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AGREEMENT BETWEEN TWO DIFFERENT FIELD METHODS USED TO MEASURE SERUM ELECTROLYTES IN CLINICAL LABORATORY

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ABSTRACT

Background: The aim of our study was to do an agreement analysis of two different laboratory methods used to measure electrolytes i.e., between the Direct ISE based ABL-800 Basic and Indirect ISE based COBAS-6000 Biochemistry auto-analyzer. **Methods**: This observational study was conducted from July 2016 to August 2016 on routine biochemistry indoor and outdoor samples. A total of 155 samples were analyzed for the Na^+, K^+ and Cl^- concentrations in the serum. The bias and variability of differences in measured values were analyzed according to Bland and Altman method and Passing-Bablok regression was conducted. **Results:** The mean difference between the two methods and 95% limits of agreement for sodium in serum was -4.9±3.2 (-5.38 to -4.36) for potassium -.05±0.1 (-0.08 to -0.03) and for Chloride was -7.8±3.1 (-8.3 to -7.3). Similarly, the mean difference between the two methods for potassium values in serum was found to be -0.25±0.75 (-1.75 to 1.25) and in urine was -5.3±38.9 (-83.1 to 72.5). Significant difference in mean values was observed between two methods for serum all three electrolytes ($P < 0.001$). **Conclusions**: Good degree of agreement was seen on comparing the two methods for measuring the potassium but not for serum sodium and chloride levels. Two instruments (COBAS-6000 and ABL-800 Basic) cannot be used interchangeably for serum electrolyte measurement. Clinicians must be aware about differences exist between the two ISE technologies. Further, in border-line clinical situations, care must be taken while interpretation of the electrolyte results and taking clinical decision.

KEYWORDS: Electrolyte measurement, Method comparison, Direct ion selective electrodes method, Indirect ion selective electrodes method.

INTRODUCTION

Electrolytes are very important for the continuation of the physiological functions of the human body. In mammals, the maintenance of osmotic pressure and water distribution in the various fluid compartments, pH maintenance, heart and muscle functioning, oxidation-reduction reactions and as a cofactors of enzymes are dependent on or affected by the four major electrolytes: Sodium (Na^+), Potassium (K^+), Chloride (Cl^-) and Bicarbonate ($HCO3^-$). Thus determination of the concentration of electrolytes is one of the most important functions of the clinical laboratory.[1]

There has been a considerable change in the methods for estimation of electrolyte in biological samples. Flame photometer, atomic absorption spectrophotometer and Direct and Indirect ISE are the available methods for estimation of body electrolytes. The regular clinical need for the availability of these electrolytes with both patients presenting to emergency departments as well as with inpatients has paved way for the development of more advanced, integrated, rapid and sensitive analyzers.[2]

Over the last several years, numerous instruments have been introduced into the clinical market for testing various critical analytes such as electrolytes; all of these systems claim to improve patient care by their simple, rapid, and fully automated user interface.[3]

In our hospital laboratory, ABL-800 Basic instrument was based upon Direction selective electrode **(**ISE) method was routinely used for electrolyte measurements. Recently, our hospital was equipped with the COBAS-6000 fully automated analyzer which has Indirect ISE system. We studied the comparability of sodium, potassium and chloride values on the Direct ISE and Indirect ISE platforms.

The aim of present study was to do an agreement analysis of two different laboratory field methods used to measure electrolytes i.e., between the laboratory

established Direct ISE method and the newer automated indirect ISE method in serum samples.

METHODS

The present prospective observational cross sectional study was conducted in Biochemistry department, Bhatia Hospital, Mumbai, India. The study protocol was approval by hospital's Ethics Committee prior of starting the study.

Study Population

Present study was conducted on routine biochemistry samples received at our laboratory over a period of one month (from July-August, 2016). One fifty five patients aged between 18-70 years, presenting to the outpatient and indoor department were included in the study.

