of Variance Table

Model Term(s)	DF	Sum of Squares	Mean Square
Mean	1	0.1785716	0.1785716
Model	2	0.1947882	0.2514067
Model (Adjusted)	1	0.01621659	0.01621659
Error	98	0.1132369	0.001155479
Total (Adjusted)	99	0.1294535	
Total	100	0.3080251	

Model Term(s)

The labels of the various sources of variation.

DF

The degrees of freedom.

Sum of Squares

The sum of squares associated with this term. Note that these sums of squares are based on Y, the dependent variable. Individual terms are defined as follows:

Mean The sum of squares associated with the mean of Y. This may or may not be a part of the model. It is presented since it is the amount used to adjust the other sums of squares.

Model The sum of squares associated with the model.

Model (Adjusted) The model sum of squares minus the mean sum of squares.

Error The sum of the squared residuals. This is often called the sum of squares error or just "SSE."

Total (Adjusted) The sum of the squared Y values minus the mean sum of squares.

Total The sum of the squared Y values.

Mean Square

The sum of squares divided by the degrees of freedom. The Mean Square for Error is an estimate of the underlying variation in the data.

Correlation Matrix of Parameters

Mean Equation - Coefficient Correlation Matrix

	A0	A1	A2
A0	1.000000	-0.965022	-0.958157
A1	-0.965022	1.000000	0.882923
A2	-0.958157	0.882923	1.000000

Standard Deviation Equation - Coefficient Correlation Matrix

	C0	C1
C0	1.000000	-0.964095
C1	-0.964095	1.000000

This report displays the correlations of the coefficient estimates.

Predicted Values and Residuals Section

Predicted Values, Residuals, and Z-Scores

Model: $y = A0+A1*x^2+A2*1/x^2$

Row No.	Gestation (X)	Response (Y)	Predicted Y	Residual of Y	Scaled Residual of y	Standard Deviation of y	Z-Score Value of y	Z-Score Prob of y
1	38.269	10.242	10.249	-0.007	-0.009	0.062	-0.12	0.4526
2	30.562	10.294	10.322	-0.028	-0.035	0.048	-0.57	0.2844
3	21.196	10.424	10.448	-0.024	-0.030	0.032	-0.74	0.2303
4	22.507	10.501	10.424	0.076	0.096	0.035	2.20	0.9861
5	33.060	10.339	10.297	0.042	0.052	0.053	0.79	0.7861
6	22.330	10.449	10.428	0.021	0.027	0.034	0.62	0.7322
7	35.606	10.342	10.273	0.069	0.087	0.057	1.21	0.8865
8	34.341	10.280	10.285	-0.005	-0.007	0.055	-0.09	0.4623
9	30.765	10.321	10.319	0.002	0.002	0.049	0.04	0.5155
10	15.666	10.593	10.605	-0.012	-0.015	0.023	-0.52	0.3012
.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.

This report shows the predicted values, residuals, and z-scores.

Row No.

The row number from the dataset.

X

The value of the covariate.

Y

The value of the response.

Predicted Y

The predicted value of the response using only the mean model.

Residual of Y

The value of the residual, the difference between Y and the predicted Y.

Scaled Residual of y

The value of the residual times the scale factor.

Standard Deviation of y

The value of the standard deviation using the standard deviation model.

Z-Score Value of y

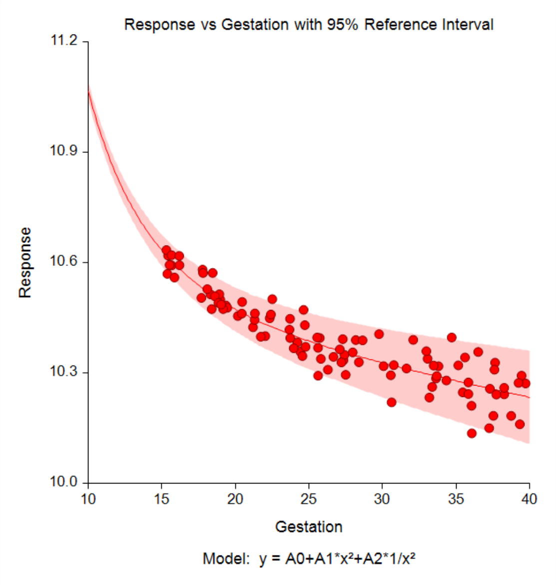
The z-score of this row. Most z-scores should be between plus and minus 2 if the data are normally distributed.

