Research Article

Impact of Total Protein and Triglycerides on the comparability of Sodium and Potassium Measurements by Direct and Indirect Ion-Selective Electrodes : An Experimental Analytical Study

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Article Info

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Keywords

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Abstract

Background: Electrolyte analysers use ion selective electrode (ISE) technologies, either 'direct'(dISE) or 'indirect'(iISE). Indirect methods are susceptible to errors in samples with high protein or lipid content. This study evaluated the individual and combined effects of elevated total proteins(TP) and triglycerides(TG) on sodium(Na^+) and potassium(K^+) measurements.

Methods: Fifty serum samples were pooled to prepare a low-protein, low-lipid matrix. Three enrichment series were generated using graded additions of 20% human albumin, 20% Intralipid, or both to achieve varying TP and TG. All samples were analysed for Na^+ and K^+ using dISE and iISE. The difference between methods (iISE–dISE) was correlated with TP and TG using regression and Bland–Altman analyses. A multivariable model assessed predictors of electrolyte bias.

Results: Albumin addition produced negative sodium bias, reaching upto 10% at TP>13g/dL, while potassium changes remained small. Triglycerides caused marked sodium underestimation at high levels (>2000 mg/dL) with minimal effect on potassium levels. When both interferents were elevated, sodium disagreement was high (bias −5.4mmol/L; limits of agreement −19.1 to +8.2), reflecting a strong cumulative effect. Multivariable regression showed TP as the strongest predictor of Na^+ bias ($R^2 = 0.67$), with minimal contribution by TG.

Conclusion: This experimental study demonstrates that increasing concentrations of total protein and triglycerides, particularly when both are elevated produce significant negative biases in sodium measured by iISE, while potassium values remain comparable. These findings become clinically relevant in critical care/ cancer patients where they receive albumin and/or lipid infusions and dISE/ iISE results are used interchangeably for patient monitoring.

Introduction

Electrolytes like sodium (Na^+), potassium (K^+), Calcium (Ca^{++}) and Chloride (Cl^-) affect neuronal excitability, cardiac conduction, fluid balance, and acid–base homeostasis. Accurate measurement is therefore essential across critical care, oncology, renal and hepatic disease, endocrine disorders, and perioperative settings, where even small deviations can alter clinical decisions [1-5].

Electrolyte analysers use ion selective electrode (ISE) technology in laboratories and point-of-care (POC) settings. ISEs may be direct (dISE) measuring undiluted samples, or indirect (iISE), where samples are diluted before measurement. Both rely on the same electrochemical principle but differ in sample handling, matrix interaction, and susceptibility to interference. dISE, used in most arterial blood gas analysers, POC Electrolyte analysers and some dry chemistry analysers (Vitros, Ortho Clinical Diagnostics, USA), measures electrolyte activity in aqueous phase of plasma, while iISE, common on high-throughput auto-analysers, measures activity in diluted samples with dilution ratios from 1:5 to 1:46 allowing lower sample volumes. iISE assumes a plasma water fraction of approximately 93% and uses it to expand the measurable concentration range [6,7]. In hyperproteinemia or hyperlipidemia, reduced plasma water fraction leads to falsely low electrolyte values by iISE (electrolyte exclusion effect). dISE remains unaffected because no dilution is performed [8]. These discrepancies may become clinically significant in marked biochemical derangements, such as multiple myeloma, macroglobulinemia, nephrotic syndrome, severe hypertriglyceridemia, parenteral nutrition, pancreatitis, and critical illness, where precise electrolyte values are crucial for therapeutic decisions [9-11]. Thus interpretation becomes difficult when dISE and iISE are used interchangeably for follow-up.

Although the effect of high protein or lipid concentrations on electrolyte measurement is well recognised [12-14], their relative and combined influence on dISE and iISE has not been well characterised. Data are limited when both abnormalities coexist, a scenario common in patients receiving lipid infusions, albumin therapy, chemotherapy, targeted therapy, or those with complex metabolic profiles.

Therefore, this experimental study was designed to simulate and quantify the effect of increasing total protein and triglyceride concentrations—individually and in combination—on Na^+ and K^+ measured by direct and indirect ISE. Using graded enrichment series and comparing measured with calculated values, we aimed to characterise matrix effects, assess method agreement, and identify conditions producing clinically meaningful deviations.

Aims and Objectives

1. To evaluate the effect of increasing total protein on the difference between direct and indirect ISE measurements of sodium and potassium.
2. To assess the impact of increasing triglycerides on the difference between direct and indirect ISE measurements.
3. To analyse the combined influence of high protein and high triglyceride levels on sodium and potassium discrepancies.
4. To develop a multivariable regression model to predict the combined effect of total protein and triglycerides on sodium and potassium measurement bias.

Methods

Study Design and Setting

This experimental study was carried out in the Department of Laboratory Medicine, All India Institute of Medical Sciences (AIIMS), New Delhi. Fifty leftover serum samples received for electrolyte investigations were identified based on low baseline concentrations of total protein (<6 g/dL) and triglycerides (<100 mg/dL), pooled, aliquoted and used for all our further experiments. The study protocol was approved by the Institutional Ethics Committee, All India Institute of Medical Sciences (AIIMS), New Delhi (Ref. No. IEC-789/07.10.2022). Supplementary Figure 1 depicts the study design.

Preparation of Enriched Samples

To generate a gradient of protein and lipid concentrations, 20% human albumin (AlbuRel, Reliance Life Sciences) and 20% Intralipid (Fresenius Kabi) were used. Intralipid is a sterile intravenous fat emulsion used as parenteral nutrition. These were added to aliquots of pooled serum in measured volumes (Supplementary Table 1) to generate samples with varying biochemical characteristics. Three enriched sample sets were prepared:

- Albumin-enriched (A-series): increasing total protein concentrations achieved using 20% human albumin
- Lipid-enriched (L-series): varying concentrations of 20% Intralipid added to increase triglyceride levels
- Combined (N-series): both 20% Albumin and 20% Intralipid added to achieve low, intermediate, and high protein-triglyceride combinations.

Biochemical Measurements

Each enriched sample was analysed for sodium and potassium levels using both dISE on the Ortho VITROS 4600 dry chemistry system (Ortho-Clinical Diagnostics Inc.) and iISE on Roche Cobas 8000 modular analyser (Roche Diagnostics International Ltd., Switzerland). Total protein, albumin, and triglyceride concentrations were measured on the Cobas analyser using manufacturer-approved reagents. The analytical measurable ranges of total protein (TP) and triglyceride (TG) were 0.2–12 g/dL and 8.5–885 mg/dL respectively. Since experimental enrichments often exceeded these linear ranges, calculated TP and TG concentrations were derived from the known volumes of pooled serum, albumin solution, and lipid emulsion added to each sample using mass-balance calculations based on their assumed concentrations. These calculated concentrations were used as the reference values for

downstream analysis (Supplementary Table 1).

