The JMP Passing Bablok Add-In as a Tool for Robust Regression and Method Comparison in the Field of Diagnostics

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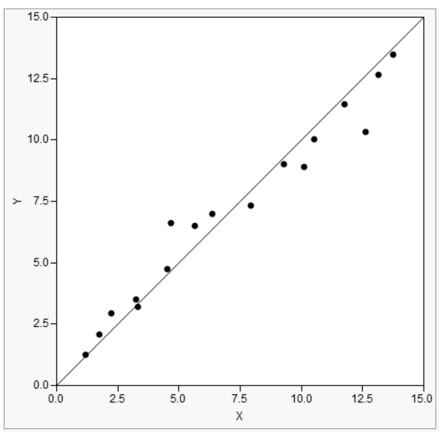
Overview

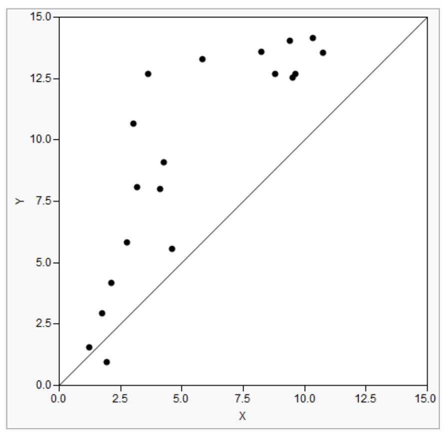
- Overview
  - Guidelines
  - Linear Model
  - Bias at the Decision Point
- Correlation coefficient
- Regression methods
- Implementation in JMP

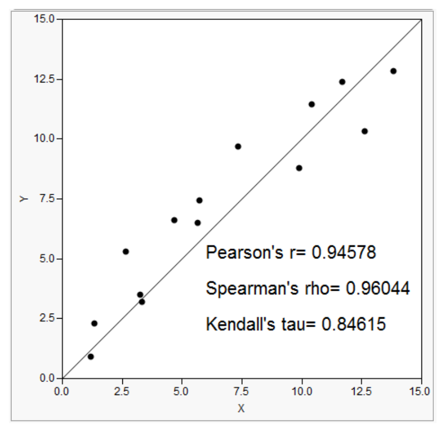
Method Comparison - Overview

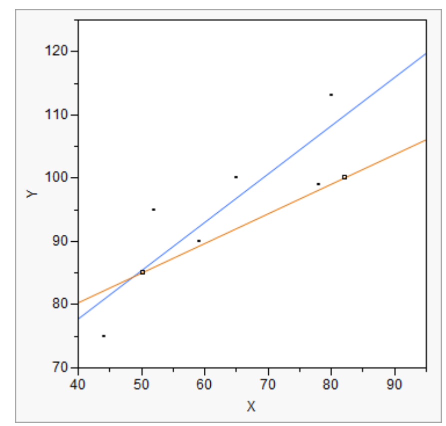
- With the help of method comparison experiments (studies), agreement between two methods of clinical measurement can be assessed

- Answer the question: „Are the methods comparable enough, that one might be replaced by the other with sufficient accuracy“









Have a look at: Correlation coefficient Regression methods

Method Comparison - Guidelines

- CLSI EP09-A2-IR: Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Second Edition (Interim Revision)
- CLSI EP09-A3: Method Comparison and Bias Estimation Using Patient Samples; Draft Guideline-Third Edition

Item – key differences	*EP09-A2*	*EP09-A3*
Level of Detail	Moderate	High
Method comparison categories	None	Establishment, Validation, Method Introduction (Verification)
Number of samples	40 laboratory (customer) 100 manufacturer	40 laboratory (customer) 100 manufacturer
Quantitative Analysis	Linear Regression	Linear Regression, Deming Passing Bablok
Bias estimation (calculation)	implemented	implemented
Characterization Method comparison studies	n/a	Tube type, samples types

Method Comparison – SOP Design

- **SOP RS0013 Method Comparison according to CLSI EP09-A3**
  - Heterogeneous quantitative assays
    - Establishment, characterization and validation
    - >120 samples to prove a specified deviation of +/- 3%
    - >100 samples to prove a specified deviation of +/- 5% or more
  - General Requirements
    - Analyte conentrations of the samples have to lie within the measurment range, as specified in the PSD or IFU for both methods
    - 90% of the measurement range have to be covered
    - Both methods have to be treated in the same way (serum or plasma)
    - To spike with analyte or to dilute the samples is allowed, if the measurment range can‘t be covered by native samples

Method Comparison – SOP Evaluation

- **SOP RS0013 Method Comparison according to CLSI EP09-A3**
  - Following statistical measures have to be evaluated
    - Correltation coefficient: r (Pearson) and tau (Kendall)
    - Regression methods: Passing-Bablok, Deming, Linear regression (Deming and linear regression can be done weighted or non-weighted)
    - Parameter: slope, intercept, bias, with 95%-confidence intervals
    - Descriptive statistics: N, mean, median, minimum, maximum
    - CUSUM-Test: results from regression analyses can only be used if the CUSUM test (99% niveau) was ok. If linearity can not be shown, further risk analysis is required.

