Original Article

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Biochemistry Section

Method-dependent Variation in Serum Electrolytes: A Cross-sectional Study Comparing Direct and Indirect Ion-selective Electrodes

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ABSTRACT

Introduction: Serum electrolyte measurement, particularly sodium, potassium, and chloride, is among the most commonly ordered investigations in routine and emergency care. Rapid and accurate reporting is essential for timely clinical decisions. Most modern electrolyte analysers utilise Ion-selective Electrode (ISE) technology, available in two forms: direct ISE (dISE), which measures electrolytes in undiluted serum, and indirect ISE (iISE), which measures them after pre-dilution. These methodological differences may influence reported values.

Aim: To compare the serum sodium, potassium, and chloride levels measured using dISE versus iISE methods.

Materials and Methods: The cross-sectional study was conducted in the Clinical Biochemistry Laboratory, Government Medical College and Hospital, Chandigarh, India, from March 2024 to and June 2024. A total of 150 serum samples were obtained from the outpatients, inpatients, and Intensive Care Unit (ICU) patients. Paired serum samples available for simultaneous direct and iISE estimation were included. Deidentified residual samples were analysed and assigned a unique code. dISE was performed using the ABL800 FLEX (Radiometer) on undiluted serum, while iISE was performed using the Beckman Coulter AU5800 on prediluted serum; both followed the manufacturer's calibration and two-level internal Quality Control (QC) protocols. Testing order was randomised, and paired measurements were completed within 30-60 minutes to avoid analyte instability. Statistical analysis included Bland–Altman plots to assess agreement and Spearman's correlation to evaluate the association between the two methods. Analysis was stratified by clinically relevant electrolyte ranges, with $p<0.05$ considered statistically significant.

Results: dISE yielded significantly higher sodium and chloride levels than iISE. Subgroup analysis using clinically relevant thresholds demonstrated significant correlations for serum sodium ≤140 mEq/L, potassium ≥4.5 mEq/L, and chloride levels across both concentration groups (>103 and ≤103 mEq/L), with correlation coefficients of r=0.546, 0.870, 0.602, and 0.703, respectively ($p<0.0001$).

Conclusion: A significant difference was observed between dISE and iISE methods for serum electrolyte measurement, particularly within specific clinical ranges. These findings highlight the need for methodological consistency in electrolyte analysis, especially in emergency and critical care settings, to prevent misinterpretation and inappropriate clinical decisions arising from analytical variability.

Keywords: Analysis, Bland-Altman plot, Blood, Electrolytes

INTRODUCTION

The measurement of serum electrolytes, particularly sodium, potassium, and chloride, is one of the most frequently ordered investigations in both routine and emergency clinical settings, where rapid and reliable results are critical for timely medical intervention [1-3]. The electrolytes are measured by ISE technology, which determines the potential difference between the sensing electrode and a reference electrode, generated by the interaction of the target ion with the ISE membrane [2]. Modern analysers typically employ ISE technology, which exists in two primary modalities: dISE and iISE [4].

In dISE systems, the serum sample comes into direct contact with the ion-selective membrane without any prior dilution. In contrast, iISE involves a pre-analytical dilution step typically at a ratio of 1:20 or higher, which facilitates analysis using smaller sample volumes and allows an extended measurement range [5]. Owing to their higher throughput, iISE systems are standard in most automated clinical chemistry analysers, whereas dISE systems are commonly used in point-of-care devices. Due to their distinct methodologies, results obtained from dISE and iISE systems may not always be directly comparable. This is particularly important in clinical scenarios involving serial monitoring, transfer between departments, or use of Point Of Care (POC) and central lab data interchangeably. Understanding whether electrolyte results from these two platforms can be reliably substituted for one another is essential to avoid potential misinterpretation and inappropriate clinical decisions [6].

The electrolyte exclusion effect is the major source of inaccuracy in iISE-based measurements [7]. Under normal physiological conditions, non aqueous components, mainly proteins and lipids, constitute about 7% of the plasma volume. In states such as hyperproteinaemia or hyperlipidaemia, the proportion of plasma water decreases. Since iISE systems are calibrated assuming a plasma water content of 0.93 kg/L, this alteration can lead to spuriously low electrolyte readings [8]. In contrast, dISE systems analyse undiluted samples and are therefore unaffected by variations in plasma composition, providing a more accurate representation of true plasma electrolyte concentrations [9].