Quality Control and Pre-Analytical Validation

Prior to sample testing, internal quality control (Levey–Jennings charts) and EQAS reports were reviewed. All analytes were within acceptable quality limits (±2 SD) on both platforms, indicating satisfactory analytical performance and readiness for the experimental procedures. To confirm comparability between instruments, two levels of SMART Lab controls were analysed on both the Cobas and VITROS platforms for Na^+ and K^+. The control results demonstrated minimal (<5%) inter-instrument variation, confirming readiness for the experimental procedures.

Data Processing and Analytical Approach

All data analyses were performed in Python (version 3.11.7) using Jupyter Notebook (version 7.0.8). NumPy (version 1.26.4) and Pandas (version 2.1.4) were used for numerical handling and array operations, while visualisations were generated using Matplotlib (version 3.8.0). Statistical analysis (paired t-tests, linear regression, Bland–Altman analysis, and multivariable modelling) were performed using SciPy (version 1.11.4) and scikit-learn (version 1.2.2).

Paired t-test were conducted for each enrichment series (A, L, and N) to compare sodium and potassium values between dISE and iISE. These tests assessed whether deviations between methods were statistically significant within each series. Linear regression assessed the agreement between measured and calculated TP and TG levels within each series. For each sample, the difference between direct and indirect ISE measurements (iISE – dISE) was calculated separately for sodium and potassium. These differences were analysed against calculated TP and TG concentrations. Agreement between dISE and iISE for Na^+ and K^+ was further evaluated using Bland–Altman analysis, yielding mean bias and 95% limits of agreement.

To visualise combined effect of TP and TG on measurement bias, two-dimensional scatter plots were created with calculated TP on the x-axis and calculated TG on the y-axis. For each sample, the size of the marker reflected the magnitude of the difference (iISE–dISE), allowing an intuitive picture of how strongly both variables contributed to interference. To make these patterns clinically meaningful, tolerance limits of ±4.0 mmol/L for sodium and ±0.3 mmol/L for potassium were applied, in accordance with CLIA allowable total error limits and biological variation–based quality specifications, as deviations beyond these thresholds are considered analytically and clinically significant [15-16].

Finally, a multivariable linear regression model was constructed using calculated TP and TG to predict measurement bias for sodium and potassium. This model evaluated whether matrix composition could predict iISE–dISE deviation and support the development of correction algorithms for samples with suspected interference.

Results

Measured sodium (Na^+) and potassium (K^+) values by direct and indirect ISE across A-, L-, and N-series are shown in Table 1. Across samples, TP ranged from 5.6–13.9 g/dL in the albumin-added A-series and 2.8–13.9 g/dL in the combined N-series, while remaining 5.7–6.3 g/dL in the lipid-only L-series. TG ranged from 105–2437 mg/dL in the L-series and 56–3602 mg/dL in the N-series, whereas A-series samples contained only baseline TG levels of 52–93 mg/dL.

Table 1: Summary of Sodium and Potassium Measurements by Direct and Indirect Ion Selective Electrodes Across Albumin-, Lipid-, and Combined-Enriched Samples.

Series	Number of samples	Na iISE (mmol/L); Mean±SD	Na dISE (mmol/L); Mean±SD	K iISE (mmol/L); Mean±SD	K dISE (mmol/L); Mean±SD	Na bias (iISE-dISE); Median (Range)	K bias (iISE-dISE); Median (Range)	Percent Na bias [(iISE-dISE)/ dISE] *100; Median (Range)	Percent K bias [(iISE-dISE)/ dISE] *100; Median (Range)
A series	17	119.54±12.4	125.21±7.8	3.3±0.8	3.37±0.8	-6.6 (-11.7, 1.2)	-0.1 (-0.2, 0.1)	-5.3 (-10.17, 0.9)	-3 (-8.3, 2.7)
L series	21	112.29±13.9	113.19±11.3	3.8±0.4	3.84±0.4	1.4 (-17.3, 2.4)	0 (-0.65, 0.15)	1.15 (-15.7, 1.9)	0 (-17.1, 3.4)
N series	33	113.56±18.9	118.98±23.5	2.41±0.6	2.56±0.6	-3.95 (-21.7, 3.25)	-0.1 (-0.4, 0)	-3.7 (-13.2, 2.8)	-4.35 (-16, 0)

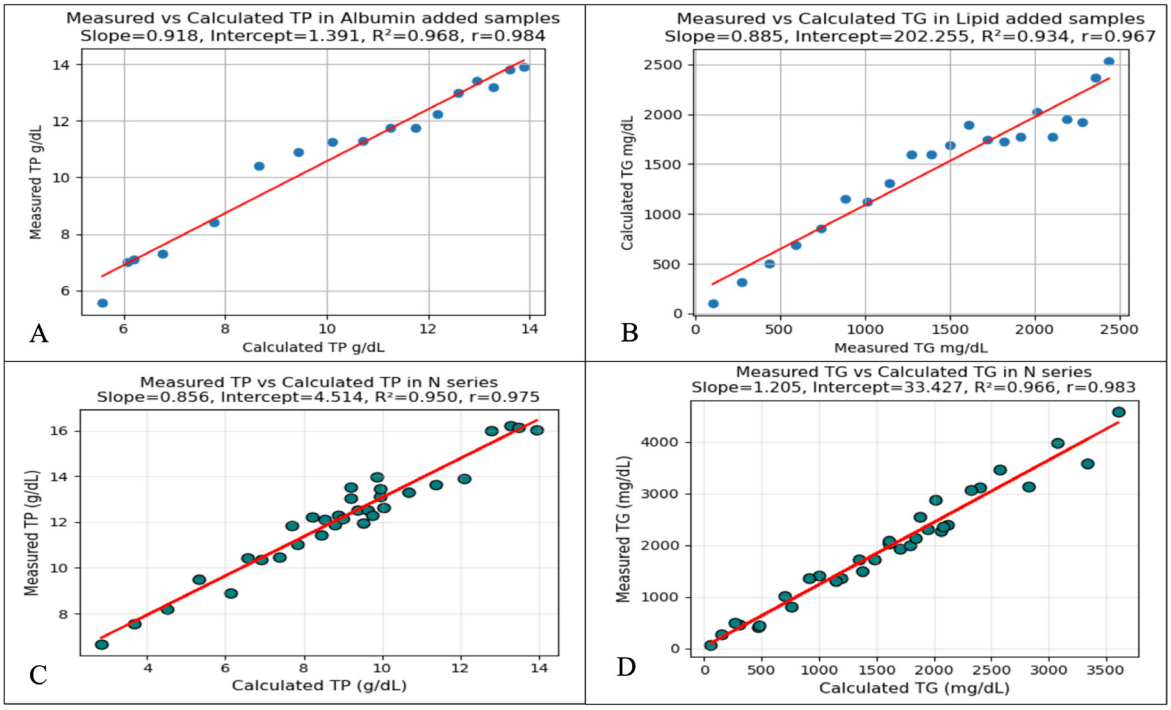
Na- Sodium; K – Potassium; dISE-Direct Ion Selective Electrodes; iISE- Indirect Ion Selective Electrodes

Data are presented as mean ± SD for measured concentrations and median (range) for differences.

