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SHORT COMMUNICATION

Evaluation of Imprecision, Bias and Total Error of Clinical Chemistry Analysers

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Abstract *Context* Two Biosystems analysers are used in our laboratory, a fully automated A25 and a semi-automated BTS-350. Internal quality control is done for both but external quality control only for A25. As BTS-350 is used for backup, it is important that the results of both analysers are not just comparable but also within predefined limits of systematic, random and total error (TE). *Aim* To evaluate the imprecision, bias and TE of the two Biosystem analysers. *Materials and Methods* Biosystems level-1 quality control sera lot number 70A was run in duplicate for 32 days on both the analysers. Between day imprecision (measured by the coefficient of variation), bias and TE were calculated for ten analytes and were checked to see whether they are within the acceptable minimum limits, desirable limits and optimum limits of allowable error based on specifications on Westgard's website updated in 2014. *Results* On both the analysers, all the analytes except alkaline phosphatase were within the acceptable minimum limits of TE and most analytes were within the desirable limits of TE. Only TG on A25 was within the optimum limit of TE. *Conclusion* The two Biosystem analysers performed comparably with errors within acceptable limits for most analytes. BTS-350 was found to be a suitable and ready backup analyser for A25.

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Introduction

In life, there is continuous fluctuation of the components in biological fluids. The biological variation (BV) of analytes is of three types, namely, variation over the life span, cyclical variation and random variation. The latter causes subtle variation around the setting point of each individual which is responsible for the within-subject or intra-individual BV, while the overall variation is responsible for the between-subject or inter-individual BV. The BV and the analytical variation both affect the test result, but while the latter can be minimised, minimisation of the former is not possible. Hence it is important to ensure that the analytical variation is kept minimised and does not contribute significant additional variation to that contributed by the BV. The analytical variability is therefore kept appropriately less than the biological variability for the test to be confidently used for clinical diagnosis and monitoring [1].

Measurement of laboratory analytical errors fall into two main categories, systematic error and random error. Systematic errors are predictable problems influencing observations consistently in one direction, while random errors are more unpredictable [2]. Systematic errors are assessed by the bias, while random errors by the imprecision measured by the coefficient of variation (CV). Imprecision affects the reproducibility and repeatability of results [3]. Reproducibility is the closeness of the results of successive measurements under changed conditions which require multicentric trials. Repeatability is the closeness of the results of at least twenty successive measurements under similar conditions. Bias is the average deviation from a true value with minimal contribution of imprecision while

Table 1 Imprecision (CV %) of Biosystem A25 & BTS-350 analysers

Analyte	A25 mean	A25 SD	BTS-350 mean	BTS-350 SD	A25 CV (%)	BTS-350 CV (%)	Desirable CV (%)	Minimum CV (%)	Optimum CV (%)
Glucose	91.8	2.29	92.4	1.87	2.5	2.0	2.8	4.2	1.4
Urea	28.8	1.54	28.3	1.52	5.4	5.4	6.1	9.2	3.1
Creatinine	1.56	0.05	1.56	0.06	3.2[a]	3.8[a]	3.0	4.5	1.5
Cholesterol	148.5	7.10	151.3	6.94	4.8[a]	4.6[a]	3.0	4.5	1.4
Triglyceride	57.9	2.96	58.0	5.21	5.1	8.9	10.0	15.0	5.0
T. Bilirubin	2.36	0.20	2.36	0.17	8.5	7.3	10.9	16.4	5.5
D. Bilirubin	0.59	0.10	0.57	0.09	16.9	15.8	18.4	27.6	9.2
ALP	168.9	13.15	168.4	11.08	7.8[a]	6.6[a]	3.2	4.8	1.6
ALT	35.2	2.94	33.9	2.48	8.3	7.3	9.7	14.6	4.9
AST	41.4	2.47	41.3	2.42	5.9	5.9	6.2	9.3	3.1

[a] Beyond limit of desirable imprecision

inaccuracy is the deviation of a single measurement from the true value with significant contribution by imprecision [4]. Multiple measurements, at least twenty and preferably forty, are therefore required for calculating imprecision as well as bias [5].

