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Short Communication

cp-R, an interface the R programming language for clinical laboratory method comparisons

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ABSTRACT

Background and objective: Clinical scientists frequently need to compare two different bioanalytical methods as part of assay validation/monitoring. As a matter necessity, regression methods for quantitative comparison in clinical chemistry, hematology and other clinical laboratory disciplines must allow for error in both the *x* and *y* variables. Traditionally the methods popularized by 1) Deming and 2) Passing and Bablok have been recommended. While commercial tools exist, no simple open source tool is available. The purpose of this work was to develop and entirely open-source GUI-driven program for bioanalytical method comparisons capable of performing these regression methods and able to produce highly customized graphical output.

Methods: The GUI is written in python and PyQt4 with R scripts performing regression and graphical functions. The program can be run from source code or as a pre-compiled binary executable. The software performs three forms of regression and offers weighting where applicable. Confidence bands of the regression are calculated using bootstrapping for Deming and Passing Bablok methods. Users can customize regression plots according to the tools available in R and can produced output in any of: jpg, png, tiff, bmp at any desired resolution or ps and pdf vector formats. Bland Altman plots and some regression diagnostic plots are also generated. Correctness of regression parameter estimates was confirmed against existing R packages.

Results: The program allows for rapid and highly customizable graphical output capable of conforming to the publication requirements of any clinical chemistry journal. Quick method comparisons can also be performed and cut and paste into spreadsheet or word processing applications.

Conclusions: We present a simple and intuitive open source tool for quantitative method comparison in a clinical laboratory environment.

Introduction

Bioanalytical method comparison is a daily activity for those who work in the field of clinical chemistry. In the clinical laboratory, commercial and in-house laboratory developed tests (LDTs) are often subject to change as new instruments are purchased, a new test becomes available, commercial reagents are reformulated or improvements are made in the workflow. Whatever the need, head-to-head regression of two analytical methods is a very common computational task.

While commercial and open-source spreadsheet programs have ordinary least squares (OLS) regression as built-in functions, the regression problem in bioanalytical method comparison does not fit a major assumption of the OLS model, namely, that there is no error in the *x*-variable. For this reason, two regression methods which do not make this assumption have become popular in the field of clinical chemistry and are recommended by the Clinical Laboratory Standards Institute (CLSI) guideline EP09-A3 [1], *Measurement Procedure Comparison and Bias Estimation Using Patient Samples*. These are Passing Bablok Regression [2–4] and so-called Deming Regression [5], an approach popularized by WE Deming but originally proposed by Adcock [6] and refined by Kummell [7].

The R statistical programming language is a freely available open-source implementation of the S-plus language which has become extremely popular among statisticians, bioinformaticians and academics because of its large user base and vast array of user-contributed packages designed for specific statistical problems [8]. At the time of writing there were two R packages that offer Passing Bablok and Deming Regression: MethComp [9] and mcr [10] packages. However, realistically most clinical laboratorians are unlikely to learn the basic functionality of R in order to take advantage of its power as a computational, statistical and graphical resource. Given the flexibility of R's plotting utilities and its ability to produce publication-quality figures in a number of popular vector and non-vector formats, the aim of this project was to create a freely available open-source GUI-driven program that would pass the necessary commands to R to perform these regression methods and would produce high quality customizeable figures for publication purposes. We have dubbed this program "Chemical Pathology R" or "cp-R" for short.