Validation of Enrichment Model: Correlation Between Measured and Calculated Concentrations

Across all three enrichment series (albumin-only, lipid-only, and combined), the measured concentrations of TP and TG correlated closely with their calculated target values (Figure 1A–D). Albumin-enriched samples displayed an excellent correlation for TP ($R^2 = 0.968$), and lipid-enriched samples demonstrated similarly high correlation for TG ($R^2 = 0.934$). In the combined enrichment series, both TP and TG continued to show strong correlation ($R^2 = 0.950$ and $R^2 = 0.966$, respectively). These findings confirmed stable and predictable enrichment gradient and justify the use of calculated values for subsequent analyses.

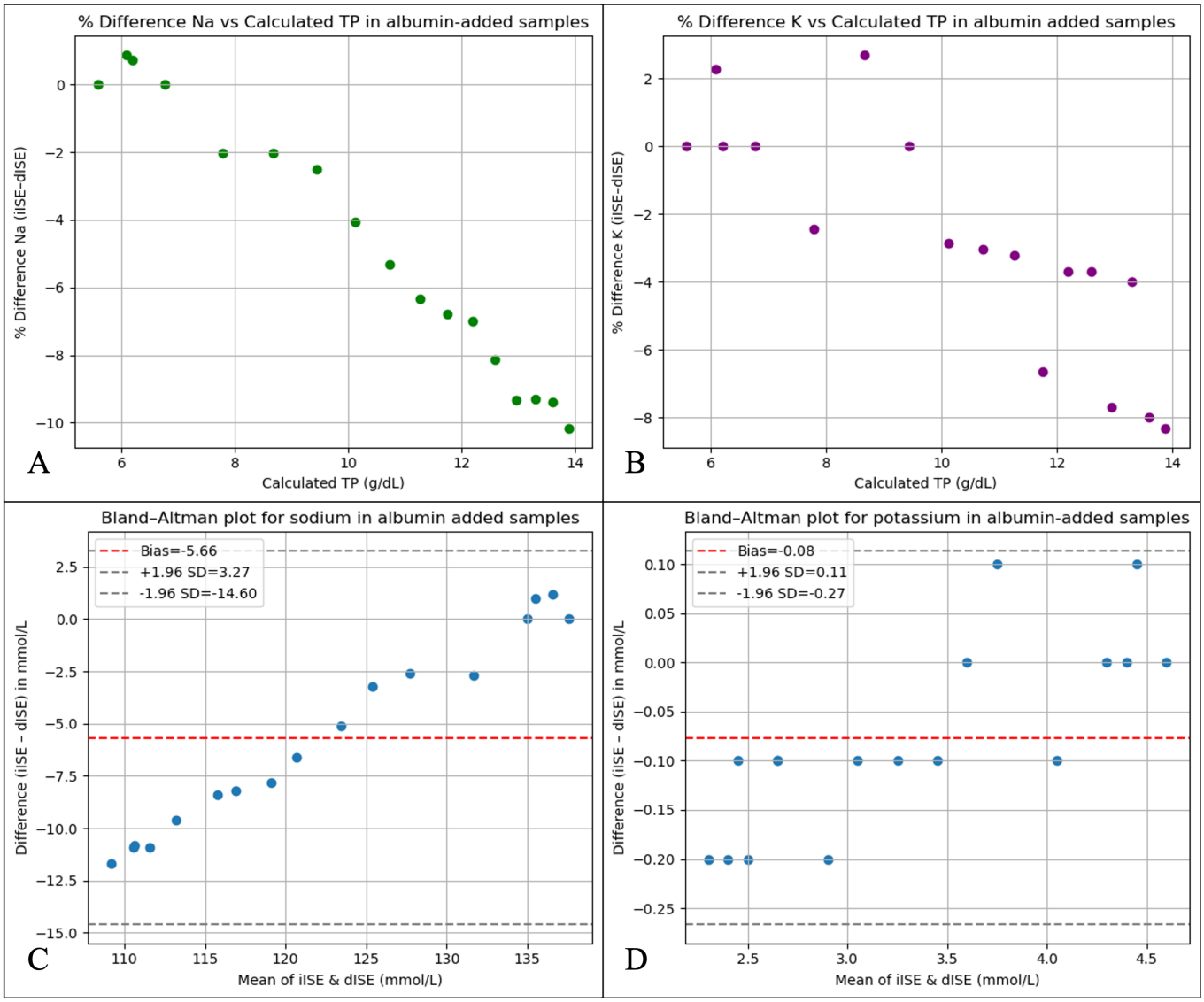
Figure 1: Linear regression analysis between measured and calculated concentrations for (A) TP in albumin-enriched samples, (B) TG in lipid-enriched samples, (C) TP in combined albumin and lipid enriched samples, and (D) TG in combined albumin + lipid enriched samples.



Effect of Albumin Addition on Sodium and Potassium Measurements (Figure 2)

In albumin-enriched samples, increasing TP levels produced progressive widening of the sodium gap between indirect and direct ISE measurements. As TP increased, sodium showed increasingly negative deviation (iISE – dISE), reaching nearly 10% underestimation at the highest protein concentrations (Figure 2A). Potassium showed a smaller downward trend (Figure 2B). Paired t-tests confirmed significant differences for both electrolytes, with sodium showing a mean bias of −5.665 mmol/L ($p < 0.05$) and potassium a smaller but significant mean bias of −0.076 mmol/L ($p < 0.05$). Bland–Altman plots supported these findings with sodium demonstrating a marked negative bias with wide limits of agreement (Figure 2C), whereas potassium remained comparatively stable, exhibiting minimal bias and tight limits (Figure 2D). Collectively, these results highlight the strong susceptibility of sodium, more than potassium, to protein-related analytical interference.