Using alpha – 0.05, beta- 0.20 with allowable difference (difference of means of control and case) of 2.46 and population variance of 10.3, sample size calculated per group was 136 (using in- house pilot study data). The formula used for sample size calculation was

Formula: $$n_1 = n_2 = \frac{2\left(z_{\alpha/2} + z_{\beta}\right)^2 \sigma^2}{\left(\mu_2 - \mu_1\right)^2}$$

However after adding 10% loss, final sample size of 155 was decided.

All venous blood samples were collected in plain gel evacuated tubes (BD Vacutainer, Franklin Lakes NJ, USA) by the trained technicians. The samples were allowed to clot and processed on same day of collection. After centrifugation at 10,000 rpm for 5 minutes, serum was separated and used for electrolyte measurements on both instruments. Lipemic, strongly hyperbilirubinemic and hemolysed samples were excluded from the study. Each sample was analyzed within a maximum of one hour after the collection.

Quality control

Electrolyte measurement by Direct ISE method was done on the bench top instrument "ABL-800 basic (Radiometer Medical ApS, Akandevej 21, 2700 Bronshoj, Denmark). This instrument was calibrated automatically for one point and 2 points in every 4 hours. The internal quality control check was performed everyday using 2 levels of controls provided by the manufacturer (AutoCheck 5^+, Aqueous quality control).
Electrolyte measurement by Indirect ISE method was done on auto-analyzer COBAS-6000 Hitachi Modular ISE system (Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305, Mannheim, Germany). The instrument was calibrated routinely every 24 hours by linear calibration and ISE electrolyte buffer; ISE electrolyte reference used according to the manufacturer's recommendations to maintained a constant ion activity on the electrodes. The internal quality control check was performed everyday by using two levels of control (PreciControlClinChem Multi 1 (PCC1) and PreciControlClinChem Multi 2 (PCC2)).

Reproducibility of the results throughout the study was evaluated via analysis of quality control samples on each of 20 days and between run precision for both methods were evaluated(Table-1).For external quality control, the lab participated in External Quality Assurance Services (EQAS) by Bio-Rad Laboratories, Irvine CA, UNITED STATES (Cycle 14;sample 12 and Cycle 15; sample 1).

STATISTICAL ANALYSIS

All data were tested for normality with the Kolmogorov-Smirnov test usingAnalyse-itsoftware Version-2.04 (Analyse-it software, Leeds, UK).All the quantitative parameters were expressed as mean and Standard Deviation (SD) and qualitative parameters were presented as frequency (%). To compare a continuous variable between groups, the Student's unpaired t-test was performed. Agreement between the 2 analyzers was assessed using the Bland-Altman approach. The linear relationship between the variables was assessed by the Pearson's correlation analyzing method. The 95% limits of agreement were estimated by mean difference ± 2standard deviation of the differences in the measurement by the two methods. Passing-Bablok regression was used to estimate analytical method agreements and possible systemic bias between them.*P* value of <0.001 was considered statistically significant.

RESULTS

A total of 155 samples were included in the study. The mean age of the patients was 36 years (18-70). Out of 155 patients, 71% were male and 29% were female. The minimum and maximum values of serum Na^+ by Direct ISE were- 123.0, 150.0, and by Indirect ISE were 120.0 and 143.0 mmol/L respectively. For K^+ minimum and maximum values by Direct ISE were-2.8, 6.7 and by Indirect ISE were- 2.6 and 6.8 mmol/L. For serum Cl^- minimum and maximum values by Direct ISE were-90.0, 117.0 and by Indirect ISE were- 81.0 and 106.0 mmol/L.

Table 2 shows the comparison of serum electrolytes between Direct and Indirect ISE method. Results showed that mean sodium, potassium and chloride levels were significantly higher in Direct ISE method compared to the Indirect ISE method (*P*< 0.0001).

