Agreement between sodium and potassium results measured on dry chemistry analyser and arterial blood gas analyser – A cross sectional study

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Abstract: *Background:* Many hospitals interchangeably use the results of electrolytes performed on Laboratory Auto-analysers (LAAs) and Arterial Blood Gas analysers (ABGs). But data on comparability and validity of these measurements were ambiguous. Most of the previous studies compared electrolyte results by LAAs and ABGs, they work on the indirect ISE and direct ISE principle respectively. In this study, we have compared electrolyte results between Dry Chemistry analyser Vs. ABG analyser both works on direct ISE. *Materials and Methods:* We analysed 120 paired samples which were collected following exclusion criteria for electrolyte analysis on Dry chemistry analyser and ABG analyser. Bland Altman test and Lins' concordance coefficient were performed to check the agreement between the two methods. Results from the two test methods were also assessed against the United States Clinical Laboratory Improvement Amendments (US-CLIA) 88 rules. *Results:* Bland Altman bias for Sodium and Potassium were -0.267(-0.88 to -0.348) and -0.097(-0.18 to 0.013) respectively. 13.3% of Sodium and 4.1% of Potassium results were outside USCLIA variation limits. Lins' concordance coefficient values for Sodium and Potassium were 0.85(0.79 - 0.89) and 0.85(0.80- 0.89). *Conclusion:* In the present study we found that the electrolytes, Sodium and Potassium measured on the Dry chemistry analyser showed bias of -0.3 and -0.1 compared to that of the Arterial Blood gas analyser. For both electrolyte values Lower limit of agreement (LLOA) and Upper limit of agreement (ULOA) were beyond the CLIA/ maximum allowable errors which indicate these methods cannot be used interchangeably.

Keywords: Electrolytes, Dry Chemistry, Arterial Blood gas Analysis, Bland Altman analysis, USCLIA.

Introduction

Serum electrolytes, serum Sodium and Potassium measurement is essential among the patients admitted in the Intensive Care Unit (ICU). Electrolyte abnormalities can represent a significant risk to life, influence the choice of treatment and prognosis of the disease [1]. Serum electrolytes are routinely measured from central laboratory auto-analysers (LAAs). However, time for reporting of results depends on many factors including samples transport, processing and analysis [2]. Any delay in reporting of electrolyte results may affect the treatment of critically ill patients. The other method for electrolyte assay is by Arterial Blood Gas analysers (ABGs) which provide quick results and thereby prompt treatment [3]. Many hospitals use these two methods interchangeably for estimation of electrolytes. But data on comparability and validity of these measurements among LAAs and ABGs are ambiguous. Earlier studies reported considerable differences in the electrolyte measurements by LAAs and ABGs [4-6].

Most of the previous studies compared electrolyte values between routine clinical chemistry analysers (wet chemistry) which are based on indirect ISE and ABG analysers which are based on direct ISE measurement [4-6]. As the indirect ISE involves pre-dilution of the samples and direct ISE will not use any dilution step, this can lead to method to method variation in the analytical results. Moreover, another factor affecting the

accuracy of the indirect ISE method results is the displacement of the plasma water by high concentrations of proteins and lipids in the blood [7-8].

Aims and objectives: In the present study we have compared the agreement between Sodium and Potassium results measured on Dry Chemistry Analyser (Vitros 4600) and Arterial Blood gas analyser (ABL 80 Flex), both works on direct ISE principle. This ensures the comparability between the same methods, and simultaneously avoids the pre-dilution step which is associated with indirect ISE and possible pre-analytical errors.

Material and Methods

Study description: A Cross sectional study was conducted to compare the electrolytes, sodium (Na) and potassium (K) results measured on Vitros 4600(Dry chemistry analyser) and Radiometer, ABL 80 Flex (ABG analyser). A total of 943 samples received from June 2019 to August 2019, we have included 120 consecutive samples which met the inclusion criteria. The present study was approved by the Institutional Ethical Committee with IEC No. IEC/2019/2/6.

Study subjects: Patients admitted to the ICUs and Causality who were advised simultaneously for ABG analysis and electrolytes by the treating physician were included in the study.