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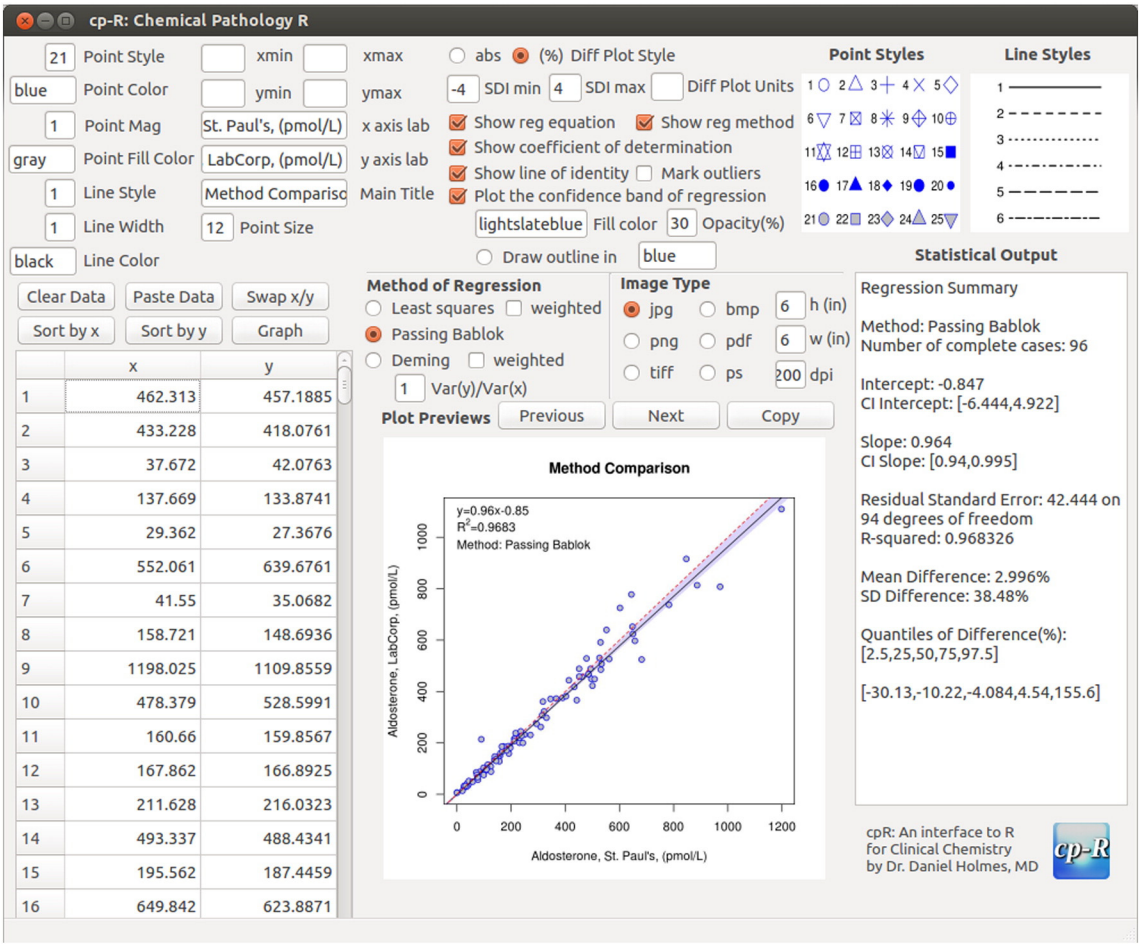

	x	y
1	462.313	457.1885
2	433.228	418.0761
3	37.672	42.0763
4	137.669	133.8741
5	29.362	27.3676
6	552.061	639.6761
7	41.55	35.0682
8	158.721	148.6936
9	1198.025	1109.8559
10	478.379	528.5991
11	160.66	159.8567
12	167.862	166.8925
13	211.628	216.0323
14	493.337	488.4341
15	195.562	187.4459
16	649.842	623.8871

Fig. 1. A screenshot of the cp-R program operating in Ubuntu Linux 12.04.

Materials and methods

Computational methods and theory

R scripts were created to perform regression tasks all necessary regression tasks: OLS, OLS-weighted, Deming, Deming-weighted, and Passing Bablok. OLS regression was performed with R's built in lm() command. Deming and weighted Deming regression were programmed using the description from Linnet [11]. Passing Bablok regression was programmed from the later of two approaches published by these authors [4], which is suited for the comparison of two different bioanalytical methods for the same analyte.

Confidence intervals (CIs) of the regression parameters (slope and intercept) are calculated using the output of lm(), by the BCa bootstrapping method [12] and by direct calculation [3,4] for OLS, Deming and Passing Bablok respectively. Confidence bands of the regression for Deming and Passing Bablok procedures were prepared using R's boot() function to produce bootstrapped regression lines ($n = 1000$ for Deming and $n = 500$ for Passing Bablok) from which 95% confidence limits could be prepared for a series of fitted $\hat{y}$ values. The piecewise continuous curve connecting all upper confidence values became the upper confidence band and the lower confidence band was similarly plotted. Just in time compiling from the R compiler() package [8] was used to speed the computationally intensive aspects of Passing Bablok regression and bootstrapping procedures. Graphical output corresponding to the user's selection of point shape, size, color, axes labels, units, resolution, and plot type were produced using the R plot() command with corresponding plot parameters appropriately assigned.

Bland–Altman plots were prepared in the standard manner [13] as were residual plots and quantile plots of the residual and all other statistical parameters.

All code was written on Ubuntu Linux 12.04 and subsequently ported to Windows and OSX platforms. The python source was written and tested in Python v 2.7.5 and v 3.3.2. The graphical user interface was created with Qt Designer v 4.8.4 [14] which is a code generator for PyQt4. The python script then passes commands to the R to perform regression and graphical procedures and output of R scripts are passed back for display in the python GUI. R scripts were written and tested in R v 3.0.1 to 3.0.3.