Linear Model

- With the help of method comparison experiments, agreement between two methods of clinical measurement can be assessed
- In order to detect systematic and proportional deviations between two methods, we choose the method comparison approach, a regression line.
- To compare method X with method Y, systematic and proportional deviations are possible, ie

$$Y = \underbrace{a}_{\text{Intercept or systematic deviation}} + \underbrace{b}_{\text{slope or proportional deviation}} \cdot X$$

- The two methods are assumed to be **equivalent** if **a=0** and **b=1**

Bias at the decision point

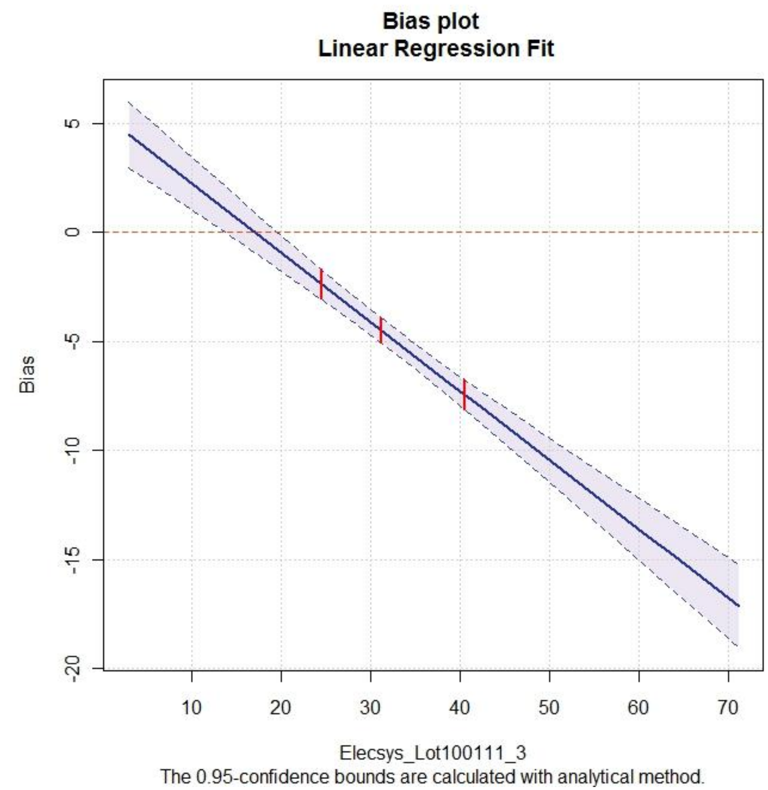
By the regression model, the result of the comparison methods is always given by two variables: intercept and slope. To convert this into a meaningful size of the bias is considered at one or more decision points.

The critical result of the comparison method is the difference between the two methods (bias) at the decision point (cut-off). The calculation is done using the appropriate regression model.

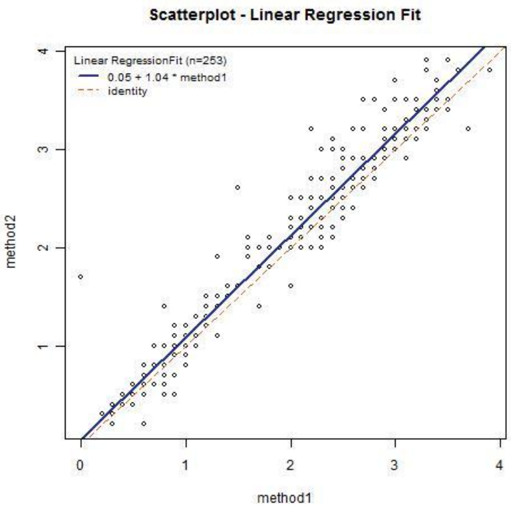
Bias is defined as:

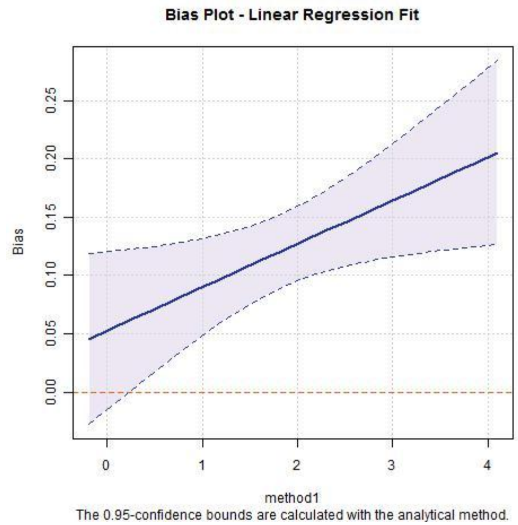
$$Bias(X_i) = a + (b-1) * X_i = \hat{Y}_i - X_i$$

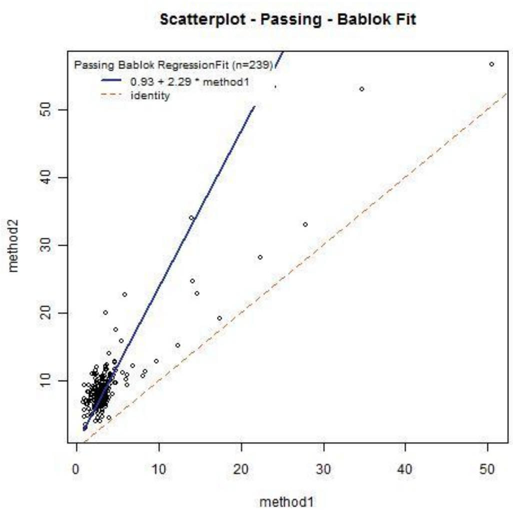
where a, b - the regression parameters, and Yi - the calculated from the regression ("estimated") values of the 2nd Present method.