Inclusion criteria: Subjects from whom arterial samples and venous samples received simultaneously for arterial blood gas analysis and electrolytes measurement with a maximum gap of 15minutes, on the same day were included.

Exclusion criteria: Samples received with a time gap of more than 15 minutes for ABG and electrolyte analysis. Missing samples, Haemolysed and lipemic samples were excluded from the study.

Sample size: With the sample correlation coefficient 0.9 and population correlation coefficient 0.94[6], power 80% and alpha error of 5%, required sample size was 114. Sample size was calculated by using then Master (2.0) software. Finally we have included 120 subjects for the study.

Instruments: Dry chemistry analyser (VITROS® 4600, Ortho Clinical Diagnostics, Raritan, NJ, USA) uses direct ISE method, not indirect ISE as in most of the wet chemistry analysers. There's no serum dilution risk with VITROS Micro slide potentiometric slides and direct ISE. On the other hand ABG analyser, ABL 80 Flex (Radiometer Medical, Copenhagen, Denmark), Point of care system determines the electrolytes based on direct ISE method. ABL 80 Flex runs automatic 1 point calibration with each measurement and 2 point calibration every 8 hours. Both the analyser were calibrated, operated and maintained according to the manufacturer's guidelines.

Analytical precision: We performed two level internal quality control for both the instruments throughout the study using Bio-Rad QC(Lot No – 26431, Lot No- 26432 for electrolytes on Vitors 4600), and Bio-Rad, Liquicheck Blood Gas Plus controls with lot No 22292, 22293 for ABG, Radiometer (ABL 80 Flex).

The reproducibility of results obtained throughout the study was evaluated via analysis of duplicate QC samples on each of 20 days, between run precision of electrolytes were shown in table 1. We have used Reagent lot numbers 277157, 273785 for ABG analysis (ABL80 Flex) and Vitors micro slides with lot numbers 42211942, 41020590 for Sodium and Potassium respectively.

Table-1: Between-run precision of Sodium and Potassium values determined by analysis of duplicate quality control materials for 20 days					
Electrolyte (mmol/L)		**Mean (Vitros 4600)**	**CV% (Vitros 4600)**	**Mean (ABL 80 Flex)**	**CV% (ABL 80 Flex)**
Sodium	L1	130.7	1.07	133.6	1.03
	L2	144.6	1.18	162.9	0.81
Potassium	L1	4.22	1.94	4.29	1.99
	L2	6.3	1.72	6.29	1.27
*L1 = low level QC, L2 = High level QC					

Sample collection & analysis: The samples received in BD vacutainer SST (Yellow top) for serum electrolytes were centrifuged within 20–30 min after clotting of blood. Serum Electrolytes were measured on Vitros 4600 analyser. ABG samples received in BD syringe Lithium Heparin, 80IU/ml, 3ml were processed on ABL 80Flex analyser immediately for electrolytes along with blood gases. Sample receiving date, time, and the Na, K results on both the analysers were noted for each sample. Electrolyte values were noted for ABG samples and venous samples received simultaneously from the same patient with a time gap of less than 15 minutes.

Table-2: Agreement between electrolyte values compared between Dry Chemistry analyser (Vitros 4600) Vs ABG analyser (ABL 80 Flex)								
Analyte	N	Mean (SD)		Bias(95% CI)	LLOA (95% CI)	ULOA(95% CI)	Coefficient of repeatability	No of pairs outside the CLIA limits
		Vitros 4600	ABL 80flex					
Na	120	134.7 ± 6.13	135 ± 6.11	-0.267 (-0.88 - 0.348)	-6.935 (-7.99 to -5.88)	6.40 (5.34 to 7.45)	6.66 (5.91 to 7.62)	16 (13.3%)
K	120	3.79 ± 0.85	3.88 ± 0.92	-0.097 (-0.18 to -0.013)	-1.01 (-1.15 to -0.86)	0.81 (0.67 to 0.96)	0.93 (0.82 to 1.06)	5 (4.1%)
*LLOA=Lower limits of agreement, ULOA= Upper limits of agreement								

Statistical analysis: Mean, Standard deviation, coefficient of variation, Frequency and Percentages were used to describe the continuous and categorical data respectively. Data was tested for normality using the Kolmogorov-Smirnov test. Bland-Altman plots were constructed to find the agreement between two methods, agreement was summarised by mean difference with Bland–Altman's 95% limits of agreement (LOA). Deming regression analysis was performed, Passing Bablok test and Lin's concordance correlation was used to find the relationship between the values measured by both the methods. The statistical significance level is considered at p <0.05. MedCalc® Statistical Software version 20 (MedCalc Software Ltd, Ostend, Belgium) was used for statistical estimations.