Table 3 shows the Bland-Altman statistics for serum electrolytes measurements using Direct ISE method compared to the Indirect ISE method. To verify the agreement between two methods, the difference (mean bias) in the measurement of electrolytes (Na^+, K^+ and Cl^-), limit of agreements and SD were calculated.The correlation coefficient for K^+ was strong (r = 0.97) whether for Na and Cl^-, the r value was less (r = 0.78 and 0.81 respectively) which suggest moderate correlation.**(Fig. 1, 2 and 3).**

Passing-Bablok regression was preferred over Deming regression in our study as the coefficient of variation (CV) and SD for 2 methods was not constant.The regression analysis of Direct and Indirect ISE for electrolytes yielded the equations which are given in table-4.The equations suggest that onlyK^+ values by Direct and Indirect ISE methods can be used interchangeably. (**Fig. 4, 5 and 6**).

The mean difference between Direct ISE and Indirect ISE for K^+ was 0.05 mmol/L, which was within the acceptable limit for K^+ defined by Clinical Laboratory Improvement Amendments (CLIA). Subsequently,the hypothesis suggesting that there was no differencebetween the 2 methods'results was rejected forNa^+ and Cl^- but accepted for K^+**.**

Table 1. Between-run precision of electrolyte assay data for both Direct and Indirect ISE methods

Analytes	Control	Indirect ISE Method on COBAS-6000			Controls (Auto- check)	Direct ISE Method on ABL-800		
		Target Value	Mean ± SD	CV%		Target Value	Mean ± SD	CV%
Sodium	PCC1	113.0	112.4 ±1.67	1.48	LEVEL 1	126.0	124.5	0.56
(mmol/L)	PCC2	134.0	132.6 ±1.51	1.13	LEVEL 2	140.0	141.3	0.40
Potassium	PCC1	3.63	3.64 ±0.06	1.64	LEVEL 1	5.5	5.4	0.73
(mmol/L)	PCC2	7.28	7.25 ±0.12	1.65	LEVEL 2	3.8	3.8	1.48
Chloride	PCC1	79.5	77.46 ±3.27	4.22	LEVEL 1	67.0	66.5	1.06
(mmol/L)	PCC2	106.0	101.34 ±2.64	2.60	LEVEL 2	98.0	96.0	0

Abbreviations: PCC1,PrecicontrolClinChem Multi 1; PCC2,PrecicontrolClinChem Multi 2.

Table 2.Comparison of serum electrolyte values obtained by Direct and Indirect ISE methods.

Analytes (mmol/L)	(Direct ISE, n=155)		(Indirect ISE, n=155)		*P* Value
	Mean ± SD	(95%CI of Mean)	Mean ± SD	(95%CI of Mean)	
Sodium	133.6 ± 5.0	137.8 – 139.4	133.8 ± 4.8	133.0 – 134.5	0.0001***
Potassium	4.27 ± .69	4.1 -4.3	4.21 ± .70	4.1 -4.3	0.0001***
Chloride	102.9 ± 5.37	102.0 – 103.7	95.1 ± 5.1	94.2 - 95.9	0.0001***

Table- 3 Comparison of the serum electrolytes results obtained by Direct and Indirect ISE methods.

Analytes	Mean bias ± SD	Acceptable limits for acceptance of difference by CLIA	(95%CI of Mean bias)	Correlation coefficient (r)	(95%CI of r)	LOA of difference
Sodium	-4.9 ± 3.2	4 mmol/L	- 5.38 to -4.36	0.78	0.71 – 0.83	-11.2- 1.4
Potassium	-0.05 ± 0.1	0.5 mmol/L	- 0.08 to -0.03	0.97	0.96 – 0.98	-0.36 – 0.24
Chloride	-7.8 ± 3.1	5% of target value	-8.3 to -7.3	0.81	0.75 - 0.86	-14.0 – - 1.6

Abbreviations: CI, confidence intervals; LOA, limit of agreement; SD, standard deviation.