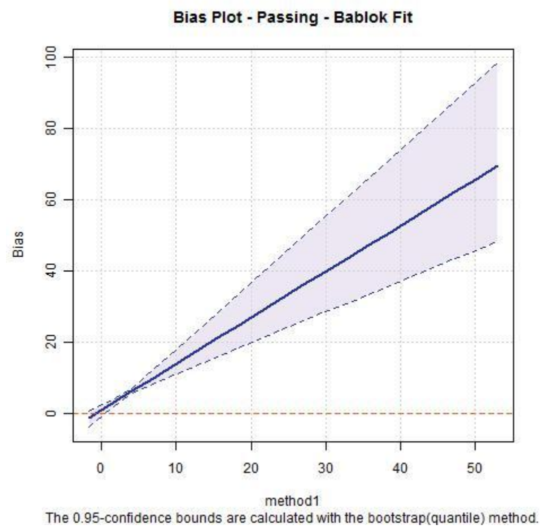


Bias at Decision Point









Relative Bias

In addition to the absolute bias, also a relative bias can be calculated, based on the Medical Decision Point:

$$RelBias(X_i) = \frac{a+(b-1)*X_i}{X_i} = \frac{a}{X_i} + (b-1)$$

Depending on the test the permitted relative bias can vary. For example a relative bias of up to 10% can be allowed at the cut-off.

For very small values of the MDPs values, the relative bias can be large.
For large MDPs values the relative bias seeks against b-1 (slope = -1).
In the lower and middle range the intercept is considered in the calculation of the bias.

Correlation Coefficient

Pearsons r

Kendalls tau

Spearmans rho

Correlation Coefficient

Pearsons r

Kendalls tau

Spearmans rho

Correlation Coefficient

Pearsons r

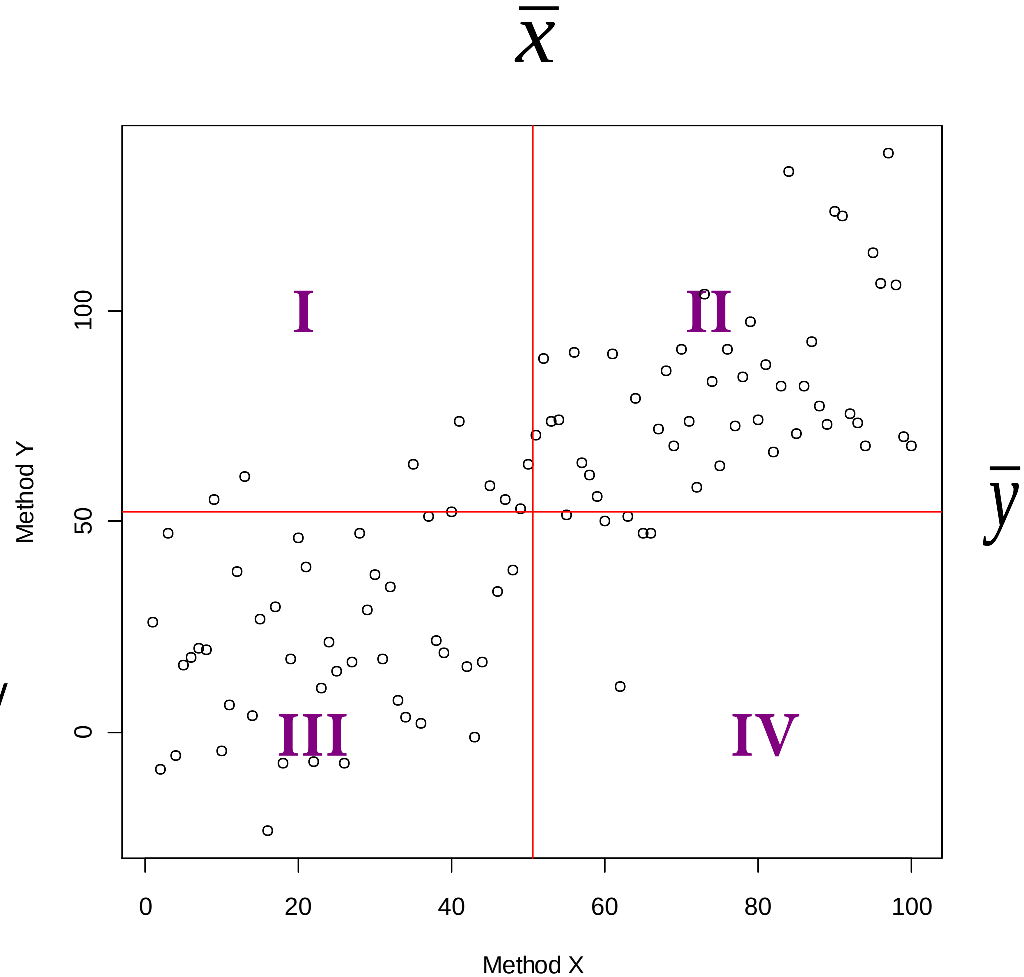
$$r = \frac{\sum (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum (x_i - \bar{x})^2 \sum (y_i - \bar{y})^2}}$$

The counter of r is in;

I	negative	(-+)
II	positive	(++)
III	positive	(--)
IV	negative	(+-)

→ r is a total> 0 since the points in I + IV prevail!

The denominator serves to normalize



Pearson's correlation coefficient indicates the degree of linear relationship between X and Y.

Correlation Coefficient
Kendalls tau

The idea of Kendall's tau is that the "strength of the relationship“ is defined by the order between two pairs, ie all possible sample pairs and their arrangement with respect to X and Y are considered.

Concordant couples: $\begin{pmatrix} & + \\ + & \end{pmatrix}$

Discordant couples: $\begin{pmatrix} + & \\ & + \end{pmatrix}$

$$\text{tau} = \underbrace{\frac{2}{n_t(n_t-1)}}_{\text{Stand.Faktor:}\tau\in[-1,1]} \cdot \underbrace{(n_c - n_d)}_{\text{\#Konk.-\#Disk.}}$$

No information about a linear relationship!