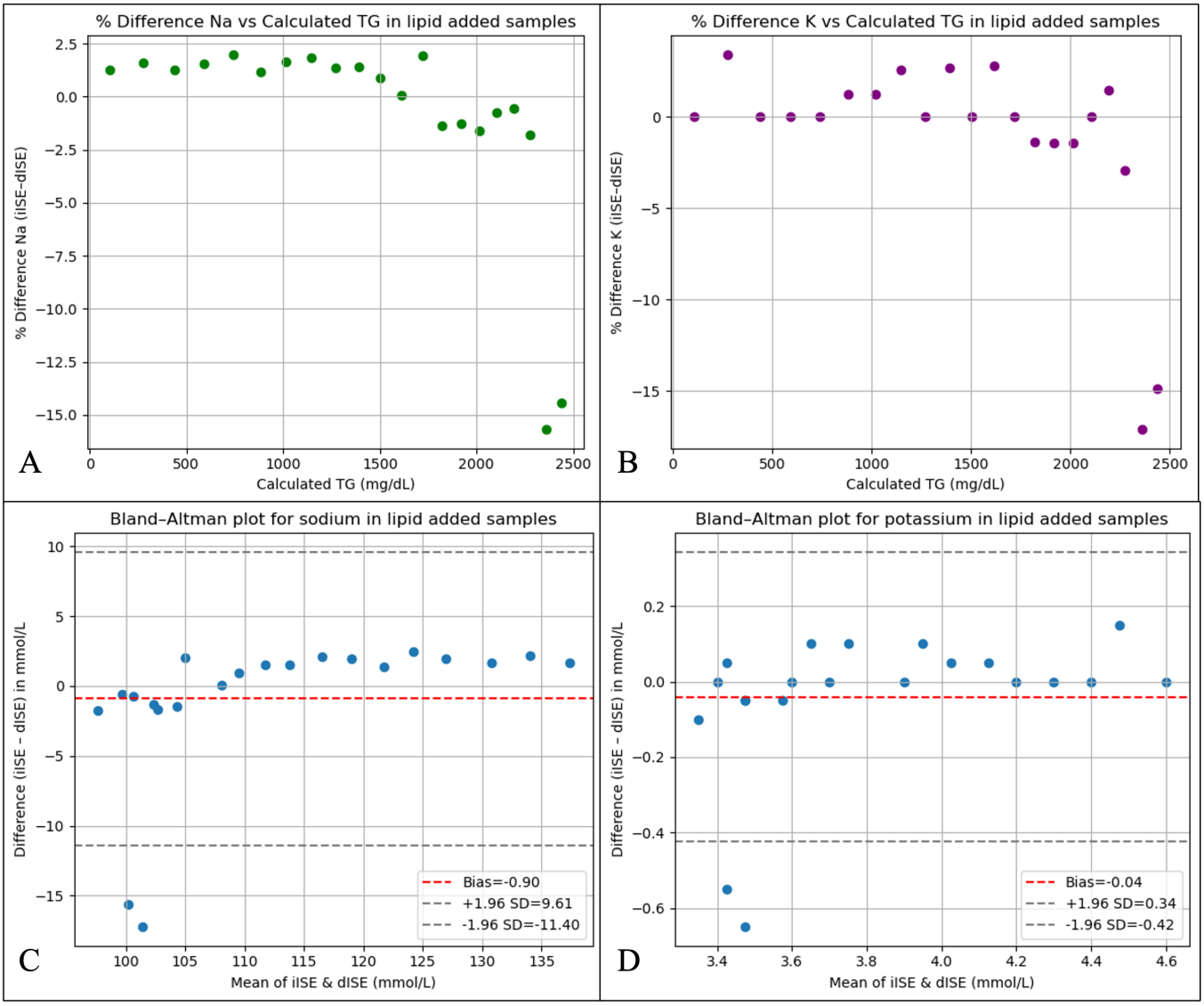
Figure 2: Effect of albumin addition on sodium and potassium measurements.
(A) %Difference in sodium (iISE–dISE) across calculated total protein levels.
(B) %Difference in potassium (iISE–dISE) across calculated total protein levels.
(C) Bland–Altman plot for sodium (iISE vs dISE) showing bias and 95% limits of agreement.
(D) Bland–Altman plot for potassium (iISE vs dISE) with bias and limits of agreement.



Effect of Triglyceride Addition on Sodium and Potassium Measurements

In the triglyceride-enriched samples, sodium values remained close to baseline but began to show a negative shift once triglyceride concentrations exceeded 2000 mg/dL (Figure 3A). Potassium values displayed only small and gradual changes with rising TG (Figure 3B). Paired t-tests showed that neither sodium (mean difference −0.895 mmol/L, p = 0.4529) nor potassium (mean difference −0.040 mmol/L, p = 0.3552) exhibited statistically significant deviation between indirect and direct ISE, indicating lipids alone had a weaker impact on measurement bias. Bland–Altman analysis supported this observation, demonstrating only mild sodium bias and negligible potassium bias across the lipid-added series (Figure 3C–D).

Figure 3: Effect of triglyceride addition on sodium and potassium measurements.
(A) %Difference in sodium (iISE–dISE) across calculated triglyceride levels in lipid-added samples.
(B) %Difference in potassium (iISE–dISE) across calculated triglyceride levels in lipid-added samples.
(C) Bland–Altman plot for sodium (iISE vs dISE) showing bias and 95% limits of agreement.
(D) Bland–Altman plot for potassium (iISE vs dISE) with bias and limits of agreement.

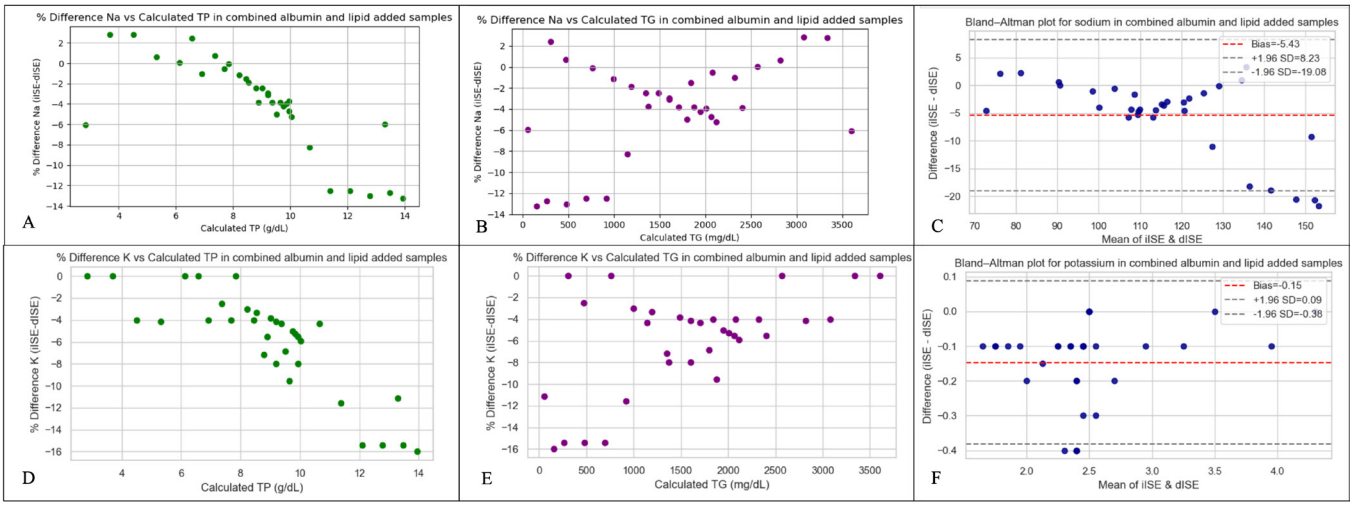


Combined Effect of Albumin and Triglyceride Addition

In samples where both TP and TG were simultaneously elevated, the impact on electrolyte measurements became more pronounced. Sodium showed progressively larger negative deviations between indirect and direct ISE measurements as both TG and TP concentrations increased (Figure 4A, 4B). The greatest underestimation of sodium was observed at the highest TP and TG levels, indicating an amplified interference. Bland–Altman analysis for sodium demonstrated a substantial negative bias, with wide limits of agreement, reflecting poor concordance between direct and indirect ISE measurements under dual interference conditions (Figure 4C). This widening of limits was greater than that observed in either albumin-only or lipid-only enrichment series.

Potassium showed increased deviation in the combined enrichment model, with negative differences becoming more evident at higher TP and TG concentrations (Figure 4D, 4E). However, the magnitude of potassium deviation remained smaller compared to sodium. Bland–Altman analysis for potassium showed a modest negative bias with relatively narrow limits of agreement, indicating better stability despite combined interference (Figure 4F).