The limits of agreement (LoA) are set as the mean difference ± 1.96 SD of differences on Bland Altman Plot. The difference in results from the two test methods was also assessed against the United States Clinical Laboratory Improvement Amendments (US-CLIA) 88 rules [9] according to which the following variations are considered as acceptable: sodium ±4.0 mmol/L; potassium ±0.5 mmol/L. The test results were considered to be interchangeable if they were within the US-CLIA variability criteria and would not alter the clinical management when compared to each other.

Results

Total of 120 paired samples were enrolled for this study. Mean Na Vitros 4600 was 134.7 ± 6.13 Vs. 135±6.11 ABL 80 flex, mean difference was 0.266, P=0.39. Pearson Correlation r=0.845(0.785-0.890) P<0.0001. Potassium mean for Vitros 4600 was 3.79± 0.85 Vs. ABLA 80 flex 3.88 ± 0.92. Mean difference was 0.97, P = 0.023. Pearson correlation r=0.864(0.811- 0.904) P < 0.0001.

Bland Altman Bias for Sodium compared between Vitros 4600 and ABL 80Flex were shown in Fig 1. Bland Altman Bias for Sodium was -0.267(-0.88 – 0.348), LLOA -6.935(95% CI -7.99 to -5.88), ULOA 6.40 (95% CI 5.34 to 7.45) with Coefficient of repeatability of 6.66 (5.91 to 7.62). 16 Sodium samples surpassed US CLIA limits of 4 mmol/L. LLOA and ULOA are beyond the CLIA/ maximum allowable errors which indicates both methods cannot be used interchangeably.

Fig-1: Bland Altman plot of Sodium compared between Vitros 4600 and ABL 80Flex
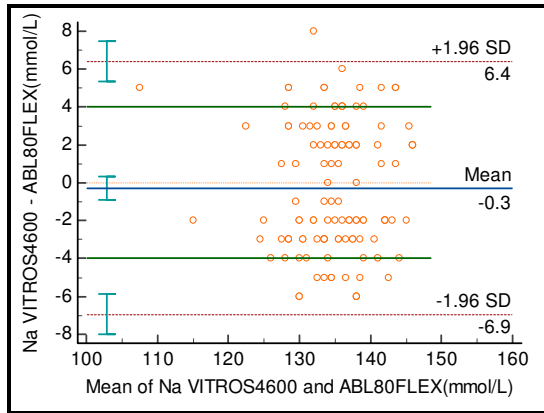
Bland Altman Bias for Potassium compared between Vitros 4600 and ABL 80Flex were shown in Fig 2. Bland Altman Bias for Potassium was -0.097(-0.18 to -0.013), LLOA -1.01(-1.15 to -0.86) ULOA 0.81 (0.67 to 0.96) with Coefficient of Repeatability 0.93(0.82 to 1.06). Five Potassium samples were beyond 1.96 SD, 34 samples surpassed US CLIA limits of 0.5 mmol/L. LLOA and ULOA are beyond the CLIA/ maximum allowable errors which indicates both methods cannot be used interchangeably. The Deming regression analysis for Sodium revealed regression equation y = 2.7251 + 0.9818 x, With Intercept 2.7251, SE = 9.23, 95% CI (-15.56 to 21.01), Slope 0.9818, SE = 0.068, 95% CI (0.84 to 1.11), Variance ratio 0.86 and Pearson correlation coefficient 0.8457 (0.78 to 0.89) Fig 3.

