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Research Article

A STUDY TO ESTABLISH THE AGREEMENT BETWEEN THE RESULTS OF ELECTROLYTES (SERUM SODIUM AND POTASSIUM) ESTIMATED BY A WET CHEMISTRY INSTRUMENT (EASYLYTE) WITH THAT OBTAINED BY A DRY CHEMISTRY ANALYZER (VITROS 350)

SHARMISTHA CHATTERJEE*, DIVYA M, KAUSHIK MAJUMDER, INDRANIL CHAKRABORTY

Department of Biochemistry, College of Medicine and Sagore Dutta Hospital, Kolkata, West Bengal, India.
*Corresponding author: Sharmistha Chatterjee; Email: sharmisthacmajumder@yahoo.co.in

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ABSTRACT

Objectives: The objective of the study was to assess the agreement between results of electrolytes (serum sodium and potassium) estimated by a wet chemistry instrument with that obtained by a dry chemistry analyzer.

Methods: It was an observational analytical cross-sectional study done in the Departmental clinical laboratory. The samples were selected randomly from the usual lab workflow. All the samples were first run on the Easylyte (wet chemistry) and then run on the Vitros 350 (dry chemistry). The paired data thus obtained were compiled and tabulated and then statistically analyzed.

Results: The agreement of the results between the two methods was evaluated using the Bland–Altman difference plot and the Passing–Bablok Regression Equation and the Deming regression studies. By analyzing the diagram of Bland–Altman, it is seen that for sodium, the average bias is of −2.22; limits of agreement being −26.12–21.77. For potassium, Bland Altman plots show a bias of −0.21; limits of agreement −0.61–0.19. Passing Bablock regression calculated an intercept of −56.86, 95% confidence interval (CI) (−100, −28) and Slope of 1.43 for sodium measurements and calculated an intercept of −0.706, 95% CI (−0.66, −0.45) and Slope of 1.2 for potassium estimation.

Conclusion: Statistical analysis revealed conflicting solutions. There is a great discrepancy between the results of the electrolyte estimation by the two methods since the methodologies are not identical.

Keywords: Measurement of electrolytes, Wet chemistry (easylyte), Dry chemistry (vitros 300).

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INTRODUCTION

Estimation of serum electrolytes, namely, sodium and potassium are among the most frequently requested tests in routine clinical practice because electrolyte homeostasis is of major physiological importance in the optimum functioning of the various organ systems and metabolic processes in the body [1]. Electrolyte measurements in blood products were traditionally performed using flame photometry which was gradually replaced by ion selective electrodes after the development of sensors capable of measuring body fluids directly, all throughout the physiological range. An ion-selective electrode develops a voltage that varies with the concentration of the ion to which it responds. The Easylyte instrument (already in use in the department) and the Vitros350 (newly acquired) both perform electrolyte measurements based on the direct ion selective electrode (direct ISE) methods. But, the novelty of the Vitros 350 is that it is a dry chemistry instrument, that is, it has completely eliminated the need of water. Since the sample volume needed in the Vitros is only 10 μL, the instrument may be advantageous for pediatric and neonatal samples. Besides, to use both the instruments simultaneously in the departmental clinical labortory, it is imperative that the two instruments should be compared in the larger interest of the patient populace attending the hospital.

Therefore, the purpose of the study was to assess the agreement between results of electrolytes (serum sodium and potassium) estimated by a wet chemistry instrument (Easylte) with that obtained by a dry chemistry analyzer (namely the Vitros 350).

METHODS

The observational analytical cross-sectional study was carried out in the departmental clinical laboratory in the department of biochemistry in a tertiary care center and a government teaching hospital catering primarily to a suburban population. The ethical clearance was obtained from the Institutional Ethics Committee. Around 120 samples were selected randomly from the usual lab workflow and tested for serum sodium and potassium. Only grossly hemolyzed, lipemic, or icteric samples were excluded from the study. All the samples were simultaneously run on the Easylyte (wet chemistry) which is already in use in the department and the newly acquired Vitros 350 (dry chemistry) instrument. Two levels of commercial third-party control were run on both the instruments everyday and standard Westgard rules of monitoring internal quality control, that is the usual warning and run rejection rules were applied. The results of sodium and potassium estimation thus obtained were compiled and tabulated in Excel sheet and statistically analyzed. The important differences in the two instruments in regard to the performance specifications are compared below in Table 1.