Uncertainty of measurement provides a quantitative estimate of the quality of a test result. Uncertainty is defined as "a parameter associated with the result of a measurement, that characterises the dispersion of the values that could reasonably be attributed to the measurand". Sources that contribute to uncertainty may include sampling, sample preparation, sample portion selection, calibrators, reference materials, input quantities, equipment used, environmental conditions, condition of the sample and changes of operator Comparison of result is made possible by an estimate of the uncertainty of measurement, calculated as the 95 % confidence interval (±1.96 CV %) [6].

The uncertainty associated with a test result due to the random errors is termed imprecision, which is determined from the data of internal quality control. The total analytical error also has to include an estimate of analytical bias. Anaytical goal setting is required to determine whether a method is producing 'fit for purpose' results. The upper acceptable limit for imprecision is taken as a proportion of the intra-individual BV of the analyte and the upper limit for analytical bias as a proportion of the overall BV, intra-individual and inter-individual. Together, this determines goal-setting for the total analytical error (bias + imprecision) [6].

In the early 1990, recommendations were made by a group of European scientists for evaluation of clinical chemistry and other analysers in terms of its imprecision, bias and total error (TE). It came to be known as the "European Biologic Goals and Calculated Biologic Allowable Total Errors" [7]. Later, Ricos et al. [8] built the biodatabase of these desirable specifications, which are periodically updated on Westgard's website [9]. The minimum and optimum limits of these specifications are also provided on Westgard's website [10, 11]. The most clinically and technically appropriate goal is taken as the minimum for imprecision and bias.

In our clinical laboratory, we are using two Biosystems analysers, a fully automated A25 analyser for routine clinical chemistry and a semi-automated BTS-350 for back-up purpose. Internal quality control with Biosystem quality control sera is carried out on both analysers to ensure that the results are within control limits by plotting the controls on the Levey Jennings chart and checking acceptability according to Westgard rules [12]. However, only the results on A25 are evaluated by the external quality assessment scheme. It is important therefore to ensure that the results of the two analysers are not just similar, but also within the predefined limits of error. Hence this study was carried out to evaluate the two Biosystem's analysers on the basis of their bias, imprecision and TE.

Methodology

We studied two analysers of Biosystem, Phillipines. The A25 is a fully automated random access analyser with following major specifications-throughput of 240 tests/h, minimum reading volume of 200 µl, filter configuration 340, 405, 505, 535, 560, 600, 635, 670 nm, measuring range 0.05–2.5 A, automatic conditioning of fluid system, Levy-Jennings QC Chart, flexibility in positioning with sample and reagents racks and unlimited STAT capabilities, temperature 10–35 °C and relative humidity <75 %. The BTS-350 is a semi-automated analyser with following specifications- LED Configuration 340, 405, 505, 535, 560, 600, 635, 670, durable and humidity proof hard coated filters, battery

Table 2 Bias (%) of Biosystem A25 and BTS-350 analysers

Parameter	QC target	A25 mean	BTS-350 mean	A25 bias (%)	BTS-350 bias (%)	Desirable bias (%)	Minimum bias (%)	Optimum bias (%)
Glucose	92	91.8	92.4	1.6	1.8	2.3	3.5	1.2
Urea	29.7	28.8	28.3	5.5	5.4	5.6	8.4	2.8
Creatinine	1.55	1.56	1.56	2.7	3.2	4.0	6.0	2.0
Cholesterol	152	148.5	151.3	4.8[a]	4.1	4.1	6.2	2.1
Triglycerides	59	57.9	58.0	4.2	7.3	9.6	14.4	4.8
T. Bilirubin	2.4	2.36	2.36	6.2	4.7	9.0	13.5	4.5
D. Bilirubin	0.6	0.59	0.57	14.6[a]	13.5	14.2	21.3	7.1
ALP	179	168.9	168.4	9.3[a]	7.4[a]	6.7	10.1	3.4
ALT	35.5	35.2	33.9	7.6	7.6	11.5	17.3	5.8
AST	41.6	41.4	41.3	4.9	4.5	6.5	9.8	3.3