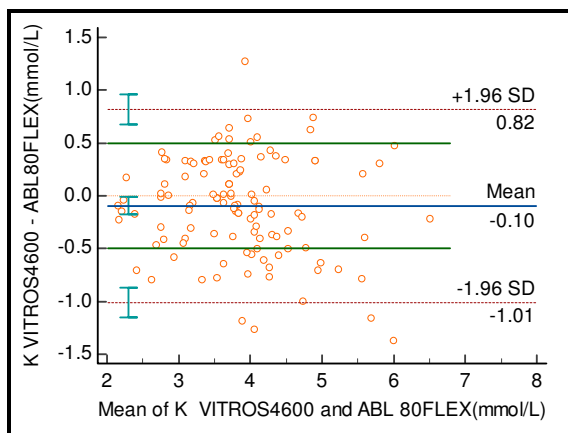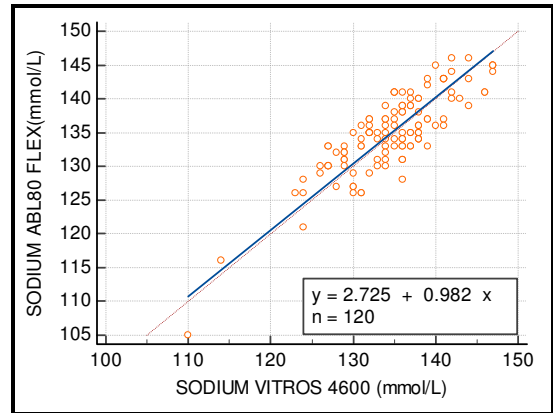**Fig-2:** Bland Altman plot of Potassium compared between Vitros 4600 and ABL 80Flex

Fig-3: Deming Regression analysis for Sodium results Vitros 4600 Vs ABL 80Flex

The Deming regression analysis for Potassium revealed regression equation y = -0.1946 + 1.0770 x with Intercept -0.19, SE 0.24, 95% CI (-0.672to 0.28), Slope 1.07, SE 0.066 (0.94 to 1.21), Variance ratio 0.8910 and Pearson correlation coefficient 0.86(0.81 to 0.90) Fig 4.

Passing Bablok test for Sodium comparison between Vitros 4600 and ABL 80Flex revealed a regression Equation y = 1.5 + 1.0 x (Fig 5). Systematic differences/ Intercept A = 1.50(1.50 to 15.83) 95% CI of Intercept A is NOT contacting 0, which indicates both methods differ from each other by a constant 1.5 Proportional differences/ Slope B = 1.0 (0.88 to 1.00), 95% CI of Slope B contains 1 which indicates there are no proportional differences between two methods.

Fig-4: Deming Regression analysis for Potassium results Vitros 4600 Vs ABL 80Flex
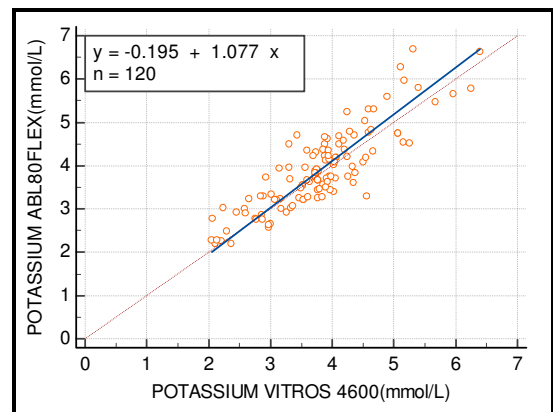
Fig-5: Passing- Bablok regression test for Sodium results on Vitros 4600 Vs ABL80Flex
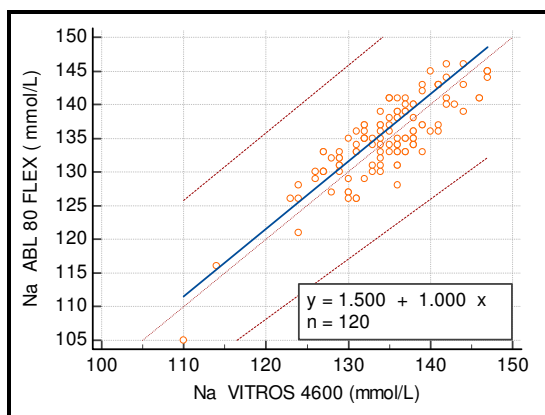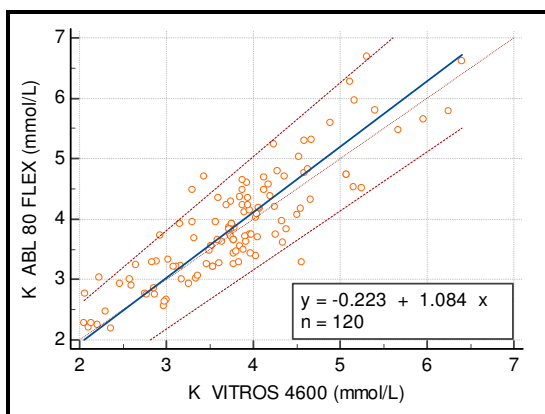