Figure 4: Effect of combined albumin and triglyceride enrichment on sodium and potassium measurements by indirect and direct ISE.
(A) % Difference in sodium (iISE–dISE) across calculated total protein concentrations
(B) % Difference in sodium (iISE–dISE) across calculated total triglyceride concentrations
(C) Bland–Altman plot for sodium showing the mean bias and 95% limits of agreement
(D) % Difference in potassium (iISE–dISE) plotted against calculated protein concentrations
(E) % Difference in potassium (iISE–dISE) plotted against calculated triglyceride concentrations
(F) Bland–Altman plot for potassium illustrating the mean bias and 95% limits of agreement

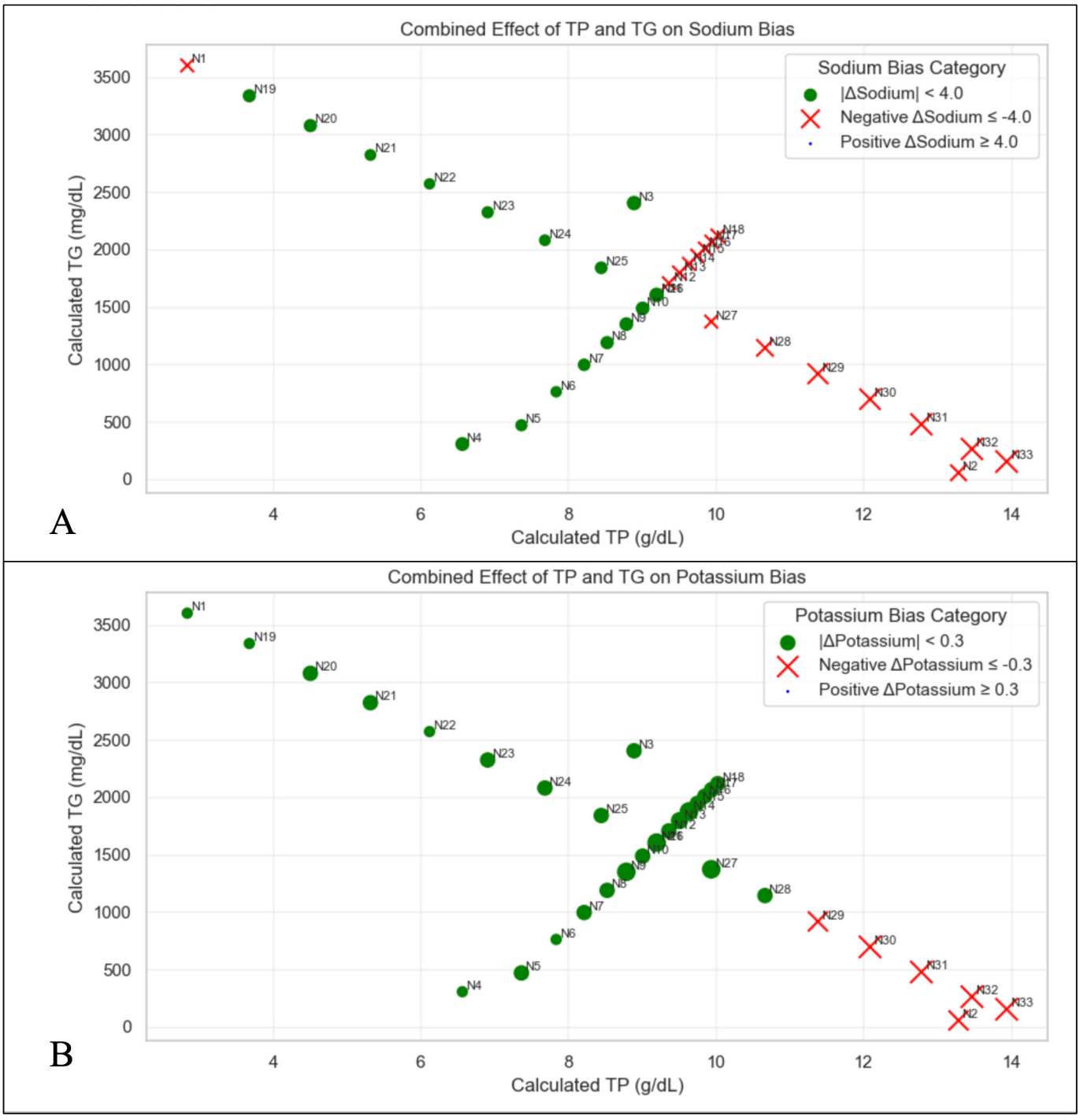


Statistical comparison confirmed that both electrolytes exhibited significant differences between indirect and direct ISE in the combined series, with sodium showing a mean difference of −5.426 mmol/L ($p < 0.05$) and potassium a mean difference of −0.147 mmol/L ($p < 0.05$), supporting cumulative interference effect.

Integrated Influence of Total Protein and Triglycerides

To assess combined effect of TP and TG on electrolyte measurement bias, calculated TP and TG concentrations were plotted simultaneously in a two-dimensional space, with marker size proportional to the magnitude of the difference between indirect and direct ISE measurements (iISE–dISE). For sodium, a clear coordinated interference pattern was observed (Figure 5A).

Figure 5: Combined effect of total protein and triglyceride levels on sodium and potassium measurement differences.
(A) Scatter plot illustrating the combined influence of calculated total protein and triglyceride concentrations on sodium bias (iISE–dISE), categorized using a clinical cut-off of ±4.0 mmol/L.
(B) Scatter plot showing the combined influence of calculated total protein and triglyceride concentrations on potassium bias (iISE–dISE), categorized using a clinical cut-off of ±0.3 mmol/L.



**The size of each marker reflects the magnitude of deviation, with larger markers indicating greater measurement discrepancies.*

Samples with concurrent elevations of TP and TG demonstrated the largest negative sodium deviations, with multiple points exceeding the clinically relevant cut-off of ±4.0 mmol/L. These high-magnitude deviations clustered predominantly at the upper extremes of both TP and TG, indicating a strong additive effect of proteins and lipids on sodium underestimation by indirect ISE.

In contrast, potassium showed substantially greater analytical stability (Figure 5B). When a clinical threshold of ±0.3 mmol/L was applied, most samples remained within acceptable limits, even at higher TP and TG concentrations. Only a small subset of samples with very high TP values demonstrated potassium deviations approaching or exceeding this cut-off, and the overall magnitude of deviation was markedly smaller than that observed for sodium.

Increasing marker size across both plots visually highlights that clinically meaningful electrolyte discrepancies arise primarily under conditions of combined biochemical elevation, with sodium being far more susceptible than potassium. These findings reinforce that simultaneous hyperproteinemia and hypertriglyceridemia pose the greatest risk for significant sodium misclassification when measured by indirect ISE, whereas potassium measurements remain comparatively valid.

Multivariable Linear Regression for Predicting Sodium and Potassium Bias

Multivariable regression analysis was performed on the combined-matrix (N-series) samples to quantify the effect of TP and TG on electrolyte deviation. For sodium, the regression model demonstrated good explanatory performance (R^2 = 0.6655) with a mean absolute error (MAE) of 3.19 mmol/L.