RESULTS AND DISCUSSION

The data obtained from the two instruments were compiled in an Excel sheet and further data analysis was carried out using MedCalcVersion 11.5.0 statistical software. The distribution of the each parameter was first determined by the Shapiro–Wilk test and was found to be non-parametric in nature. The descriptive statistics are tabulated in Table 2.

When Sodium measurements obtained by Easylyte were compared with that obtained by Vitros, the Wilcoxon rank sum test revealed a significant difference in the means, Z value=−9.14 ($p<0.00001$). In case of potassium values, the Wilcoxon rank-sum test revealed a significant difference in the means, Z value=8.84 ($p<0.00001$) (vide Figs. 1 and 2).

Table 1: Performance specifications of Easylyte and Vitros 350 for estimation of electrolytes compared

Performance indicator	Easylyte		Vitros 350	
	Sodium	Potassium	Sodium	Potassium
Method	Potentiometry-direct ISE	Potentiometry-direct ISE	Potentiometric microslides, direct ISE	Potentiometry- direct ISE
Sample volume	100 µL serum	100 µL serum	10 µL serum	10 µL serum
Analytical measurement	20–200	0.2–40	75–250	1.0–14.0
Reference range	135–145	3.5–5.0	137–145	3.5–5.1
Reproducibility within run (%)	<1	<2	0.7	0.02
Reproducibility between run (%)	<2	<2.5	0.7	0.71

ISE: Ion selective electrode

Table 2: Descriptive statistics

Statistical parameters	Sodium (electrolyte) (meq/l)	Sodium (Vitros) (meq/l)	Potassium (Easylyte) (meq/l)	Potassium (Vitros) (meq/l) r
Mean	139.1	141.1	5.37	4.5
Median	139.2	143	4.36	4.5
Mode	139.2	142	3.99	4.7
Standard deviation	2.99	3.88	0.36	0.45
Normality (Shapiro Wilk)	Reject normality	Reject normality	Reject normality	Reject normality

Correlation between the two methods was further examined by Kendall's correlation coefficient. A strong correlation between the sodium measurement values obtained by the two instruments as Kendal coefficient was 0.50, (>0, p<0.05). In case of potassium, Kendall's correlation coefficient was 0.83, (>0, p<0.05). Both the correlation coefficients are Table 3.

Interpretation:
- Tau ≥ 0.07 weak association
- ≥ 0.21 medium association
- ≥0.35 strong association.

The agreement of the results between the different techniques was further evaluated using the Bland–Altman difference diagram and the Passing–Bablok and Deming regression line.

Bland Altman plots show a bias of −2.22; (limits of agreement −26.12–21.77) for sodium measurements by Easylyte versus Vitros (Fig. 3). In case of potassium, Bland–Altman plots show a bias of −0.21; limits of agreement −0.61–0.19 (Fig. 4).

Passing–Bablock regression calculated an intercept (β0)=−56.86 (95% CI=lower confidence limit (LCL)=−100, upper confidence limit (UCL)=−28. and a slope(β1)=1.43. (LCL=1.22, UCL=1.73) for sodium values (Fig. 5). In case of potassium, Passing–Bablock regression returned an intercept of (β0)=−0.66 (LCL=−0.86, UCL=−0.45), and a slope (β1) of 1.23, (LCL=1.15, UCL=1.25) (vide Fig. 6).

Nest, the Deming regression was carried out for sodium values of Easylyte versus Vitros and the results obtained are as follows. Deming regression revealed a intercept of −31.02 and a Slope 1.24. In case of potassium, Deming regression revealed a intercept of −31.02 and a Slope 1.24 (Figs. 7 and 8, respectively).

Table 3: The correlation –Kendall's Tau correlation of the sodium and potassium values measured by the two instruments

Parameters	Kendall Tau correlation	95% CI lower limit	95% CI upper limit
Na (Easylyte) versus Vitros	0.50	0.41	0.58
K (Easylyte vs. Vitros)	0.83	0.79	0.86

CI: Confidence interval

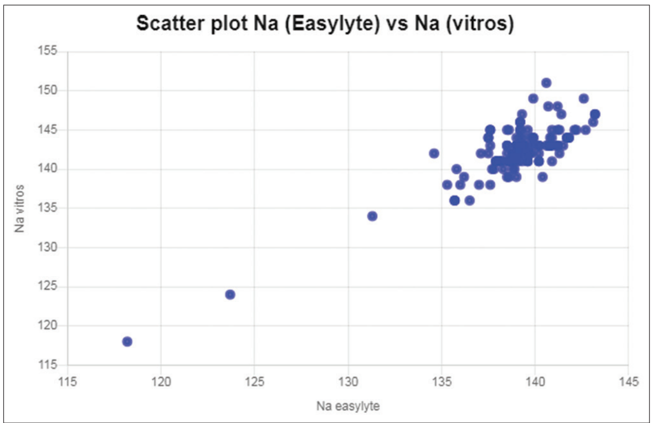

Fig. 1: Scatter plot of sodium estimated by Easylyte versus that estimated by Vitros