Fig-6: Passing- Bablok regression test for Potassium results on Vitros 4600 Vs ABL80Flex

Random differences/Residual Standard Deviation (RSD) = 2.57(-5.04 to 5.04), as the 95% CI of the RSD is beyond CLIA criteria 4mmol/L, random differences between the two methods is more than allowable errors indicating two methods may not be in agreement. Cusum test for linearity model validity showed No significant deviation from linearity (P=0.80). Passing Bablok test for Potassium comparison between Vitros 4600 and ABL 80Flex revealed a regression equation y = -0.22 + 1.08 x (Fig 6).

Systematic differences/ Intercept A = -0.22(-0.74 to 0.15) 95% CI of Intercept A is contacting 0, which indicates there are no systematic differences between the both methods. Proportional differences/ Slope B = 1.08(0.97 to 1.22), 95% CI of Slope B contains 1 which indicates there are no proportional differences between two methods. Random differences/ Residual Standard Deviation (RSD) = 0.32(-0.64 to 0.64) as the 95% CI of the RSD is beyond CLIA criteria 0.5 mmol/L, random differences between the two methods is more than allowable errors indicating two methods may not be in agreement. Cusum test for linearity model validity showed No significant deviation from linearity (P=0.11)

Lin's Concordance correlation analysis which describes the relationship between paired samples showed that there was a poor strength of agreement between the methods with Precision ρ < 0.90. It shows there was good accuracy with Cb=0.99 for both Na and K measured on two equipment (Table 3).

Table-3: Lin's Concordance correlation between electrolytes measured on Dry Chemistry analyser Vs ABG analyser					
Variables	**Comparison**	**N**	**Lin's rcc (95% CI)**	**Precision ρ**	**Accuracy Cb**
Na	Vitros 4600 Vs ABL 80 Flex	120	0.85(0.79 - 0.89)	0.85*	0.99
K	Vitros 4600 Vs ABL 80 Flex	120	0.85(0.80- 0.89)	0.86*	0.99
*Values of Precision ρ < 0.90 indicates poor strength of agreement between the methods. Accuracy is good, but not the precision.					

Discussion

The present study investigated whether there was an agreement between serum electrolytes measured on Dry chemistry analyser and ABG analyser. If so, results obtained by these two methods can be used interchangeably in clinical practice. In our study we found the Bland Altman bias for Sodium compared between Vitros 4600 and ABL 80Flex was -0.267(-0.3) and for Potassium it was -0.097(-0.1). The bias was within the USCLIA limits

(4 and 0.5 mmol/L for Sodium and Potassium). However both Sodium and Potassium LLOA and ULOA are beyond the CLIA/ maximum allowable errors which indicates both methods cannot be used interchangeably (Fig 1A & 1B).

Passing Bablok test for Sodium comparison between Vitros 4600 and ABL 80Flex revealed both methods differ from each other by a constant 1.5 and there are no proportional differences between two methods. But random differences between the two methods are more than allowable errors indicating two methods may not be in agreement. Passing Bablok test for Potassium comparison between Vitros 4600 and ABL 80Flex revealed there are no systematic differences between the both methods and there are no proportional differences between two methods. But random differences between the two methods are more than allowable errors indicating two methods may not be in agreement.