As it is frequently the case that Clinical Pathologists and Clinical Chemists do not have administrative rights on their workplace computer and can therefore not install the python or R interpreters, a "portable" version of cp-R was prepared by compiling the python code to a binary executable using cx_freeze v 4.3.2 [15] for Windows platforms. This allows cp-R to run by means of the simple inflation of a zip file to a folder or flash media drive.

Results

A screenshot of cp-R is shown in Fig. 1.

Example graphical output is shown in Fig. 2. The data is taken from a comparison of liquid chromatography and tandem mass spectrometric analysis of serum aldosterone ($n = 96$ samples) between St. Paul's Hospital Laboratory at the University of British Columbia and the Laboratory Corporation of America [16]. Results of various forms of regression analysis are shown in Table 1. Direct comparison with the results of the

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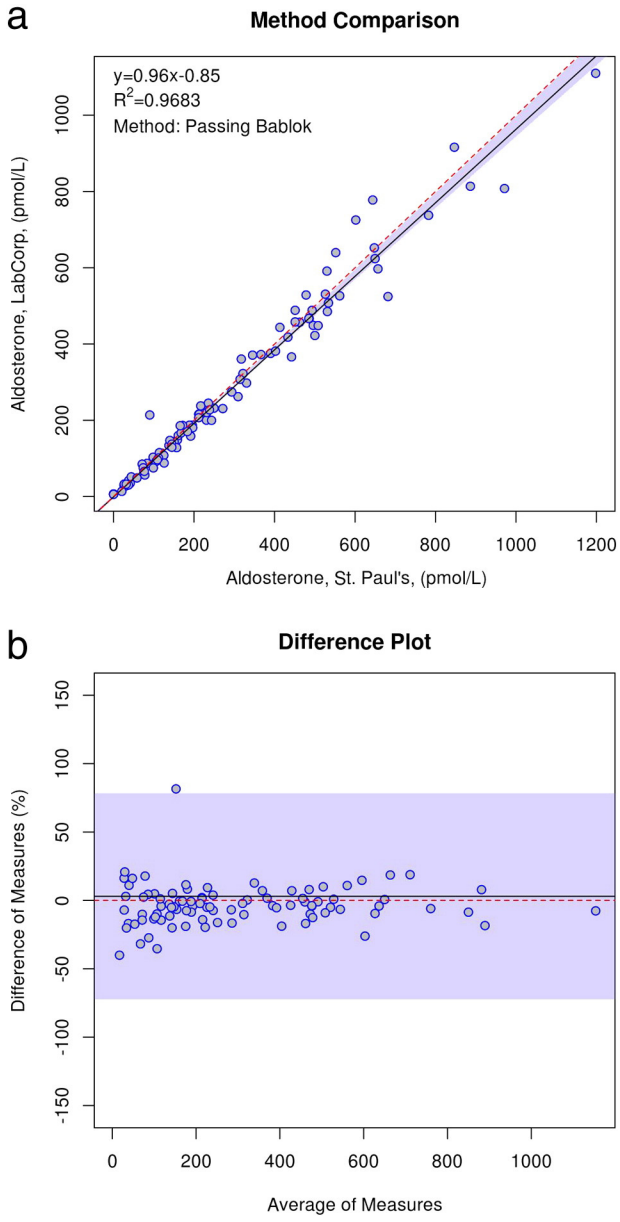

Fig. 2. Example graphical output of regression and difference plots. The black solid line is the regression line while the red dashed line represents the line of identity. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

MethComp and mcr packages is also displayed. For Deming regression, the ratio of variances, was set to its default value of 1 in all cases. For weighted Deming regression, paired results with x values equal to 0 were omitted (n = 3). The Deming method in the MethComp package did not offer weighting at the time of writing, accounting for omissions in Table 1. Bootstrapped CIs were reported as the mean of 100 replications of each 1000-fold bootstrap resampling procedure. As the mcr package offers several bootstrapping algorithms, the BCa method was chosen to match that of cp-R. The MethComp package, on the other hand, uses direct resampling with replacement using the sample() function for its bootstrapping procedure and so BCa could not be specifically selected. Therefore, for Deming regression, modest differences in CIs are expected on the basis of random resampling and calculation algorithms. In contrast, CIs for Passing Bablok were determined by direct calculation rather than bootstrapping [2,4].

Hardware specifications

The cp-R program runs on any laptop or desktop computer as would be typically found in an office environment and has been tested on Ubuntu 12.04 to 14.04 inclusive and both Python 2.7 and 3.0. In the Windows environment, all source code and pre-compiled binaries have been tested on WinXP, Vista, and Win7. In the Mac environment, source code and pre-compiled binaries have been tested on OSX 10.9 (Mavericks).