This observational analytical and cross-sectional study was carried out in the Department of Biochemistry, College of Medicine and Sagore Dutta Hospital among the patients attending the Department of Biochemistry for measurement of serum sodium and potassium. From time to time, state of art instruments based on latest technologies are introduced for better evaluation of patients' samples and seamless functioning of the clinical laboratory. However, introduction and subsequent usage of a newly installed instrument and integration into the daily laboratory workflow must follow a protocol [2,3]. This is necessary to establish confidence in both the laboratory technicians and the customer for lessening the overall measurement and diagnostic uncertainty. Electrolyte measurements are extremely important for a number of reasons. They are one of the most common reversible causes of morbidity and mortality in hospitalized patients [4]. Usually, around 50–60 samples are received everyday in the departmental clinical lab which includes a considerable number of pediatric and neonatal samples where the sample volume received is very little. Both the Easylyte instrument (already in use) and the Vitros 350 (newly procured) work on the same principle, that is, direct ISE. However, the advantage of the Vitros is that it requires only 10 µL of the sample and hence is considered to be more useful in pediatric and neonatal sample where the sample volume received is very low. The sample volume required in the Vitros 350 is only around 10 µL and it is for this

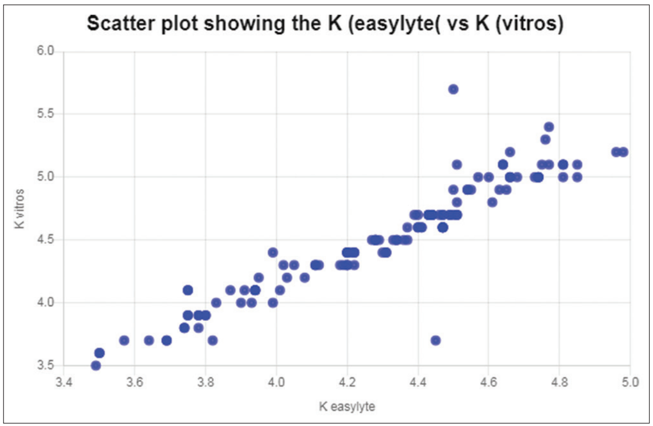

Fig. 2: Scatter plot of potassium estimated by Easylyte versus that estimated by Vitros

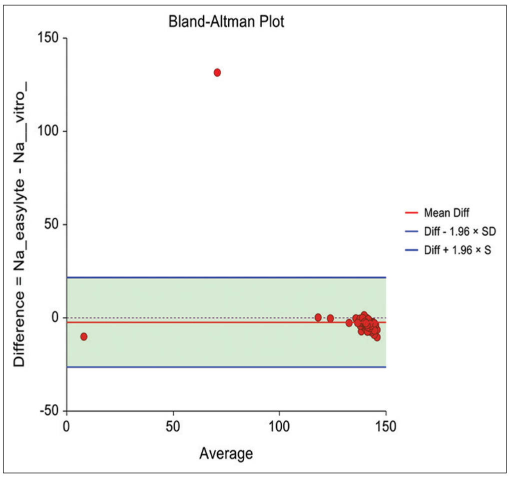

Fig. 3: Bland–Altman analysis for method comparison: Bias and limits of agreement (difference-means plot)

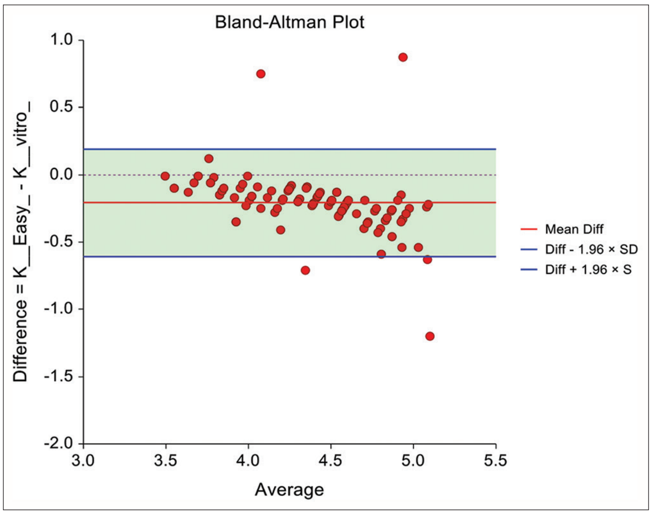

Fig. 4: Bland–Altman analysis for method comparison: Bias and limits of agreement (for potassium)

very advantage that the instrument was procured in the laboratory. To include the instrument into the day-to-day operation of the laboratory, this study was carried out to assess the the agreement between results of electrolytes (serum sodium and potassium) between the two instruments.