Jain et al. [10] conducted a study on 200 paired samples and found no significant difference between the potassium values measured by Laboratory Autoanalyzer (LAA) and Blood gas analyser (BGA), while there was a significant difference between the sodium values. A study conducted by Uysal E et al [11] showed good correlation between Sodium and Potassium measured on LAA and ABG. A study conducted by Gavala et al. [12] reported that serum sodium and potassium measured on BGA were significantly lower than LAA values. They found more than 30% of the samples showed more bias than USCLIA recommendations.

A study conducted by Solak [13] which compared serum Sodium values of different Sodium(high, low, normal) groups between LAA and BGA showed BGA systematically and consistently measured low sodium values compared to LAA and all the Sodium groups showed higher differences than USCLIA recommendations. Mirzazadeh et al. [14] found good agreements between the BGA and LAA results for sodium, potassium and calcium and concluded that BGA can be accepted as a POCT for critically ill patients. Johnston and Murphy [15] observed higher levels of potassium in arterial samples when compared to venous samples.

Very few studies have compared Sodium and Potassium values measured on Dry Chemistry analyser and ABG analyser. A study conducted by Jian Bo Zhang [16] which compared Sodium and Potassium values between Dry Chemistry analyser and ABG analyser showed ABG measured values were lower than that of Dry Chemistry values. Another study conducted by Garcia-Pachon E [17] which compared Sodium and Potassium values between Dry Chemistry analyser and ABG analyser showed there was no significant bias between Sodium values measured whereas serum potassium values measured by Dry chemistry analyser showed higher values than ABG analyser. Their study included patients with respiratory acidosis.

In contrast to the above mentioned studies our study results showed Sodium and Potassium values measured on Dry chemistry analyser were slightly lower than that of ABG analyser. The differences in the measured values can be explained by the fact that two machines use different chemical reactions. LAA measures electrolytes in serum whereas Blood gas analyser measures in whole blood which was anticoagulated. Anticoagulant Heparin present in the sample collection tubes may increase the volume of the sample and dilutes the plasma portion of the blood. This can lead to lowering the value of the measured electrolytes on the blood gas analyser. High heparin in the tubes may itself bind to the electrolytes present in the sample thereby lowering the concentration of measured electrolytes on the blood gas testing [18].

Pre-analytical errors that can lead to erroneous results of electrolytes measured on LAA and ABG are due to use of different anti-coagulants, sampling from catheters, hemolysis of the sample and improper storage. Clinical and laboratory standards institute (CLSI) recommends the exclusive use of pre-heparinized dry balanced heparin sample tubes or syringes for electrolyte measurements on an ABG analyser. However some bias in electrolyte estimation has been reported even with use of electrolyte balanced Heparin [19-20]. In our study we have used balanced heparin syringes for whole blood collection.

Although there were many studies conducted on the same topic, our study has the following strengths;

a) Our study is a cross-sectional study and not a retrospective study.
b) We have ensured both the arterial and venous blood samples were drawn from trained staff simultaneously and we have followed our sample rejection criteria in case of hemolysis, insufficient samples, transport delay etc.
c) We have compared electrolyte measurements on machines which use a similar method-direct ISE. This ensures the comparability between the same methods, and simultaneously avoids the pre-dilution step which is associated with indirect ISE and possible pre-analytical errors.
d) We have used balanced heparin syringes to ensure there will not be any pre-dilution effect by heparin.
e) Statistical methods used by our study were appropriate than simply using student t- test, Pearson correlation, ANOVA etc. We have used Bland Altman's 95% of limits of agreement to find the agreement between two methods, other statistical tests used were Passing Boblok test, Deming regression analysis and Lin's concordance correlation.

Conclusion

In the present study we found that the electrolytes, Sodium and Potassium measured on the Dry chemistry analyser showed bias of -0.3 and -0.1 compared to that of Arterial Blood gas analyser. Though the bias is found to be within the CLIA limits for both Na and K, LLOA and ULOA are beyond the CLIA/ maximum allowable errors which indicates both methods cannot be used interchangeably. There are many factors that can influence electrolyte measurements like different types of instruments, calibrators and other pre-analytical variables. Hence we suggest that each laboratory should check the agreement between different methods before they use the electrolyte results interchangeably.

Conflicts of interest: There are no conflicts of interest.

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