Understanding the differences between these two methods is crucial, as discrepancies between dISE and iISE, particularly for sodium, can lead to misinterpretation and inappropriate clinical decisions. Variations in plasma water content are the primary cause of such inconsistencies. Because iISE involves a dilution step, it fails to account for the actual plasma water fraction, resulting in misleading sodium values such as pseudohyponatremia, pseudonormonatremia, or pseudohypernatremia. In contrast, dISE measures undiluted samples and therefore yields accurate results

even when plasma water volume is altered [10]. With the increasing adoption of POC testing, there is a growing tendency among clinicians to use results from dISE and iISE methods interchangeably. However, this practice may introduce clinical uncertainty. There is limited established consensus regarding the correlation between these two analytical approaches. The study aimed to compare serum sodium, potassium, and chloride measurements obtained using dISE and iISE methods. The primary objective was to assess the level of agreement between the two techniques, while the secondary objective was to further evaluate the correlation between dISE and iISE measurements across different electrolyte concentration ranges.

MATERIALS AND METHODS

The cross-sectional study was conducted in the Clinical Biochemistry Laboratory, Government Medical College and Hospital, Chandigarh, India, between March 2024 and June 2024.

This study utilised leftover serum samples received in the laboratory for routine electrolyte analysis. These samples were additionally analysed using the dISE solely for the purpose of method comparison. No additional sample collection, patient contact, or financial transactions were involved.

Sample size: A total of 150 samples were included, aligning with the Clinical and Laboratory Standards Institute (CLSI) guidance for method comparison and approaching the sample precedent of a previous study that detected protein-related differences in Na^+/K^+ [4,11].

Inclusion criteria: Samples having adequate serum volume for paired analysis on both analysers, and processed within two hours of collection were included.

Exclusion criteria: Samples with insufficient volume, presence of gross interferents such as haemolysis, lipaemia, or icterus, or those processed beyond the acceptable stability time were excluded.

Study Procedure

Blood was collected in plain vacutainers, allowed to clot for 20-30 minutes, and centrifuged at 3000 rpm for 10 minutes. For dISE, Na^+, K^+, and Cl^- were measured undiluted on the ABL800 FLEX (Radiometer) using direct potentiometry. For iISE, the same electrolytes were measured on prediluted serum using the Beckman Coulter AU5800 via indirect potentiometry. All serum samples were analysed on both instruments within 30 minutes of each other to minimise any effect of storage or evaporation on electrolyte concentrations. Samples were kept at room temperature and were not subjected to freezing or prolonged storage. Both analyses were performed on the same aliquot, ensuring uniform sample handling conditions. Calibration and two-level Quality Control (QC) (normal and pathological) were performed daily; results were accepted only if QC was within limits. Each sample was tested once per method, with 10% duplicate testing for repeatability, and paired measurements were performed within 30-60 minutes.

The standard reference range of the electrolytes taken was sodium levels 135-145 mEq/L, potassium levels 3.5-5.0 mEq/L and chloride levels 98-106 mEq/L [12].

For the purpose of subgroup analysis, midpoint values within the normal reference range were selected as cut-offs (<140/≥140 mEq/L for sodium, <4.5/≥4.5 mEq/L for potassium, and <103/≥103 mEq/L for chloride).

STATISTICAL ANALYSIS

Data processing and statistical analyses were carried out using Microsoft Excel (Microsoft Corp., USA, Microsoft Excel 2019). Bland–Altman analysis and additional statistical verification were performed using MedCalc®. Statistical Software (version 22.0). Statistical analysis included paired t-test, Wilcoxon signed-rank test, Bland-Altman analysis, and Spearman's correlation.

RESULTS

Median serum levels of sodium and chloride were found to be higher when measured using the dISE method with values of 140 mEq/L and 107 mEq/L, respectively. These differences were statistically significant when compared to measurements obtained via the indirect method. In contrast, mean potassium levels were comparable between the two methods, with no statistically significant difference between the two modalities [Table/Fig-1].