[a] Beyond limit of desirable bias

power pack, photometric range (0.0–3.5 A) for all wavelengths, flow cuvette 18 µl and Levy-Jennings QC Chart. Both analysers were calibrated at installation using Biosystems calibrators and Biosystems reagent kits. Calibration was done thereafter whenever internal quality control results failed westgard's rules. Biosystems level-1 (human) quality control sera lot number 70A suitable as an accuracy control was run in duplicate for 32 days on both the analysers and the average of the two values was noted. The control sera complies with the directions set by the ISO 15189. It's values are traceable to international certified reference materials: C-RSE/IFCC, SRM927 c, SRM 909 b, ERM-DA470, ERM-AD455, BRM 97/662, RM W1066, BCR 470. As the A25 analyser does not have onboard cooling, we analyse only ten analytes on it that are most frequently ordered. The mean values and standard deviations were calculated for these ten analytes i.e. glucose, urea, creatinine, total cholesterol, triglycerides (TG), total bilirubin, direct bilirubin, alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST) for each machine. The controls were plotted on the Levey Jennings chart to check acceptability according to Westgard rules. Student t test was applied to ensure that there was no significant difference in the means of any analyte measured by the two analysers. Between day imprecision, bias and (TE) were determined for each analyte on each analyser as follows-

$$CV\% = (SD/\text{Mean}) \times 100$$

CV (%) is the coefficient of variation for measuring between day imprecision, SD is the standard deviation

$$\text{Bias}\,\% = (\textit{Average}\ \text{absolute deviation from the target value}/\text{Target}) \times 100$$

The TE (%) was calculated as *1.65 × CV (%) + Bias (%)* [13]. The factor 1.65 implies that 95 % of the results will fall within the TE limit, given a Gaussian distribution.

The between day imprecision, bias and TE for the two instruments were checked for each analyte to see if they were within the limits of minimum, desirable and optimum specifications updated in 2014 respectively [9–11]. These analytical goals are derived from BV [6] as follows-

(a) There are three levels of analytical goal for imprecision derived from intra-individual BV:

$$\text{Optimum: } CV_A = < 0.25 \times CV_I$$

$$\text{Desirable: } CV_A = < 0.50 \times CV_I$$

$$\text{Minimum: } CV_A = < 0.75 \times CV_I$$

where: CV_A = Coefficient of variation (analytical) and CV_I = Coefficient of variation (intra-individual), derived from the intra-individual BV

(b) There are three levels of analytical goal for bias derived from intra-individual and inter-individual BV:

$$\text{Optimum: } B_A = < 0.125\left(CV_I^2 + CV_G^2\right)^{1/2}$$

$$\text{Desirable: } B_A = < 0.250\left(CV_I^2 + CV_G^2\right)^{1/2}$$

$$\text{Minimum: } B_A = < 0.375\left(CV_I^2 + CV_G^2\right)^{1/2}$$

where: B_A = analytical bias, CV_I = CV of within-subject (intra-individual) BV and CV_G = CV of between—subject (inter-individual) BV.

(c) The two parameters are conveniently combined as total error allowable (TEa), for which three levels of analytical goal are set:

$$\textit{Optimum: } \text{TEa} = < 1.65(0.25CV_I) + 0.125\left(CV_I^2 + CV_G^2\right)^{1/2}$$

$$\textit{Desirable: } \text{TEa} = < 1.65(0.50CV_I) + 0.250\left(CV_I^2 + CV_G^2\right)^{1/2}$$