Regression Methods

Linear Regression

Weighted Linear Regression

Deming Regression

Weighted Deming Regression

Passing/Bablok Regression

Regression methods

Linear Regression

Error-free method is compared with a faulty method.

The error-free method is used as the independent variable (X variable) in the linear model.

Intercept and slope are optimized in regard to minimization of the sum of the squares of the vertical distances between measured Y values and the estimated regression line.

$$\hat{b}_{\text{LinReg}} = \frac{S_{xy}^2}{S_{xx}^2} = \frac{\sum_i (x_i - \overline{x}) \cdot (y_i - \overline{y})}{\sum_i (x_i - \overline{x})^2}$$

$$\hat{a}_{\text{LinReg}} = \overline{y} - \hat{b}_{\text{LinReg}} \cdot \overline{x}$$

JMP LinReg

Regression Methods

Weighted linear Regression

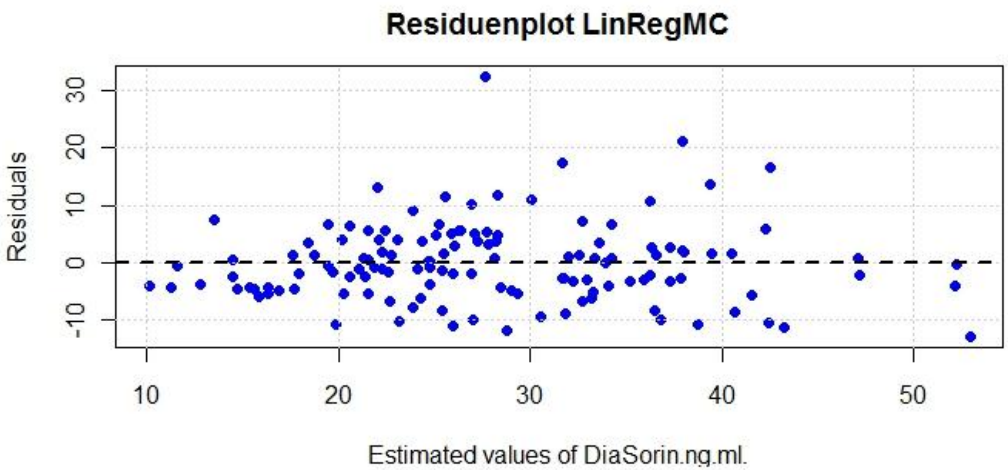
Weighted linear regression: if the assumption is violated on the homoscedasticity (the measurement error increases with the concentration of the sample).

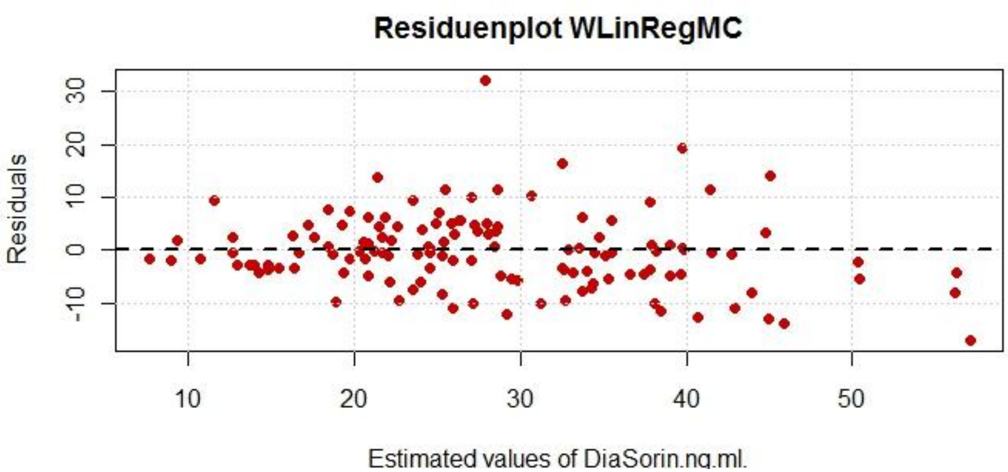
In this case, each pair (X_i, Y_i) is normalized by the corresponding weight w_i i = 1,2 … n The transformed data $(X_i * w_i, Y_i * w_i)$ have then identically distributed variances, and the regression coefficients are estimated in the same way as in the case of the simple regression.