Electrolyte	Method	Min	Max	Central tendency	p-value
Sodium (mEq/L)	Direct ISE	118	222	Median: 140	<0.00001*
	Indirect ISE	112	180	Median: 138	
Potassium (mEq/L)	Direct ISE	2.1	6.6	Mean: 4.54±1.10	0.469**
	Indirect ISE	2.3	6.1	Mean: 4.50±1.01	
Chloride (mEq/L)	Direct ISE	85	193	Median: 107	<0.00001*
	Indirect ISE	80	168	Median: 103	

[Table/Fig-1]: Comparison of electrolyte levels using Direct (dISE) and Indirect ISE (iISE) methods.
Na, Cl: median (min–max), *Wilcoxon signed-rank test; K: mean±SD, **paired t-test.
A p-value of <0.05 was considered significant

A significant difference between dISE and iISE measurements was observed for sodium in the normonatraemic and hypernatraemic groups and for chloride in the normochloraemic and hyperchloraemic groups (p<0.001), whereas no significant difference was noted for potassium across its subgroups [Table/Fig-2].

Electrolyte (mEq/L)	Number	Mean difference (mEq/L)	p-value*
Sodium (N=150)			
<134	08	2.6	0.19
135-145	119	3.2	<0.001
>145	23	12.1	0.006
Potassium (N=150)			
<3.5	01	**	**
3.5-5.0	82	0.0	0.419
>5.0	67	0.09	0.086
Chloride (N=150)			
<98	02	**	**
98-106	65	3.5	<0.001
>106	83	6.4	<0.001

[Table/Fig-2]: Comparison of electrolyte measurements by dISE and iISE.
*paired t-test, **sample size too small to detect a difference

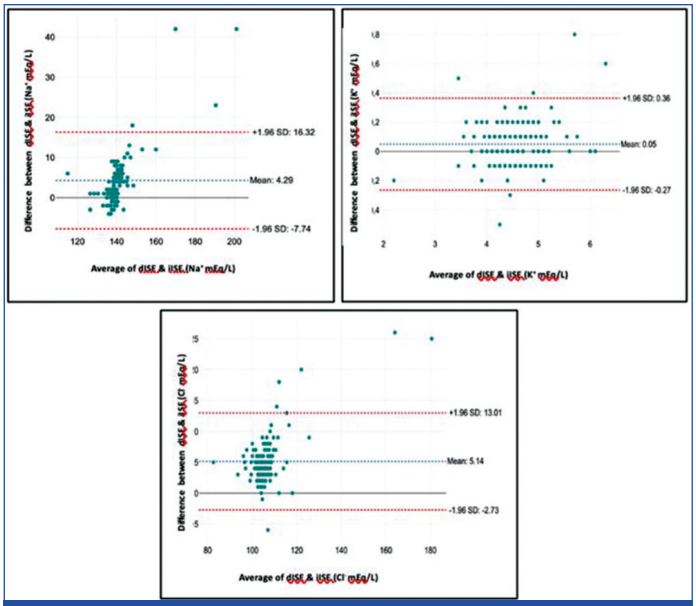
Bland-Altman analysis demonstrated a positive bias for sodium and chloride, with dISE reporting higher values than iISE (mean differences: +4.29 and +5.0 mEq/L, respectively), along with wider limits of agreement and outliers, suggesting limited interchangeability. In contrast, potassium showed minimal bias (+0.05 mEq/L) and narrow limits of agreement, indicating good agreement between the two methods [Table/Fig-3,4].

The correlation between dISE and iISE methods varied with analyte concentration [Table/Fig-5].

DISCUSSION

The present study compared serum electrolyte concentrations, i.e., sodium, potassium, and chloride, measured using dISE and iISE methods. Significant differences were observed between the two methods, with dISE yielding higher sodium values, while potassium values were comparable between dISE and iISE methods. These method-dependent variations underscore that the two techniques are not interchangeable. Subgroup analysis further revealed notable discrepancies in samples with sodium ≤140 mEq/L, potassium ≥4.5 mEq/L, and for chloride across both low and high ranges.