Diff_Na (iISE − dISE)= −2.4383(TP) − 0.0012(TG) + 18.2809

From the model, a corrective equation for estimating direct ISE sodium from indirect ISE measurements is:

Na dISE-equivalent =

Na(iISE)+2.4383(TP)+0.0012(TG)−18.2809

For potassium, the model also showed moderate predictive strength (R^2 = 0.6652) with a very small MAE of 0.059 mmol/L.

Diff_K (iISE − dISE) = −0.03733(TP) − 0.0(TG) + 0.18966

Based on the multivariable regression model, a corrective equation for estimating direct ISE potassium from indirect ISE measurements is:

K dISE-equivalent =K (iISE)+0.03733(TP)−0.18966

Across both electrolytes, TP was the strongest driver of deviation, whereas triglycerides contributed modestly to sodium bias and had no measurable impact on potassium. These models indicate that errors can be predicted using TP and TG levels. However, external validation is required before clinical use.

Discussion

This experimental study evaluated how progressively increasing TP and TG concentrations added individually and in combination affect the differences between direct and indirect ion-selective electrode (ISE) measurements for sodium and potassium. By experimenting with a graded and controlled enrichment model that mimicked the biochemical extremes seen in real clinical practice, matrix effects responsible for ISE discrepancies were characterised. A key strength of the study was the use of a pooled, low interference serum matrix with stepwise enrichment with albumin and lipid. Strong correlation between measured and calculated TP and TG concentrations across the A-, L-, and N-series confirmed analytical validity of the model. These findings give confidence that the experimental matrix accurately represented progressive elevations similar to those seen in real clinical scenarios. Clinically extreme TP and TG levels occur in conditions such as multiple myeloma, Waldenström macroglobulinemia, severe dehydration, and certain inflammatory states, familial chylomicronemia, poorly controlled diabetes, pancreatitis, and patients receiving parenteral nutrition.

The results from albumin-enriched samples align with prior studies demonstrating pseudohyponatremia in hyperproteinemia, with underestimation becoming more pronounced at high protein concentrations ~>8 g/dL. Dimeski and colleagues, 2012 [12] showed similar behaviour using paraprotein-rich clinical samples, and Fortgens & Pillay, 2011[14] emphasised that this effect persists even with modern analysers. Potassium showed only minimal change, consistent with prior literature suggesting that potassium measurement is less susceptible to water-displacement error due to its smaller absolute concentration and tighter physiological buffering. Paired t-test comparing sodium and potassium values between dISE and iISE gave significant p-values ($p < 0.05$ for both sodium and potassium). This supports earlier clinical observations published by Chopra & Datta, 2020 [17] in their direct–indirect ISE comparison study.

In the triglyceride-enriched samples, the results were consistent with prior evidence of lipid-related underestimation of sodium by iISE [18-20]. Koch et al. 2021 [18] demonstrated that Intralipid®-based testing underestimates lipid interference, with minimal sodium bias up to lipemic indices approaching 2000, whereas patient samples showed significant pseudohyponatremia (≥4 mmol/L) at lipemic indices exceeding ~700, reflecting poor correlation between lipemic index and true interference. They also reported a poor correlation between lipemic index and triglyceride concentration. Sen, 2016 [19] similarly showed a progressive negative bias in iISE sodium levels with increasing TG concentration, while potassium remained comparatively stable. Hou et al, 2025 [20] confirmed concentration-dependent sodium bias with rising TG concentrations using controlled lipemia models where potassium deviations remained small and clinically insignificant. These findings align closely with our lipid-enriched series, where sodium bias increased at higher triglyceride concentrations while potassium remained relatively robust. Notably, in our lipid-only series, differences between indirect and direct ISE were not statistically significant

for either sodium or potassium indicating that moderate hyperlipidemia alone may not consistently result in measurable analytical disagreement.

The combined enrichment model (N-series) demonstrated how simultaneous elevations of TP and TG amplify electrolyte measurement bias beyond the effect of either interferent alone (Figure 4A–C). Bland–Altman analysis also showed negative sodium bias with markedly widened limits of agreement, indicating poor agreement under combined interference conditions. On the other hand, potassium had increased deviation in the combined series as compared to albumin-only or lipid-only models. These findings extend prior observations of pseudohyponatremia in hyperproteinemia or hyperlipidemia studied in isolation by showing a clear cumulative and potentially synergistic electrolyte exclusion effect when both matrix components are elevated together.

In the combined enrichment model, TP was the dominant driver of bias, particularly for sodium. Although rising TG levels did contribute to increasing sodium deviation, the largest negative biases were seen when high TG concentrations occurred together with elevated TP. This suggests that proteins are the main driver of the electrolyte exclusion effect, with lipids acting to further intensify the error rather than causing it independently.

Potassium deviations increased in the combined series than in the A or L series, but the overall magnitude remained small. Only a few samples exceeded a clinically relevant cut-off of ±0.3 mmol/L. Sodium, however, frequently crossed the ±4.0 mmol/L allowable error limit, underscoring its greater susceptibility to exclusion effects when measured by iISE. Overall, TP is the primary determinant of clinically significant sodium bias with TG acting as an amplifier. This difference in susceptibility helps explain why pseudohyponatremia is commonly encountered in complex biochemical states, whereas clinically meaningful potassium errors are far less frequent.

With a two-dimensional visual framework incorporating the clinical thresholds, this study provides a practical method for identifying biochemical conditions under which iISE results may become diagnostically misleading. To our knowledge, this visual–analytical approach to evaluating combined matrix interference has not been previously reported and represents a novel methodological contribution to the study of electrolyte measurement interference. These findings emphasise the need for heightened laboratory vigilance and support preferential use of dISE or confirmatory testing in patients with complex biochemical abnormalities. Multivariable regression analysis of the combined enrichment (N-series) samples showed how TP and TG together influence electrolyte bias. TP emerged as the primary driver of sodium deviation, while TG contributed modestly and did not influence potassium. This behaviour is in line with the electrolyte exclusion mechanism described by Dimeski et al. 2012 [12], who identified displacement of plasma water by non-aqueous solids causes pseudohyponatremia, while potassium remains relatively resistant to clinically meaningful error. Recent modelling studies have similarly shown that sodium bias can be estimated from matrix parameters, supporting algorithm-based interpretation or automated flagging in high-risk samples [12,20]. Our dual-interference model demonstrates that combined protein and lipid measurements can predict sodium bias under complex biochemical conditions, supporting multivariable approaches to improve interpretation of indirect ISE results in routine practice. The most novel and clinically relevant aspect of this study is the evaluation of the combined enrichment model (N-series), which remains largely unexplored. Earlier studies typically examined hyperproteinemia or hyperlipidemia in isolation; however, many real-world settings - such as nephrotic syndrome, myeloma, Waldenström macroglobulinemia, parenteral nutrition, chemotherapy-related metabolic derangements, and critical illness - feature simultaneous elevations of both proteins and lipids. In this context, our controlled enrichment model provides important new insight into their combined effects. The findings are clinically relevant because sodium measurement directly influences management in hyponatremia. Misclassification may lead to inappropriate hypertonic saline therapy, inadequate treatment, or risk of osmotic demyelination if true sodium levels are higher than reported. There are, however, limitations to consider. Although the enrichment model mimics clinical conditions, added albumin and Intralipid do not perfectly replicate endogenous proteins and lipoproteins. The study used a single direct and single indirect ISE platform; thus, results may differ with other analyser architectures. Furthermore, extreme concentrations achieved experimentally, particularly triglycerides >3000 mg/dL, may not occur in all practice settings. Finally, regression models derived from artificial samples require validation in real patient populations before clinical use. Overall, this study provides an experimental evaluation of how proteins and lipids - alone and together - affect sodium and potassium measurement by direct and indirect ISE. The findings highlight the importance of recognising matrix effects when interpreting electrolytes in complex biochemical states and support the use of direct ISE or blood-gas-based technologies in high-interference scenarios. Future clinical studies should validate these correction models and explore automated laboratory warning systems for samples at risk of pseudohyponatremia.