Table 4. Passing-Bablok Regression Equation for serum electrolyte measurements by Direct and Indirect ISE methods

Analytes	Passing-Bablok Regression Equation
Sodium	$Na^+$by Indirect ISE method (mmol/L)= -5 + 1 $Na^+$by Direct ISE method (mmol/L)
Potassium	$K^+$by Indirect ISE method (mmol/L)= 0 + 1 $K^+$by Direct ISE method (mmol/L)
Chloride	Cl^- by Indirect ISE method (mmol/L)= -7 + 1 Cl^- by Direct ISE method (mmol/L)

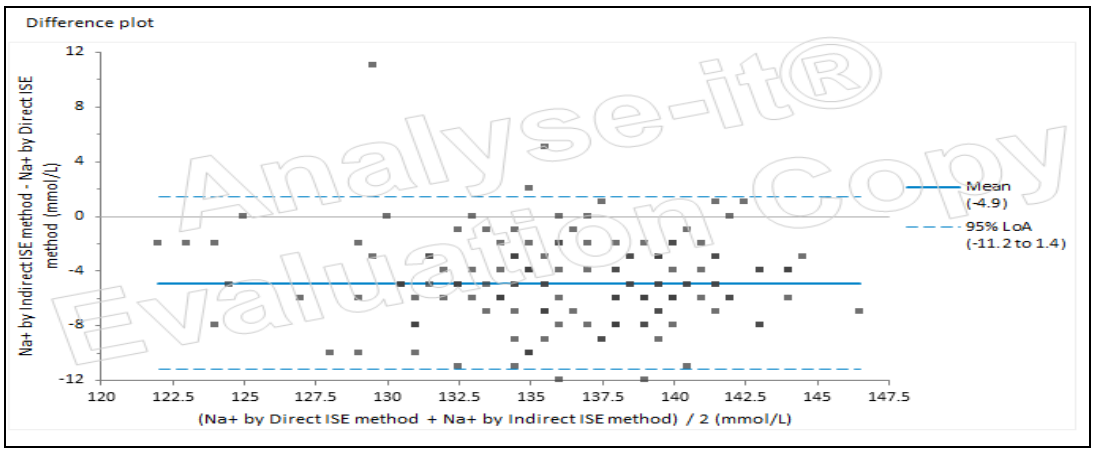

Figure 1. Bland and Altman bias plot for serum Sodium levels

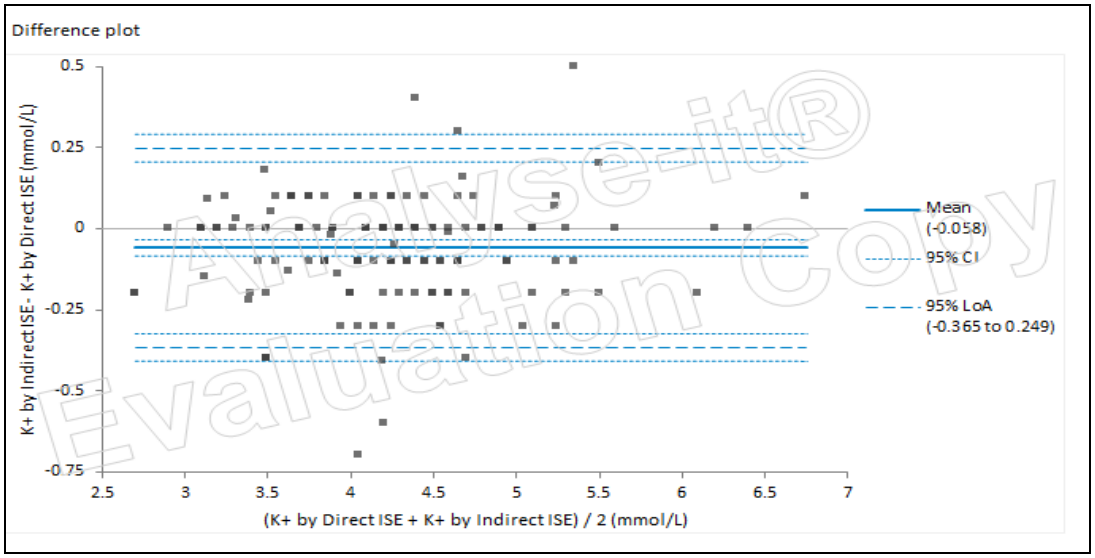

Figure 2. Bland and Altman bias plot for serum Potassium levels

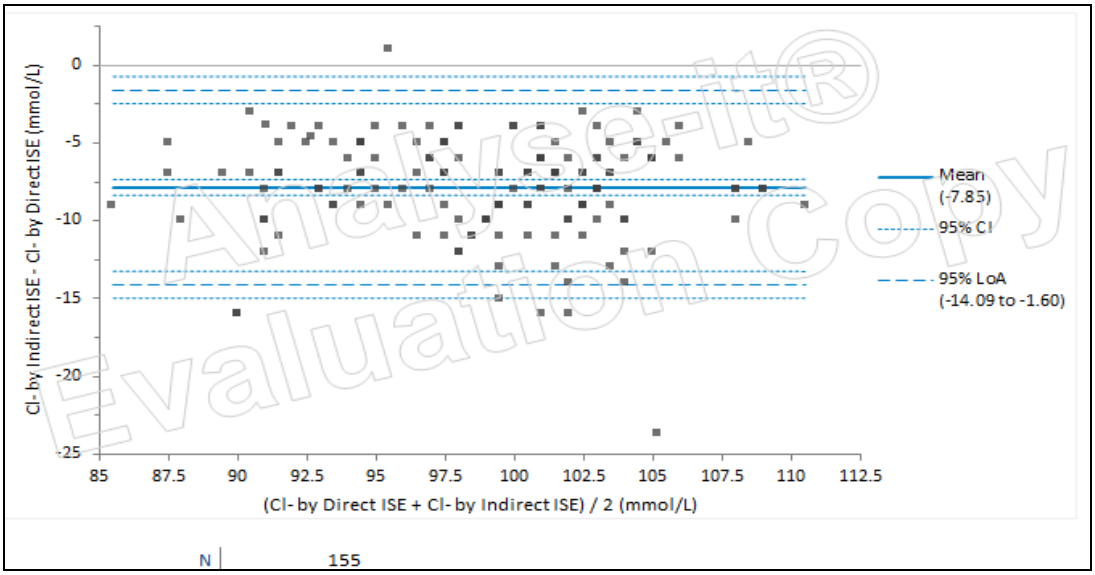

Fig 3. Bland and Altman bias plot for serum Chloride levels