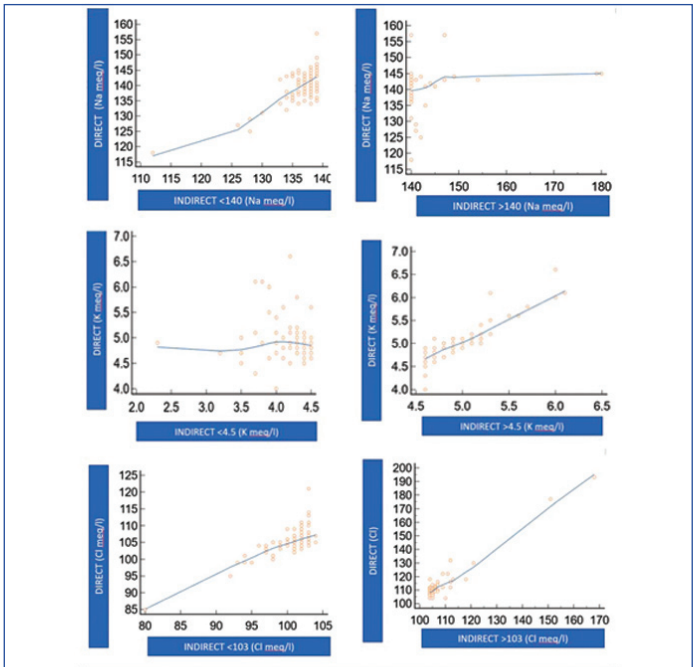
A study demonstrated that intra-individual sodium levels differed significantly between dISE and iISE, even in the absence of acute



[Table/Fig-3]: Bland-Altman plot comparing serum sodium, potassium and chloride levels measured by direct ISE (dISE) and indirect ISE (iISE) methods.
*The X-axis represents the average values from both methods, while the Y-axis shows the difference between the direct and indirect measurements. The dotted line indicates the mean difference (bias), and the dashed lines represent the 95% limits of agreement (mean±1.96 SD).

Electrolyte	dISE value	iISE value	Bias direction	Clinical agreement
Sodium	Higher	Lower	+4.29 mEq/L	Not interchangeable
Potassium	Slightly lower	Slightly higher	+0.05 mEq/L	Largely interchangeable
Chloride	Higher	Lower	+5.0 mEq/L	Not interchangeable

[Table/Fig-4]: Comparative summary of serum electrolyte measurements obtained by direct (dISE) and indirect ISE (iISE) methods.



Analyte	Level	Spearman's ρ (r)	P value	95% CI
Sodium	>140 mEq/L	0.268	0.131	-0.082 to 0.560
	≤140 mEq/L	0.546	<0.0001	0.403 to 0.663
Potassium	>4.5 mEq/L	0.870	<0.0001	0.801 to 0.923
	≤4.5 mEq/L	0.045	0.724	-0.029 to 0.205
Chloride	>103 mEq/L	0.602	<0.0001	0.420 to 0.738
	≤103 mEq/L	0.703	<0.0001	0.575 to 0.797

[Table/Fig-5]: Correlation between Direct (dISE) and Indirect ISE (iISE) methods for serum sodium, potassium, and chloride, stratified by analyte concentrations.
For sodium, a moderate correlation was observed at lower levels (≤140 mEq/L), whereas potassium showed excellent correlation only at higher levels (≥4.5 mEq/L). Chloride demonstrated moderate-to-strong correlation at both concentration ranges, with slightly better agreement at lower levels.

illness, with levels being higher using iISE. On average, iISE values were 1.9 mEq/L higher than dISE, with 95% confidence limits ranging from –3.2 to 6.9 mEq/L (p<0.001) [13]. Comparable findings have been reported by another study, wherein mean sodium concentrations measured by dISE were 136.1±6.3 mmol/L, compared with 137.8±5.4 mmol/L by iISE, with the difference reaching statistical significance (p<0.001). Bland-Altman analysis demonstrated wide agreement limits, with 95% limits of agreement ranging from -9.4 to 12.6 mmol/L, indicating clinically relevant variability [14]. Similar observations have been reported by other investigators [15,16].