Conclusion

This study shows that increases in TP and TG, especially when they occur together, can substantially distort sodium results measured by indirect ISE, while direct ISE measurements remain largely reliable. TP was the main driver of sodium error, with triglycerides adding further bias only at very high levels. When both were elevated, the largest discrepancies were observed. Potassium measurements were far less affected across all conditions. These findings highlight the need for

caution when interpreting indirect ISE sodium in patients with complex biochemical abnormalities and support the use of direct ISE or confirmatory testing to prevent clinically meaningful errors.

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Conflicts of Interest
The authors declare that they have no competing interests.

Author Contributions
Conceptualization: SKD, PC; Sample collection, experiments: GB, PC; Formal analysis: PC; Supervision: SKD, SP; Writing original draft: PC, AG; Writing, review & editing: PC, AG, GB, SKD,SP; Approval of final version: PC, AG, GB, SKD,SP
All authors have read and approved the final manuscript.

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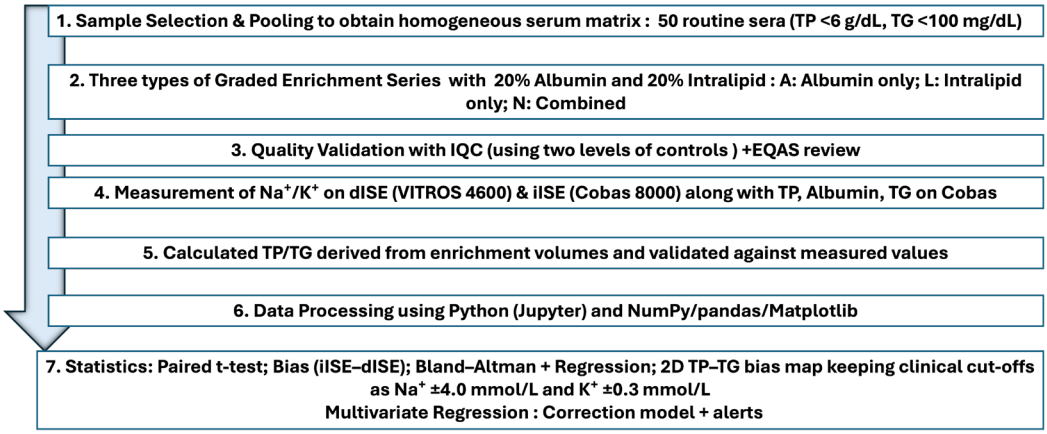
Supplementary files

Supplementary Figure 1: Overview of the study workflow and analytical strategy.



Supplementary Table 1: Composition of experimentally enriched serum samples and calculation of theoretical total protein (TP) and triglyceride (TG) concentrations used for validation experiments.

Supplementary Table 1A. A series: Samples spiked with 20% human albumin							
Label	Pooled serum added (µL)	Amount of albumin added (µL)	Measured TP levels (g/dL)	Mass of TP (g) in serum (600µL)	Mass of TP(g) in Albumin added	Total TP Mass (g)	Calculated TP (g/dL)
A1	600	0	5.58	0.03	0.00	0.03	5.58
A2	600	20	7.00	0.03	0.00	0.04	6.08
A3	600	25	7.11	0.03	0.01	0.04	6.20
A4	600	50	7.30	0.03	0.01	0.04	6.77
A5	600	100	8.40	0.03	0.02	0.05	7.78
A6	600	150	10.40	0.03	0.03	0.06	8.66
A7	600	200	10.90	0.03	0.04	0.08	9.44
A8	600	250	11.25	0.03	0.05	0.09	10.12
A9	600	300	11.30	0.03	0.06	0.10	10.72
A10	600	350	11.75	0.03	0.07	0.11	11.26
A11	600	400	11.75	0.03	0.08	0.12	11.75
A12	600	450	12.25	0.03	0.09	0.13	12.19
A13	600	500	13.00	0.03	0.11	0.14	12.59
A14	600	550	13.40	0.03	0.12	0.15	12.95
A15	600	600	13.20	0.03	0.13	0.16	13.29
A16	600	650	13.80	0.03	0.14	0.17	13.60
A17	600	700	13.90	0.03	0.15	0.18	13.88

TP mass added from albumin was calculated assuming a TP concentration of 21 g/dL in the albumin preparation, using the formula (21 000 / 100 000) × albumin volume (µL). Total TP mass was used to derive the calculated TP concentration after volume correction.

Supplementary Table 1B. L series: Samples spiked with 20% Intralipid							
Label	**Pooled serum added (µL)**	**Amount of LIPID (1:5 dilution of 20% Intralipid) added (µL)**	**Measured TG levels (mg/dL)**	**Mass of TG (mg) in Serum (800µL)**	**Mass of TG (mg) in LIPID added**	**Total TG Mass (mg)**	**Calculated TG levels (mg/dL)**
L1	800	0	104.85	0.84	0	0.84	104.85
L2	800	20	316.1	0.84	1.42	2.26	275.46
L3	800	40	500.1	0.84	2.84	3.68	437.95
L4	800	60	686.25	0.84	4.26	5.10	592.88
L5	800	80	857.45	0.84	5.68	6.52	740.77
L6	800	100	1146.65	0.84	7.1	7.94	882.09
L7	800	120	1126.4	0.84	8.52	9.36	1017.26
L8	800	140	1308.1	0.84	9.94	10.78	1146.68
L9	800	160	1598.3	0.84	11.36	12.20	1270.71
L10	800	180	1596.5	0.84	12.78	13.62	1389.67
L11	800	200	1688.2	0.84	14.2	15.04	1503.88
L12	800	220	1895.2	0.84	15.62	16.46	1613.61
L13	800	240	1749.05	0.84	17.04	17.88	1719.12
L14	800	260	1730.25	0.84	18.46	19.30	1820.64
L15	800	280	1769.45	0.84	19.88	20.72	1918.41
L16	800	300	2025.8	0.84	21.3	22.14	2012.62
L17	800	320	1775.25	0.84	22.72	23.56	2103.46
L18	800	340	1945.35	0.84	24.14	24.98	2191.12
L19	800	360	1920.6	0.84	25.56	26.40	2275.76
L20	800	380	2369.55	0.84	26.98	27.82	2357.53
L21	800	400	2534.8	0.84	28.4	29.24	2436.57

TG mass added from lipid was calculated assuming a TG concentration of 7100 mg/dL in the lipid preparation, using the formula (7100/100000) × lipid volume (µL). Total TG mass was used to derive calculated TG concentration after volume correction.