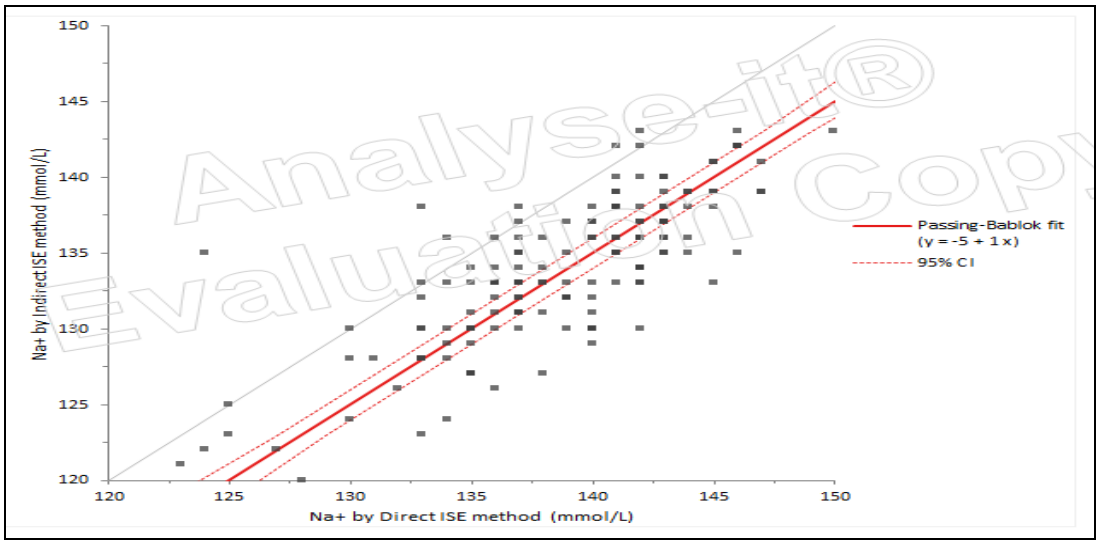

Figure 4. Passing-Bablok regression with 95% confidence interval for Direct ISE sodium results *vs* Indirect ISE sodium results

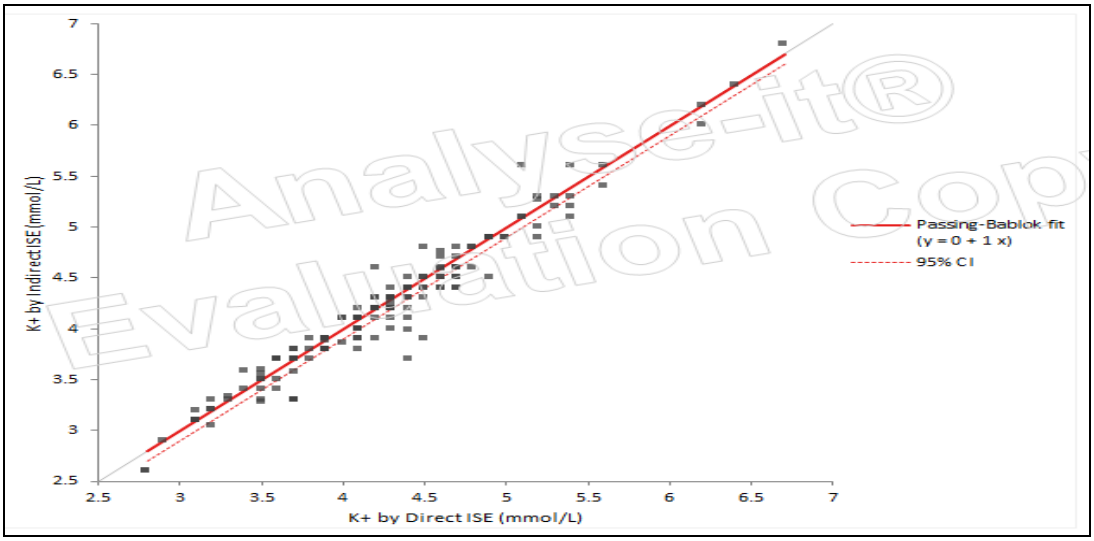

Figure 5. Passing-Bablok regression with 95% confidence interval for Direct ISE potassium results *vs* Indirect ISE potassium results.