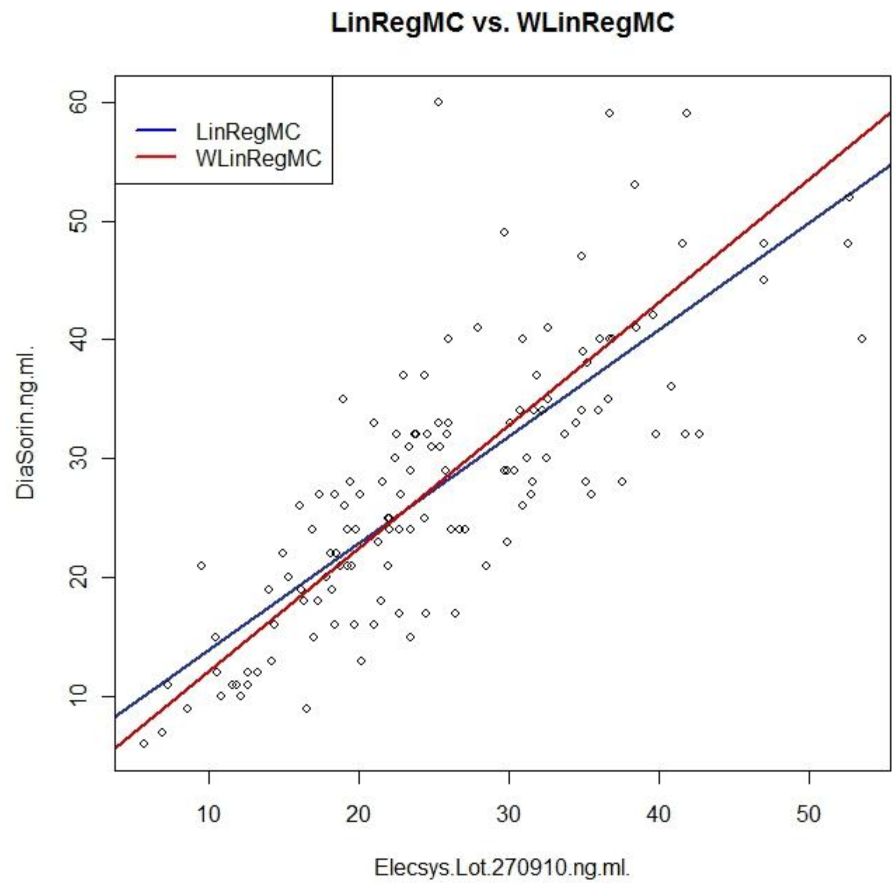
If the growth of the variance is uniformly (the coefficient of variation will remain constant over all ranges), the weighting $w_i = \frac{1}{{X_i}^2}$ is taken.

Regression Methods

Weighted lin. Regression vs. Simple lin. Regression







Regression methods

Errors in both variables

Two measurement methods will be compared by a regression line, which are both subject to error.

In this case, the estimates of the linear regression underestimated the intercept and overestimate the slope.

To what extent this happens, depends on the ratio of the distribution of the concentrations of the method X from the measurement error of the X method.

Regression Methods

Deming Regression

Both methods are deviate from their unobservable "true" value s X^*_i nd Y^*_i ie
Following relations will be assumed:

$$X_i = X^*_i + \delta_i \quad \text{where} \quad \delta_i \sim N(0, {\sigma_x}^2)$$

$$Y_i = Y^*_i + \varepsilon_i \quad \text{where} \quad \varepsilon_i \sim N(0, {\sigma_y}^2)$$

In the next step, the regression line for the "true" values of both methods is given:

$$Y^*_i = a + b * X^*_i$$

JMP Orth_Reg

Regression Methods
Deming Regression

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JMP Orth_Reg

Regression Methods
Deming Regression – Error Ratio

The choice of the error ratios has an influence on the estimation of the regression line and thus on the bias calculation.

All lines pass through the centroid of the data distribution. The further away the bias of this emphasis has to be calculated, the results differ more.

However, the choice of unweighted\weighted has also a significant influence on the regression results.

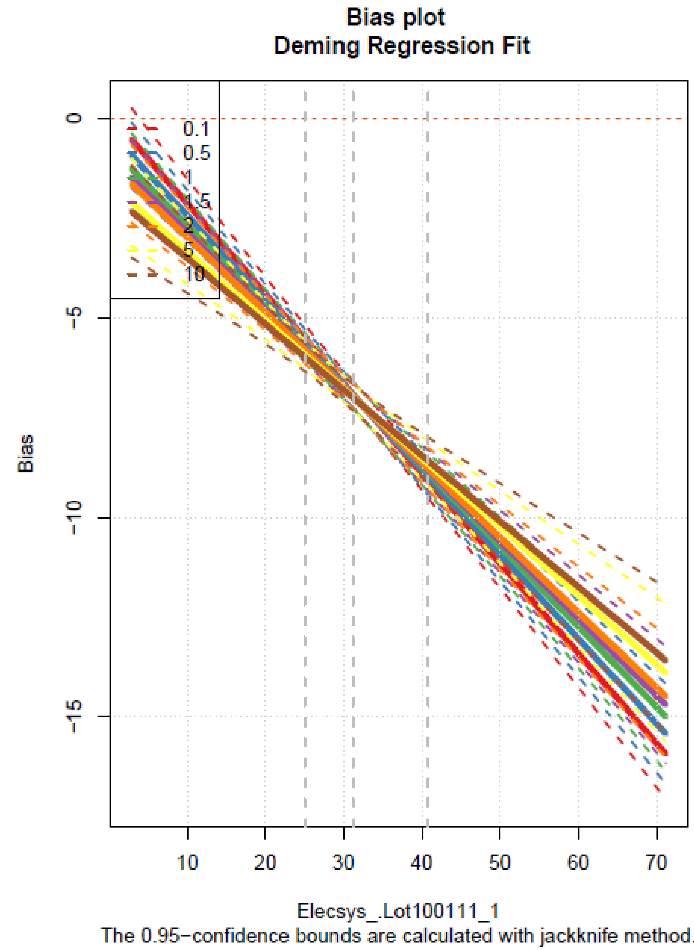
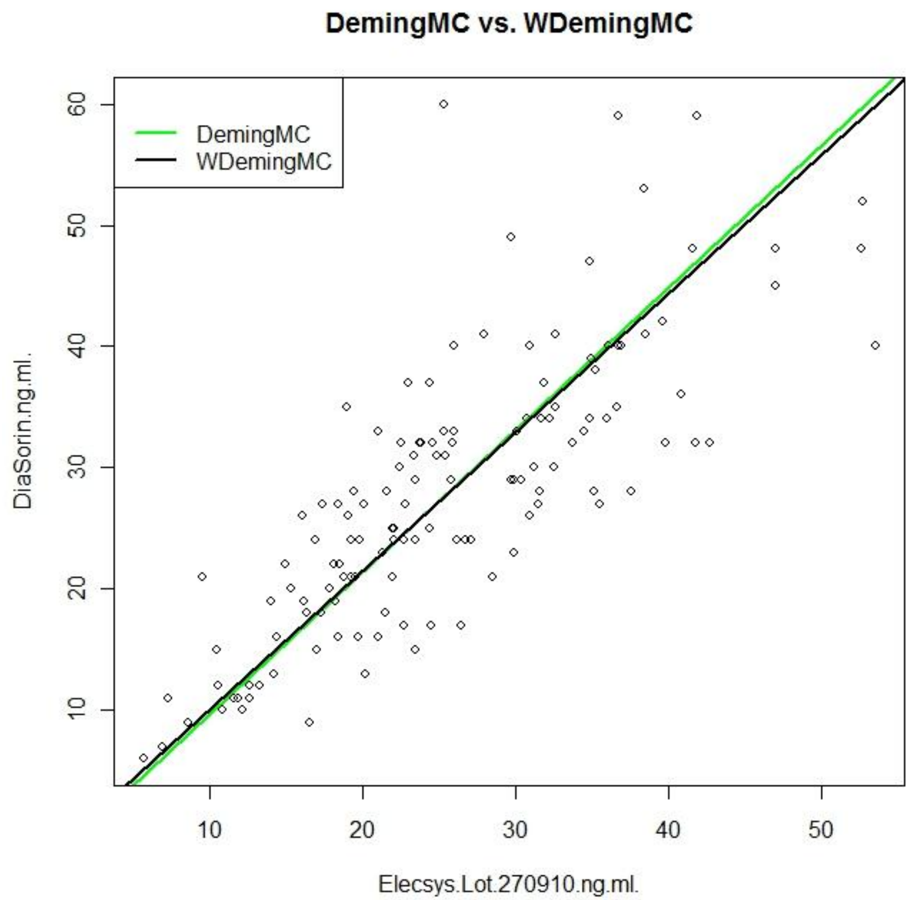