Z-Score Prob of y

The probability level of the above z-score assuming the normal distribution.

Y vs X with Reference Interval

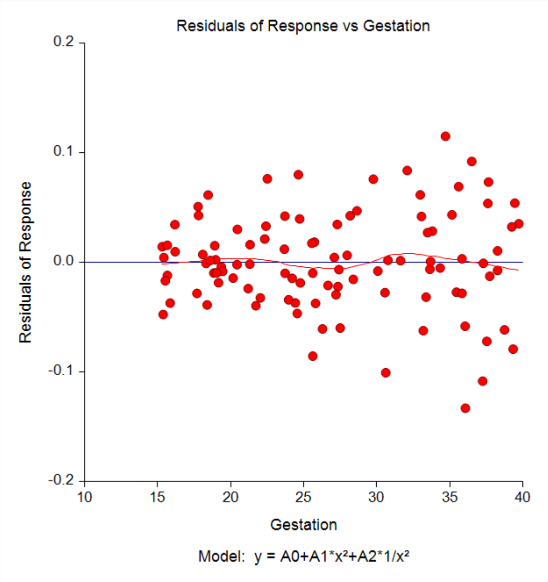
Plot of Y vs X with Reference Interval



This plot displays the data along with the estimated function and reference interval. It is useful in deciding if the fit is adequate and the reference interval is appropriate.

Residuals of Y vs X

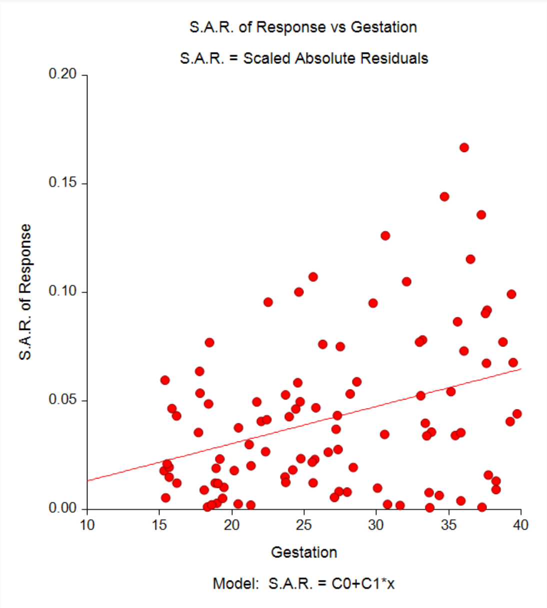
Plot of Residuals of Y vs X



This is a scatter plot of the residuals versus the independent variable, X. The preferred pattern is a rectangular shape or point cloud. Any nonrandom pattern may require a redefining of the model. A loess curve is overlaid to give you a better understanding of the trends in the data.