The results of the present study showed no statistically significant difference between potassium concentrations measured using iISE and dISE methods. This finding is consistent with previous report indicating that potassium values estimated by colorimetric methods are comparable to those obtained by both dISE and iISE across all concentration ranges [14,17-20].

In the present study, chloride levels measured by the dISE method were found to be higher compared to those obtained using the iISE. Similar results have been forwarded by another study that also reported significantly higher chloride levels when measured with dISE [21]. Another study observed higher serum sodium and potassium concentrations with iISE (140.0±5.0 and 4.5±0.6, mEq/L respectively) compared to dISE (136.5±5.2 mEq/L and 4.5±0.6, respectively). Moreover, the difference between the two methods for sodium widened as total protein concentration decreased, demonstrating the susceptibility of iISE to protein status [12].

Further evidence highlights the non interchangeability of dISE and iISE results, especially in patients with hyperproteinemia (≥8 g/dL) or hypercholesterolaemia (≥300 mg/dL). Significant differences in sodium (p=0.007) and potassium (p=0.002) were reported between samples with normal and elevated protein levels. Similarly, sodium discrepancies varied significantly across cholesterol categories (low vs. normal, p=0.002; high vs. normal, p=0.031), while potassium also differed significantly between low and normal cholesterol groups (p=0.009) [4]. A study from India recommended that POCT-based methods and iISE should not be used interchangeably [22]. Conversely, another comparison of dISE and iISE reported no significant difference in potassium measurements between the two techniques, although sodium values remained significantly different [23].

Additionally, a study found in ICU samples, up to 25% demonstrated a ≥4 mmol/L difference in sodium between methods, particularly in hypoproteinaemia cases where iISE tended to overestimate sodium levels [24]. These discrepancies largely arise from the inherent assumptions of iISE, which requires sample dilution and is influenced by abnormal plasma water fractions. Supporting this, another investigation concluded that direct and iISE methods are not comparable, with significant differences in sodium values noted especially in hypoproteinaemia patients [25].

A study focused on critically-ill patients further highlighted an absolute difference of 3 mmol/L between dISE measurements in lithium-heparin plasma versus whole blood, with higher values in plasma. A linear association was also observed between sodium discrepancies and estimated water content. The authors advocated for whole-blood dISE as the preferred method, particularly in acutely ill patients, to ensure greater accuracy. This approach reduces the risk of misclassification of electrolyte disturbances and improves decision-making in critical care, emergency, and routine clinical practice [26].

From a clinical perspective, it is essential that clinicians consistently use either dISE or iISE for serial monitoring of patients, particularly in critical care units where treatment decisions often rely on small electrolyte variations. Interchanging methods may lead to spurious results that may compromise clinical decision-making and jeopardise patient safety. Therefore, laboratories and healthcare teams need to

establish standardised protocols specifying the preferred measurement technique for consistent and reliable electrolyte monitoring.

Limitation(s)

The study has certain limitations. It was conducted at a single tertiary care centre with a relatively modest sample size, which may limit the generalisability of the findings to other settings or populations. Information regarding patient demographics, potential confounding variables such as serum albumin, lipid profile, and hydration status, was not available, preventing adjustment for these factors that may influence electrolyte measurements. The study did not include a comparison with a reference or gold-standard method, such as flame photometry, which could have further validated the observed differences. Despite these limitations, the study provides valuable insights into the non interchangeability of dISE and iISE techniques in routine laboratory practice.

CONCLUSION(S)

The results of the present study demonstrate significant differences between electrolyte values obtained by dISE and iISE methods, with sodium, potassium, and chloride showing analyte-specific variations. These discrepancies highlight that the two methods are not reliably interchangeable, particularly in critical care settings or in patients with altered protein or lipid levels. Consistent use of a single method for serial monitoring in individual patients is therefore essential to minimise misclassification of electrolyte disturbances and to ensure accurate clinical decision-making. Standardisation of measurement practices can improve diagnostic reliability and support better patient management. Nevertheless, larger multi-centre studies are warranted to confirm these findings and to develop harmonised guidelines for electrolyte assessment across diverse clinical contexts.

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