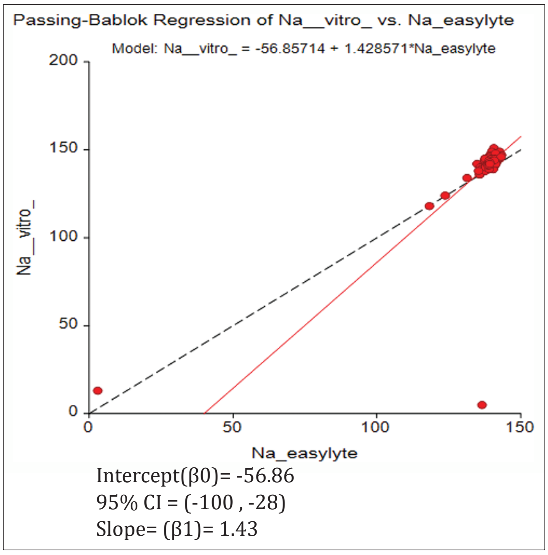

Fig. 5: Passing -Bablok Regression of Na estimation on Vitros vs Easylte

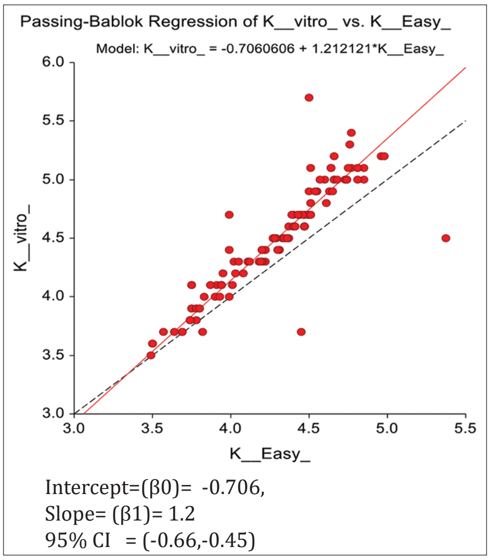

Fig. 6: Passing Bablok regression of Potassium estimation on Vitros vs Easylyte

A detailed study of the obtained results reveals conflicting statistical solutions. Let us consider them one by one. First, we take up the results of sodium measurements by the Easylyte versus Vitros. The Wilcoxon rank-sum test revealed a significant difference in the means in the sodium measurements, Z value=−9.14 ($p<0.00$). Kendall's correlation coefficient (t) came to be 0.50 (95% confidence intervals [CI] =0.41–0.58), $p<0.05$, suggesting a strong correlation. Linear regression studies revealed a correlation coefficient of 0.85 and "r" value 0.62, p=0.0. By normal standards, the association between the variables would be considered to be statistically significant. The Passing Bablock regression calculated an intercept ($\beta 0$)=−56.86 (95% CI=(−100, −28) slope($\beta 1$)=1.43. The Passing–Bablock is normally utilized to calculate the 95% CI of the intercept and the slope. The intercept represents the systematic bias or the difference between two methods and the slope represents the proportional bias between the two methods under consideration in the Passing Bablock equation. If the 95% CI for the intercept includes zero and the 95% CI of the slope include one, only then two methods are considered to be comparable. But here, zero is not included in the 95% CI of the intercept and one does lie within the 95% CI of the slope. This suggests that there is a systematic bias and the methods are not