Scaled Absolute Residuals vs X

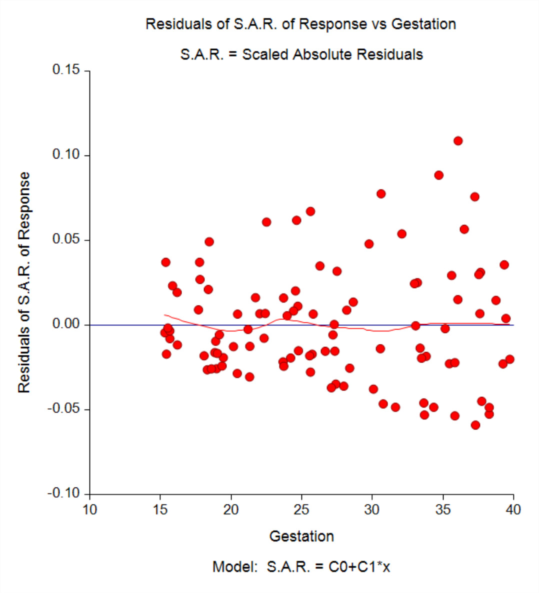
Plot of Scaled Absolute Residuals vs X



This a scatter plot of the scaled absolute residuals versus X. The line is the model of the standard deviation.

Residuals of Scaled Absolute Residuals vs X

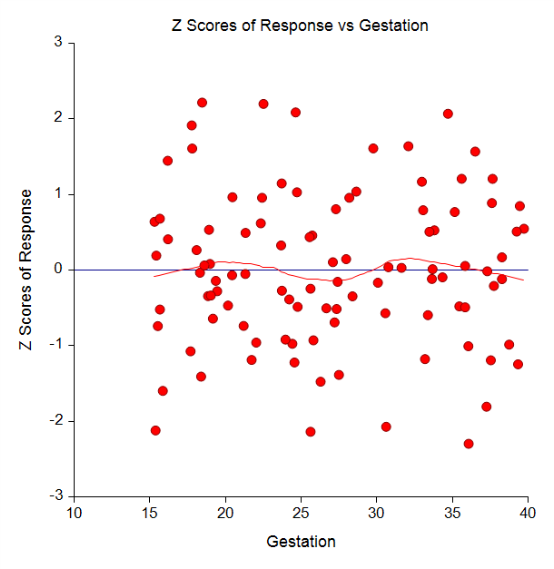
Plot of Residuals of Scaled Absolute Residuals vs X



This is a scatter plot of the residuals from the S.A.R. fit versus the independent variable, X. Often, the plot will exhibit a funnel shape indicating the changing nature of these residuals. This is to be expected. A loess curve is overlaid to give you a better understanding of any patterns that should be modelled.

Z-Score vs X

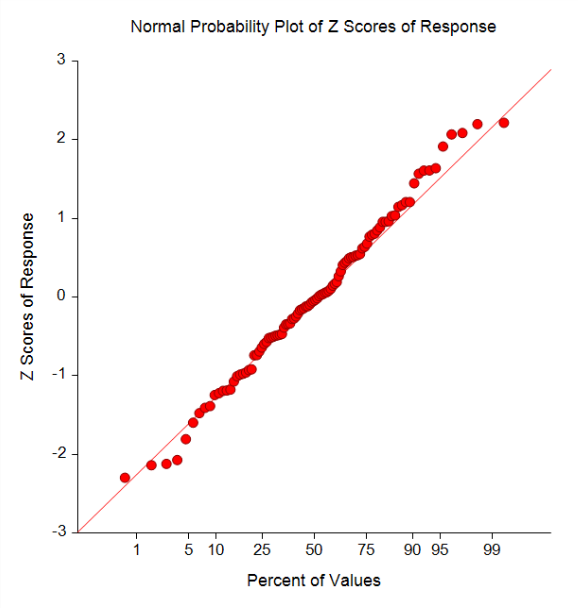
Plot of Z-Score vs X



This scatter plot displays the z-scores versus the covariate, X. If all has gone well, this plot should show a random pattern.

Normal Probability Plot of Z-Scores

Normal Probability Plot of Z-Scores



If the z-scores are normally distributed, the data points of the normal probability plot will fall along a straight line. Major deviations from this ideal picture reflect departures from normality. Stragglers at either end of the normal probability plot indicate outliers, curvature at both ends of the plot indicates long or short distributional tails, convex or concave curvature indicates a lack of symmetry, and gaps or plateaus or segmentation in the normal probability plot may require a closer examination of the data or model.