Table 3 Total Error of Biosystem A25 & BTS-350 analysers

Parameter	A25 total error (%)	BTS-350 total error (%)	Desirable total error (%)	Minimum total error (%)	Optimum total error (%)
Glucose	5.7	5.1	7.0	10.5	3.5
Urea	14.3	14.2	15.6	23.4	7.8
Creatinine	7.9	9.6[a]	8.9	13.4	4.5
Cholesterol	12.7[a]	11.7[a]	9.0	13.5	4.3
Triglycerides	12.6	22.1	26.0	39.0	13.0
T. Bilirubin	20.2	16.8	26.9	40.4	13.5
D. Bilirubin	42.6	39.5	44.5	66.8	22.3
ALP	22.1[a]	18.2[a]	12.0	18.0	6.0
ALT	21.4	19.7	27.5	41.3	13.8
AST	14.8	14.2	16.7	25.1	8.4

[a] Beyond limits of desirable total error

$$\textit{Minimum:}\ \mathrm{TEa} = <1.65(0.75\mathrm{CV_I}) + 0.375\left(\mathrm{CV_I^2} + \mathrm{CV_G^2}\right)^{1/2}$$

Results

All the analytes on both the analysers except ALP were within the minimum limits of between day imprecision. Three analytes on both analysers had CV (%) outside the limits of desirable imprecision—total cholesterol, creatinine and ALP. No analyte had CV(%) within the limits of optimum imprecision (Table 1).

All the analytes on both analysers were within the minimum limits of bias (%). However on A25, three analytes had bias (%) outside the desirable limits—total cholesterol, direct bilirubin and ALP, while on BTS-350 there was one—ALP. Only TG on A25 had bias (%) within optimum limits (Table 2).

All the analytes on both analysers except ALP were within the minimum limits of TE (%). However, analytes with TE (%) outside the desirable limits on A25 were two—total cholesterol and ALP, while on BTS-350 there were three—creatinine, total cholesterol and ALP. Only analyte within optimum limit of TE (%) was TG on A25 (Table 3).

Discussion

It is a medical need to have some preset quality specification of laboratory test results. This ensures quality and uniformity not just across laboratories but also within a laboratory with multiple analysers. Evaluation of performance with preset quality specifications based on BV has been going on since many years, [14] but lack of consensus on the published recommendations makes it difficult for authors to select the most appropriate method. In our study, we checked the level of error on our two analysers in comparison to the limits specified on Westgard's website.

On both the analysers, all analytes except ALP were within minimum limits and most within desirable limits of imprecision, bias and TE. Cholesterol was outside desirable limits of both imprecision and bias while outliers affected creatinine, affecting only its precision. Only TG on A25 was within the stringent optimum limit of TE.

Several authors have reported analysis of results on their analyser following one or the other guideline with variable results. Coudene et al. evaluated 32 common analytes on the ABX Pentra 400 according to the National Committee for Clinical Laboratory Standards and Valtec protocols and reported imprecision within acceptable limits with moderate influence of interfering substances [15]. Miler et al. compared Olympus AU2700 with Olympus AU640 with guidelines from the Croatian Society of Medical Biochemists [16] The results were comparable but control samples with low concentrations did exceed their allowable biases with conjugated bilirubin having the maximum bias (16.48 %).

In our study, the two analysers provided comparable results. Lack of automation did not decrease the performance of BTS-350 although automation is necessary for handling large sample loads. BTS-350 proved to be an effective back-up analyser for correct diagnosis and longitudinal follow up. Although laboratory errors are frequently pre-analytical or postanalytical [17], ensuring minimisation of analytical error on both analysers ensures two ready analysers standardized for comparable reporting.

There were two major limitations of our study. It used only level one quality control sera. The imprecision is better recorded at more than one level of quality control depending on the range of reportable values and clinical use of the test. We could have also used patient data to calculate the BV by collecting and storing a number of

samples from a number of individuals to analyse them simultaneously. The within-subject and between-subject variance could be determined by analysis of variance (ANOVA) of obtained results [1]. Controlling pre-analytical factors, the results of BV estimates in adults are similar. The estimates of BV however, is not only made available but updated every 2 years on westgard's website [9].

Conclusion

The two Biosystem analysers performed comparably with all analytes except ALP within the minimum specified acceptable limits and most analytes within the desirable limits. However, only TG on A25 met the most stringent optimum limit for all the errors. BTS-350 was thus found to be a suitable and ready backup analyser for A25.

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