Supplementary Table 1C: N series: Samples spiked with both 20% human albumin and 20% Intralipid.

Label	Pooled serum added (µL)	Amount of albumin added (µL)	Amount of LIPID (1:5 dilution of 20% Intralipid) added (µL)	Measured TP levels (g/dL)	Measured TG levels (mg/dL)	Mass of TP (g) in serum	Mass of TP (g) in albumin added	Mass of TP (g) in LIPID added	Total TP Mass (g)	Calculated TP (g/dL)	Mass of TG (mg) in Serum	Mass of TG (mg) in LIPID added	Mass of TG (mg) in Albumin added	Total TG Mass (mg)	Calculated TG levels (mg/dL)
N1	600.00	0.00	600.00	6.67	4587.10	0.03	0.00	0.00	0.03	2.84	0.63	42.60	0.00	43.23	3602.43
N2	600.00	600.00	0.00	16.21	75.00	0.03	0.13	0.00	0.16	13.29	0.63	0.00	0.05	0.68	56.43
N3	600.00	600.00	600.00	12.28	3122.90	0.03	0.13	0.00	0.16	8.89	0.63	42.60	0.05	43.28	2404.28
N4	600.00	50.00	20.00	10.43	467.60	0.03	0.01	0.00	0.04	6.57	0.63	1.42	0.00	2.05	306.43
N5	600.00	100.00	40.00	10.47	418.60	0.03	0.02	0.00	0.05	7.37	0.63	2.84	0.01	3.48	469.88
N6	600.00	150.00	80.00	11.02	813.20	0.03	0.03	0.00	0.07	7.84	0.63	5.68	0.01	6.32	761.58
N7	600.00	200.00	120.00	12.23	1408.70	0.03	0.04	0.00	0.08	8.22	0.63	8.52	0.02	9.17	996.21
N8	600.00	250.00	160.00	12.10	1353.30	0.03	0.05	0.00	0.09	8.53	0.63	11.36	0.02	12.01	1189.02
N9	600.00	300.00	200.00	11.87	1718.50	0.03	0.06	0.00	0.10	8.79	0.63	14.20	0.02	14.85	1350.28
N10	600.00	350.00	240.00	12.15	1714.80	0.03	0.07	0.00	0.11	9.01	0.63	17.04	0.03	17.70	1487.15
N11	600.00	400.00	280.00	13.53	2032.70	0.03	0.08	0.00	0.12	9.20	0.63	19.88	0.03	20.54	1604.77
N12	600.00	450.00	320.00	12.53	1936.90	0.03	0.09	0.00	0.13	9.36	0.63	22.72	0.04	23.39	1706.94
N13	600.00	500.00	360.00	11.94	1992.40	0.03	0.11	0.00	0.14	9.51	0.63	25.56	0.04	26.23	1796.51
N14	600.00	550.00	400.00	12.53	2545.60	0.03	0.12	0.00	0.15	9.64	0.63	28.40	0.04	29.07	1875.68
N15	600.00	600.00	440.00	12.28	2305.90	0.03	0.13	0.00	0.16	9.75	0.63	31.24	0.05	31.92	1946.16
N16	600.00	650.00	480.00	13.98	2878.80	0.03	0.14	0.00	0.17	9.85	0.63	34.08	0.05	34.76	2009.31
N17	600.00	700.00	520.00	13.13	2274.40	0.03	0.15	0.00	0.18	9.95	0.63	36.92	0.06	37.61	2066.21
N18	600.00	750.00	560.00	12.63	2403.40	0.03	0.16	0.00	0.19	10.03	0.63	39.76	0.06	40.45	2117.75
N19	600.00	50.00	560.00	7.57	3575.90	0.03	0.01	0.00	0.04	3.68	0.63	39.76	0.00	40.39	3338.27
N20	600.00	100.00	520.00	8.17	3989.40	0.03	0.02	0.00	0.06	4.51	0.63	36.92	0.01	37.56	3078.45
N21	600.00	150.00	480.00	9.49	3138.60	0.03	0.03	0.00	0.07	5.32	0.63	34.08	0.01	34.72	2822.85
N22	600.00	200.00	440.00	8.89	3472.10	0.03	0.04	0.00	0.08	6.12	0.63	31.24	0.02	31.89	2571.38
N23	600.00	250.00	400.00	10.35	3063.00	0.03	0.05	0.00	0.09	6.91	0.63	28.40	0.02	29.05	2323.93
N24	600.00	300.00	360.00	11.84	2367.40	0.03	0.06	0.00	0.10	7.69	0.63	25.56	0.02	26.21	2080.40
N25	600.00	350.00	320.00	11.42	2137.90	0.03	0.07	0.00	0.11	8.45	0.63	22.72	0.03	23.38	1840.72
N26	600.00	400.00	280.00	13.04	2088.20	0.03	0.08	0.00	0.12	9.20	0.63	19.88	0.03	20.54	1604.77
N27	600.00	450.00	240.00	13.44	1503.30	0.03	0.09	0.00	0.13	9.94	0.63	17.04	0.04	17.71	1372.49
N28	600.00	500.00	200.00	13.29	1315.70	0.03	0.11	0.00	0.14	10.67	0.63	14.20	0.04	14.87	1143.78
N29	600.00	550.00	160.00	13.64	1359.00	0.03	0.12	0.00	0.15	11.38	0.63	11.36	0.04	12.03	918.56
N30	600.00	600.00	120.00	13.91	1020.00	0.03	0.13	0.00	0.16	12.09	0.63	8.52	0.05	9.20	696.75
N31	600.00	650.00	80.00	15.99	442.60	0.03	0.14	0.00	0.17	12.79	0.63	5.68	0.05	6.36	478.28
N32	600.00	700.00	40.00	16.15	507.10	0.03	0.15	0.00	0.18	13.47	0.63	2.84	0.06	3.53	263.07
N33	600.00	750.00	20.00	16.03	271.50	0.03	0.16	0.00	0.19	13.94	0.63	1.42	0.06	2.11	153.95

For the N-series, TP contributed by each component was first converted to mass using the component volume (µL) and assumed TP concentration (serum 5.58 g/dL; albumin 21 g/dL; lipid 0.1 g/dL) as: TP mass = volume × concentration / 100000. Total TP mass was obtained by summing component masses, and calculated TP (g/dL) was derived as: (total TP mass × 100000) / total volume (µL).

For the N-series, TG mass contributed by each component was calculated from its volume (µL) and assumed TG concentration (serum 104.85 mg/dL; lipid 7100 mg/dL; albumin 8 mg/dL) using TG mass (mg) = concentration × volume / 100000. Total TG mass was obtained by summing component masses, and calculated TG (mg/dL) was derived as (total TG mass / total volume) × 100000.