Figure 5.9: Comparison of bias confidence intervals for Deming Regression, for different lambdas

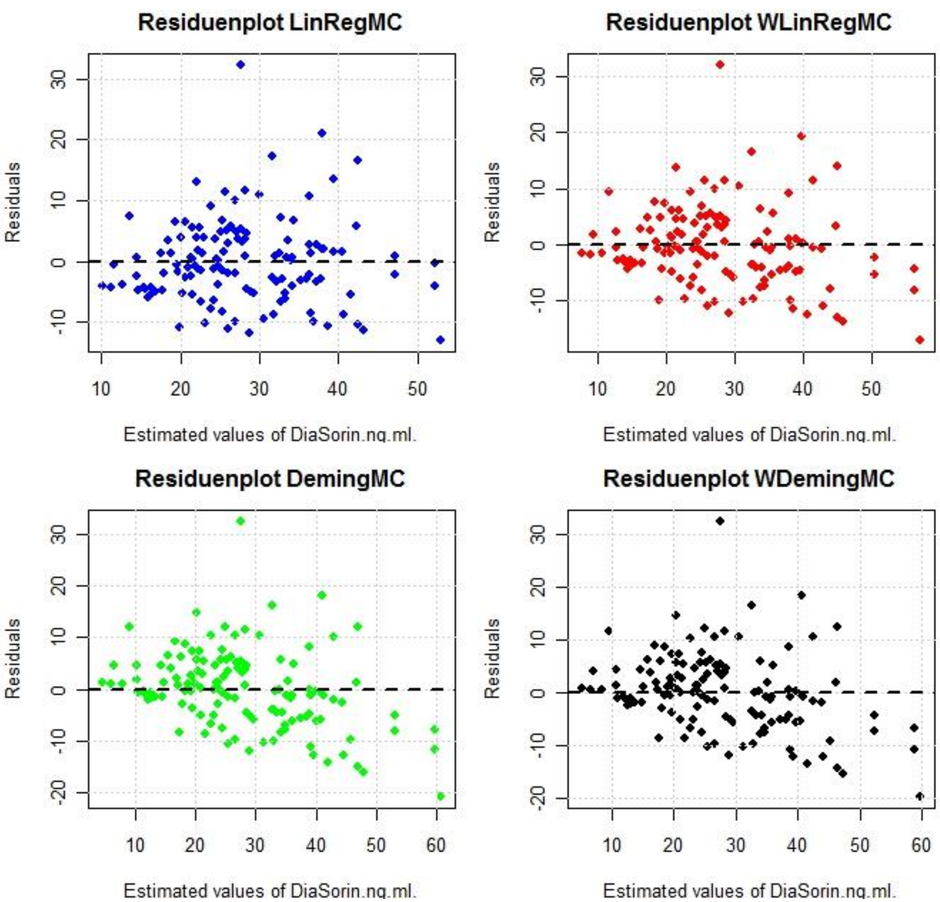
Regression Methods
Weighted Deming Regression