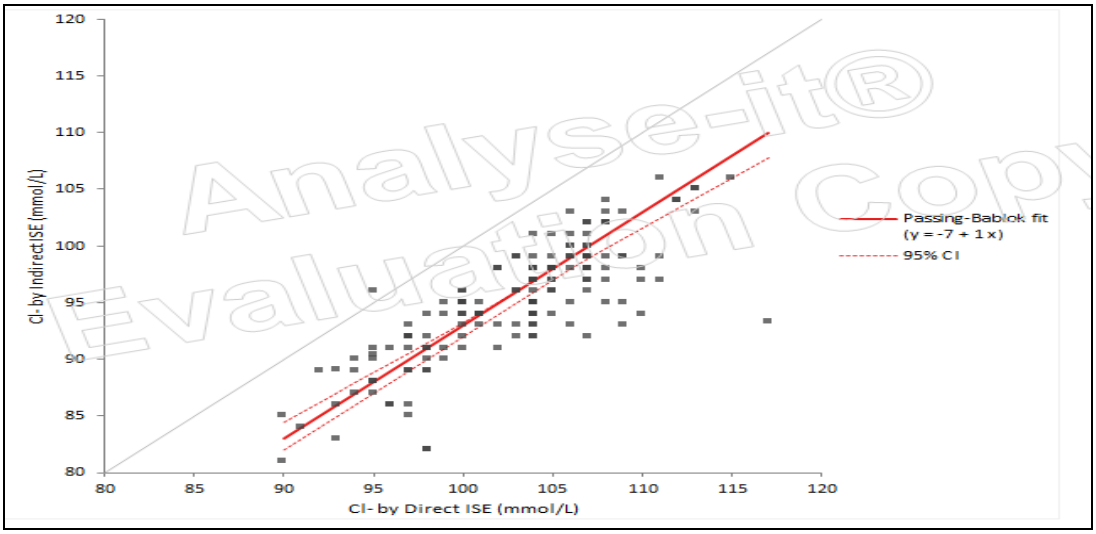

Figure 6. Passing-Bablok regression with 95% confidence interval for Direct ISE chloride results *vs* Indirect ISE chloride results.

DISCUSSION

The great clinical importance and common ordering of electrolyte studies in patients require the use of rapid, simple and valid methods for electrolyte's measurements. In the present study, we evaluated serum Na^+, K^+ and Cl^- levels using two different field methods and equipment to investigate whether the results were equivalent and values could be employed interchangeably. The COBAS 6000 by Roche autoanalyser was introduced in our laboratory to decrease the turnaround time by running electrolytes along with other clinical biochemistry parameters in one shot for individual patients.

The COBAS 6000 by Roche electrolyte measurements was based upon Indirect ISE method whether the ABL-800 Basic was based upon Direct ISE method. In our laboratory we were continuing with the same RIs which we were using for the ABL-800 Basic even after installing the new COBAS 6000 instrument. There was a big question before us that whether analytical performance of newly installed Indirect ISE method was comparable with the older Direct ISE method and whether two different methodology based ISE analyzer can be used simultaneously in one central laboratory with a common RI.

So, the need to check the comparability of two different instruments with different methods for ISE measurements was felt and the study was planned.

The serum electrolyte data obtained in the present study was evaluated by applying Bland and Altman agreement analysis and Passing-Bablok regression. The 95% limits of agreement estimated by mean difference ±2.0 SD of the differences provided an interval within which 95% of differences between measurements by the two methods were found to lie. The results of our study indicated that the Na^+ and Cl^- values measured on two instruments (COBAS 6000 and ABL-800) with different ISE

methods (Indirect and Direct ISE) were not comparable with each other only K^+ values were applicable interchangeably.

Though it is known fact that Direct ISE technologies measure electrolyte activity in the water phase of serum or plasma only, not the concentration in the total volume in which; the cells, proteins and lipids do not influence the analysis. In Indirect ISE, a fixed volume of plasma is aspirated and diluted with a diluent for analysis, which means the electrolytes are measured in the volume of total plasma: The plasma sample volume consists of solids (e.g. proteins and lipids) and water (which contains the dissolved electrolytes) and not the plasma water phase.[4] In a normal plasma sample, solids represent approximately 7% of the total plasma volume.