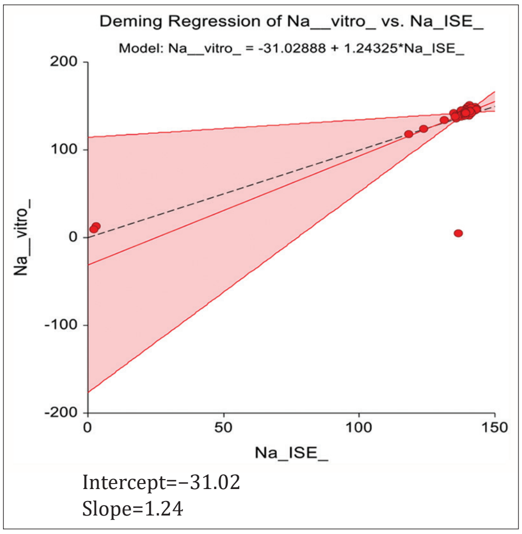

Fig. 7: Deming regression of sodium estimation on Vitros vs Easylyte

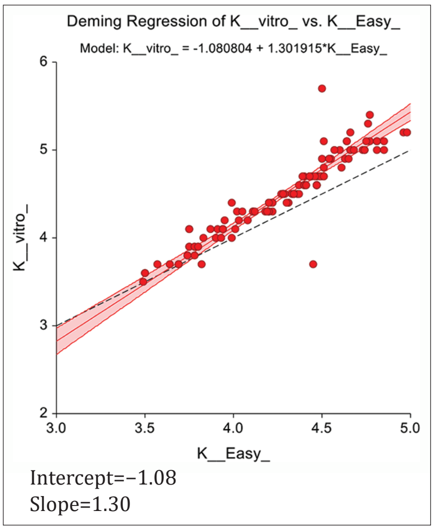

Fig. 8: Deming regression of Potassium estimation on Vitros vs Easylyte

comparable within the investigated comparable range [5,6]. The Bland–Altman analysis revealed a bias of −2.22 with the limits of agreement being −26.2–21.7 [7-9]. This means that on an average, the Vitros measures 2.22 units more than the Easylyte. The limits of agreement are too wide for clinical purpose and so the results may be ambiguous [8]. Finally, the Deming regression returned an intercept of −31.02 and a Slope 1.24. While the linear regression considers that the two methods have no random measurement errors, the Deming regression assumes that both the methods have some inherent measurement errors.

Next, we discuss about potassium measurements by the Easylyte versus Vitros. Wilcoxon rank-sum test revealed a significant difference in the means, Z value=−8.84 ($p<0.00$). Kendall's correlation coefficient =0.83(>0, $p<0.05$) suggesting a strong correlation. Passing–Bablock regression calculated an intercept of (β0)=−0.66 (95%=−0.86–−0.45) and a slope (β1) of 1.23, (95%=1.15–1.25). Once again, there is a systematic bias and the methods are not comparable. Deming regression revealed an intercept of −31.02 and a Slope 1.24. Bland–Altman plots show a bias of −0.21; limits of agreement being −0.61–0.19.

Thus, extensive statistical analysis revealed conflicting results for both the parameters on both instruments. This is compliant with other studies where the authors note that since the methodologies used by the two analyzers differ greatly, it is useless to compare the two because the methodologies may have some effect on the level of agreement of the measurements. Therefore, no comments are possible on clinical decision limits [10]. The basic surmise of the study is that electrolyte measurements on these two instruments cannot be used interchangeably.

A primary pre-requisite of method comparison studies is to take a minimum of 40 samples selected to cover the entire range of the method, to be run over a period of minimum 5 days and preferably within 2 h of each other. The same protocol is outlined in CLSI EP09-A3 document also [11]. It is also important that the samples are accurately measured and the distribution of the values adequately reflect the entire range of analyzed values to ensure a good quality of the study. In our study, we took 120 samples run over a period of roughly a month and analyzed simultaneously. But even then, it was impossible to establish linearity trends due to the small sample size.

Further, dry chemistry has several advantages over wet chemistry like requirement of a small sample volume and a reduced problem of carryover (a systemic error that affects measured value obtained) is reduced in dry chemistry [12]. Apart from infants and neonates, small sample volumes are also received in case of in debilitated patients like cancer survivors where alternate therapies may have to be considered [13].

CONCLUSION

This study was carried in a medical college to establish the agreement between two instruments with regard to estimation of serum electrolytes. Extensive statistical analysis of the data obtained revealed conflicting results. Hence, it may be concluded that the values of serum electrolytes obtained from two different kinds of analyzers cannot be used interchangeably.

AUTHORS CONTRIBUTION

1. Idea and Concept and development of the research question-Dr Indranil Chakraborty
2. Sample collection and assay- Dr Kaushik Majumder and Divya M
3. Statistical analysis, write up and corresponding author-Dr Sharmistha Chatterjee.

CONFLICTS OF INTERESTS

None.

AUTHORS FUNDING

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