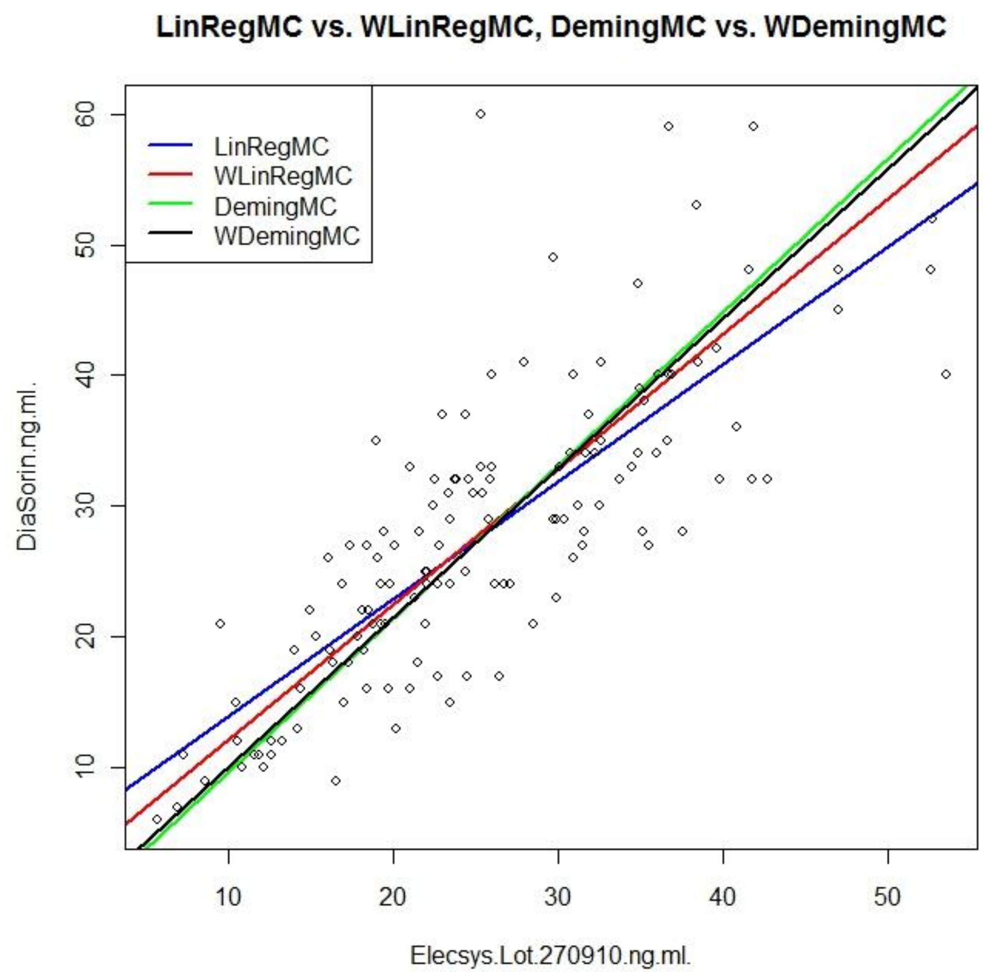
In the case that the measurement errors of the methods is not constant but proportional to the magnitude of the measured concentrations, the weighted Deming regression is used (similar to the weighted linear regression).



Regression Methods

Weighted Deming vs. Simple Deming Regression





Regression Methods

Passing/Bablok Regression

Robust method, based on the idea of the slope calculation between two points.

For every 2 points i and j the slope is calculated as the quotient of the differences :

$$b_{ij} = \frac{Y_i - Y_j}{X_i - X_j}\ ,\ \ 1 \leq i < j \leq n$$

Number of combinations (gradients):

$$N = \binom{n}{2} = \frac{n * (n-1)}{2}$$

Regression methods

Passing/Bablok Regression

The slopes are sorted in ascending order.
It is not the median of the slopes used as the pitch estimator, but it is searched for an offset value K, by which the pitch estimator is shifted from the median ("Median correction")

$$K = (Anzahl\ der\ Steigungen\ b_{ij} < -1)$$

The offset is currently selected in a way, that a meaningful estimate can be given for slopes near by 1. Would you expect different slopes, one would have to choose different offset values.

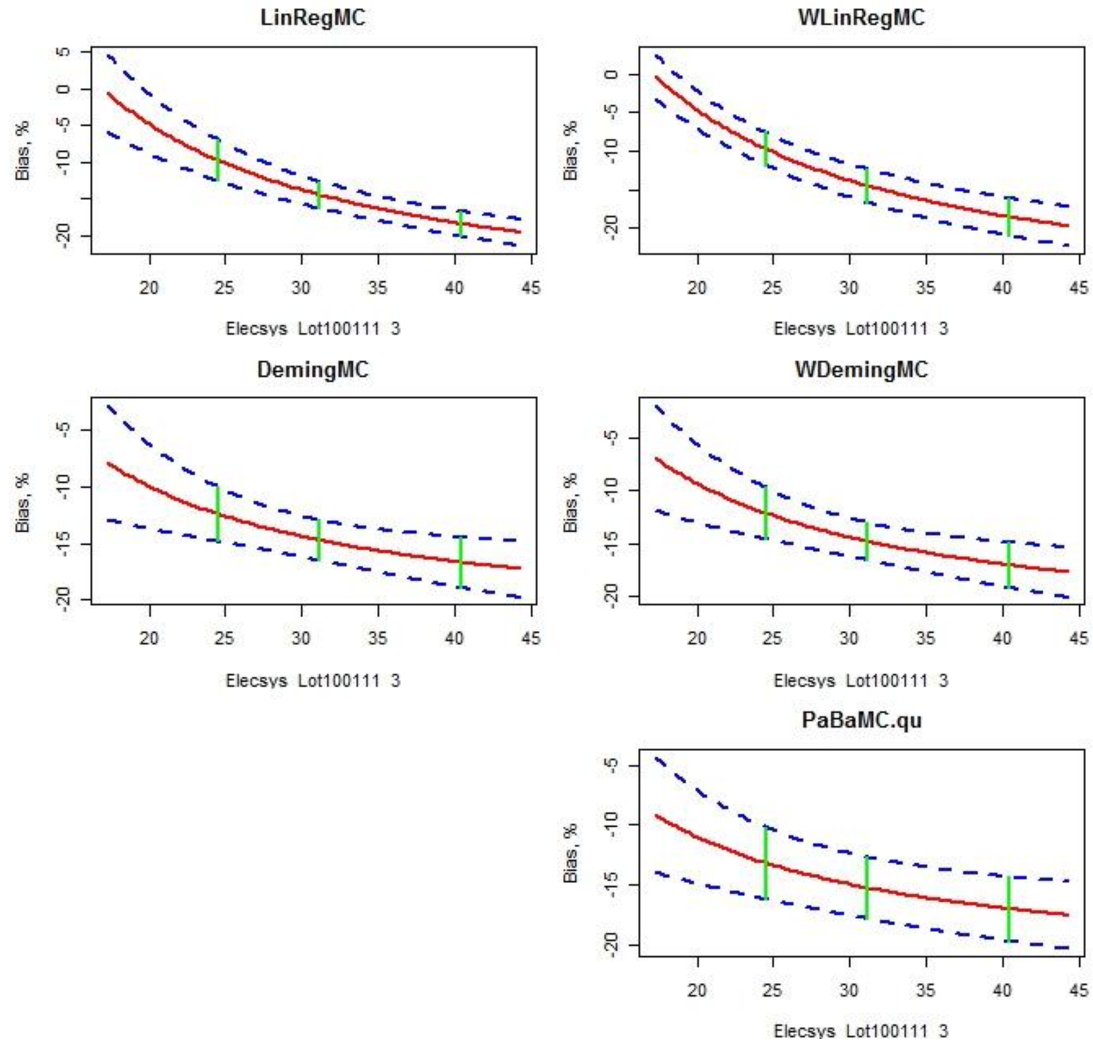
With an odd number of slopes, the slope estiamte is the slope with the rank:

$$\underbrace{\frac{N+1}{2}}_{\text{Rang des Medians}} + \underbrace{K}_{\text{Verschiebung um K Ränge}}$$

Regression Methods

Regression models and relative Bias

The choice of regression model has an influence on the estimate of the absolute and relative bias.



Comparison of methods - action

Regression methods

- **Linear Regression:** One of the methods is error free, this method is used as an independent variable in the linear model (variable X). Measurement error (SD) of the Y method is constant over the measurement range.
- **Weighted Linear Regression:** One of the methods is error free, this is used as an independent variable in the linear model (variable X). Measurement error (SD) of the Y method increases over the measurement range.
- **Deming Regression:** Both methods have errors (X and Y). Ratio of the measurement error of both methods is known. Measurement error (SD) of both methods are constant over the measurement range.
- **Weighted Deming Regression:** Both methods have errors (X and Y). Ratio of the measurement error of both methods is known. Measurement error (SD) of both methods to increase the measurement range.
- **Passing/Bablok:** No specific distribution assumptions about the error variances, however, the ratio of the measurement errors should be about 1. Data are positively correlated. Slope is around 1, Errors in both variables and tolerance in the presence of outliers.

Confidence Intervals "Analytical CI"

Confidence interval for 95% (99%) level: possible small areas in which the true parameters falls with a high probability (95%, 99%).

If the estimator of an unknown parameter $\hat{p}$ is normally distributed, we obtain a confidence interval for the 95% level for the unknown paramete p by

$$[\hat{p} - 2 \cdot \sqrt{Var(\hat{p})}, \hat{p} + 2 \cdot \sqrt{Var(\hat{p})}]$$

This formula can be used as an approximation formula when the variance Var (p) can be calculated. But this is not always the case, for example for the bias based on the Passing-Bablok regression.

JMP_ConfInt

Confidence Intervals
“Bootstrap CI”

If the variance of the estimated parameters can not be calculated, confidence intevals must be calculated using the bootstrap method.

This method is used in the Passing-Bablok regression. Since this regression method is already individually time-consuming, it is slowed by the bootstrap method more, and thus have longer processing times for large data sets.

Test of Linearity
Cusum Test

Each data point is assigned a score - positive value if the point lies above the regression line, a negative value if it is below.

The maximum cumulative sum of the scores is calculated. If this is greater than a certain threshold, then the linearity assumption is rejected.

In this cases, a risk assessment has to be done, to what extent the deviation from linearity affects the evidence in the analysis.

JMP Cusum

Implementation

Demo JMP

Literature

- [1] Bablok, W. & Passing, H. (1983) A new biometrical procedure for testing the equality of measurements from two different analytica methods – Application of linear regression procedures for method comparison studies in Clinical Chemistry, Part I. *J. Clin. Chem. Clin. Biochem*., **21**, 709-720.
- Theil
- [2] Altman,D.G., Bland, J.M.: Measurement in medicine: the analysis of method comparison studies. Statistician (1983); 32; 307-317
- [3] Bland,J.M., Altman, D.G.: Statistical methods for assessing agreement between two methods of clinical measurement. Lancet (1986) i: 307-10
- [4] Linnet,K., Bruunshuus,I.: HPLC with enzymatic detection as a candidate reference method for serum creatinine. Clinical Chemistry 1991; 37 1669-75.
- [5] Buser, M.:Handbuch Methodenvergleich Validierungsdokument UMTS0001, Version 4 (1996).
- [6] Hawkins, D.M., Diagnostics for conformity of paired quantitative measurements, Statistics in Medicine, 21:1913-1935, 2002
- [7] Feldmann et al. (1981) A multivariate approach for the biometric comparison of analytical methods in clinical chemistry. *J. Clin. Chem. Clin. Biochem*., **19**, 121-137.
- [8] Cheng, C-L., Van Ness J, Statistical Regression with Measurement Error, Oxford University Press Inc., New York, 1999

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