To make results from Direct ISEs equivalent to Indirect ISEs, most ISE method operate in what is commonly referred to as the "flame mode". In this mode, the directly measured concentration in plasma water is multiplied by the average water volume fraction of plasma (0.93). So normally Indirect ISE systems are standardized to this percentage assuming a 'normal concentration' of solid.[1]

In our case, COBAS 6000 ISE system was not standardized for this factor. Instead of standardization, instrument manufacturer have provided separate RIs for Indirect ISEs method which are as follows-Sodium- 136-145mmol/L, potassium-3.5-5.1mmol/L and Chloride-98-107mmol/L for adults. Though these RIs were slightly lower to the RIs which are being used in the laboratory i. e.Sodium- 134-145 mmol/L, potassium-3.6-5.4 mmol/L and Chloride-98-110 mmol/L for adults.As our results suggested that two instruments and ISE methods for electrolyte measurements in our laboratory cannot be used interchangeably and thus the common RIs cannot be applied to both the methods, there is the need for using separate RIs for two different methods. The use of two different RIs for the same analytes may create confusion to the clinicians so we suggest that two comparable methods/instruments should ideally be used in one laboratory to provide valid and reliable results in favor of patients benefit.

Further, the standardization of Indirect ISE system is done assuming that a 'normal concentration' of solid in serum/plasma but the fact is that large changes in plasma lipids, proteins and other solids often occur in relatively common clinical conditions and in therapies, such as parenteral alimentation with lipid emulsions. Study by *Dimeski and Barnett*have shown that the plasma sodium, potassium and chloride measurements are affected by changes in plasma protein concentration when measured by Indirect ISE systems. In this study, Bayer Diagnostics Australia; using Direct ISE technology and Hitachi Modular ISE system (Roche Australia) using Indirect ISE technology instruments were used for electrolytes measurements .Using Indirect ISE, low plasma protein concentrations caused a 'pseudohyper' effect in Na, K and Cl^- analytes and a 'pseudohypo' effect with high plasma protein concentrations. The variation in total protein concentration had the greatest effect on plasma sodium measurement. The relationship was non-linear and no accurate predictive value could be calculated for the plasma electrolytes with changes in plasma protein concentrations.[4]

This alteration in electrolytes values can be explained by "Electrolyte Exclusion Effect". Electrolyte Exclusion Effect is the exclusion of electrolytes from the fraction of the total plasma volume that is occupied by solids. In pathological conditions, plasma water volume is altered and this Electrolyte Exclusion Effect becomes problematic because the concentration of the solids in plasma (more often protein than lipids) is changed by > 7% or < 7 %, the ratio of solids to water alters; the water content is decreased or increased causing the observed discrepancies between Direct and Indirect ISE measurements.[1]

In our hospital most of the samples for serum electrolyte's measurement come from the ICU and other wards whose other serum biochemical parameters are mostly not within normal limits. Though in our study, we didn't quantified the serum lipids and proteins levels, but as most of the samples were from ICU and other wards, it can be hypothesized that the difference in electrolyte values by Direct and Indirect ISE method may be due to the altered solids and water ratio in serum of patients.

Our study illustrates the importance of determining the consistency of serum electrolyte values obtained by two different methods in the individual hospital laboratories. As instrument type and calibration methods may differ among laboratories, it is advisable to conduct an in-house study for comparison of two instruments/ methods in the one laboratory before installation of new instrument to check their compatibility. It would not only help to check their compatibility but will help to evaluate the clinical significance of any difference between data yielded by two instruments. It should be ensured that the results provided by Direct and Indirect ISE method must not differ to a clinically relevant extant.

CONCLUSION

We conclude that the serum sodium and chloride levels were not comparable when using Direct and Indirect ISE methods in our laboratory. Only serum potassium values were found to be used interchangeably for two ISE methods in our laboratory. Alterations in electrolyte concentrations are unpredictable If the two different methodology based instruments are being used in one laboratory, the clinicians must be informed about the method used for electrolyte measurement and they should be aware about differences exist between the two ISE technologies. Further, in border-line clinical situations, care must be taken while interpretation of the electrolyte results and taking clinical decision.

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