Obtaining the program

The platform independent cp-R source code and pre-compiled binaries (Win and OSX only) are available at: http://sourceforge.net/projects/cprchempath/.

Discussion

In the unweighted Deming procedure, if we denote the true value of a measurand as X_i and the measured value as x_i, we may write:

$$x_i = X_i + \varepsilon_i$$

$$y_i = Y_i + \delta_i$$

where ε_i and δ_i are normally distributed random variables with expected values of 0. The unweighted Deming model assumes that var(ε) and var(δ) are respectively constant and that their ratio $\lambda = \text{var}(\varepsilon)/\text{var}(\delta)$ is known [11]. Practically speaking, var(ε) and var(δ) are constant only when one is measuring over narrow analytical ranges, as is the case with electrolytes for example [1] and estimated values would be generally known from within-run precision experiments. If their values are not known, estimates may be obtained by performing duplicate analytical determinations of both x and y followed by direct calculation [1]. In the case of cp-R we have not implemented this feature because, in our experience, the necessary experiment incurs reagent expense that is perceived as unjustified. For this reason, we have left it to the user to estimate λ. Similarly, in the weighted Deming procedure, the user can

Table 1
A comparison of regression parameters, y-intercepts, a, and slopes, b, for the cp-R program and the mcr and MethComp packages. Confidence intervals for Deming and weighted-Deming regression are the average of 100 bootstrap procedures (1000 resamplings each). Confidence intervals for Passing Bablok procedures are directly calculated. Abbreviations a = intercept, b = slope, CI = confidence interval, NA = not available.

Regression	Package	a	CI of a	b	CI of b
Deming	cp-R	0.802	[−11.33,12.28]	0.972	[0.920,1.034]
Deming	MethComp	0.802	[−12.11,11.48]	0.972	[0.921,1.035]
Deming	mcr	0.802	[−11.04,12.46]	0.972	[0.919,1.033]
w-Deming	cp-R	−1.745	[−5.82,2.84]	0.980	[0.947,1.026]
w-Deming	MethComp	NA	NA	NA	NA
w-Deming	mcr	−1.745	[−6.11, 2.53]	0.980	[0.948,1.027]
Passing Bablok	cp-R	−0.854	[−6.473,4.922]	0.964	[0.940,0.996]
Passing Bablok	MethComp	−0.853	[−6.473,4.922]	0.964	[0.940,0.996]
Passing Bablok	mcr	−0.853	[−6.444,4.922]	0.964	[0.940,0.995]

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estimate λ from the coefficients of variation (CVs) of *x* and *y* obtained from QC results or precision experiments [1]. In our program, the default value is 1.

The MethComp package implements the original formulation of the Passing Bablok procedure [2] and the mcr offers both the original and various modifications thereof [10]. In their original publication, Passing and Bablok's method comparison procedure was not scale-invariant as pointed out by the authors [3]. That is, if results on one of the axes were reported in a different system of units (eg ng/L vs pmol/L), the slope, *b*, would not proportionally change by the conversion factor between the two units of measure. For this and other reasons the authors discuss, the original approach [2] was not recommended for use in the comparison of two different methods for the same analyte. In our personal experience, this comparison of two chemically distinct methods is more often required in the clinical context which is why we implemented the later (scale invariant) approach [4]. However, as shown in Table 1 the earlier and later Passing Bablok methods may yield virtually identical regression parameters. Any tangible difference will generally be more conspicuous in smaller data sets.

As a matter of convenience to the user, the default dots per inch (DPI) is 72 and 6 × 6 inches which allows users to quickly cut and paste images into their spreadsheet or word-processing files at a size that typically occupies about 1/2 page. The user can quickly change colors by entering either their name, the corresponding integer, or the hexidecimal color code [17]. Semitransparent plot points can be achieved by setting the color to its hexidecimal value followed by a 2-digit hexidecimal number from 00 (0% transparency) to FF (100% transparency). For example, the color "darkviolet" (hex #9400D3) at 50% transparency would be obtained by entering the parameter "Point Color" as #9400D380. Images can be produced in a variety for formats in required dimensions and DPI or in postscript or PDF vector formats as required. This allows the user to meet the image format resolution requirements of any journal. Users can save their series of plots, the corresponding plot parameters, raw data (for future reloading) and regression statistics in a folder.

At the time of writing, cp-R has over 1000 downloads from 53 countries. It is our hope that the availability of a simple free program will allow users at sites with restricted budgets, particularly those in developing world, to be able to produce scientifically sound and aesthetically pleasing method comparison analyses.

Conclusion

We present a simple intuitive graphical user interface to the R statistical programming language to perform OLS, Deming and Passing Bablock regression for comparing bioanalytical methods, particularly in Clinical Chemistry.

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