Research Article

Evaluating the Interference of Residual 'Teepol' Detergent on Serum Electrolytes, Protein, and Cholesterol: An In-Vitro Study

Kavindya Fernando[1,2*], Dilini Jayasekara[2], Chiranthi Welhenge[3], Mihilie Kulasinghe[2], Piumi Silva[2], BKTP Dayanath[2]

[1]*Department of Biochemistry and Clinical Chemistry, Faculty of Medicine, University of Kelaniya, Sri Lanka*
[2]*Department of Chemical Pathology, Colombo North Teaching Hospital, Ragama, Sri Lanka*
[3]*University Medical Unit, Colombo North Teaching Hospital, Ragama, Sri Lanka*

Article Info

[*]***Corresponding Author:***
Kavindya Fernando
Department of Biochemistry and Clinical Chemistry, Faculty of Medicine, University of Kelaniya, Sri Lanka
E-mail: kavindyam@kln.ac.lk
Tel: +94777907465
Address: PO BOX 06, Thalagolla Road, Ragama, Sri Lanka

Keywords

Detergent, Teepol, ISE, Interference, Electrolytes

Abstract

Introduction: In low-resource settings, reusing blood collection tubes cleaned with detergents such as 'Teepol' is common due to economic constraints. However, residual detergent may interfere with biochemical assays. This study evaluated the effect of residual 'Teepol' on serum electrolyte, total protein, and cholesterol, with emphasis on direct ion-selective electrode (ISE) methods.

Method: A controlled interference experiment was conducted using pooled human serum spiked with increasing concentrations of 'Teepol' (0%, 0.2%, 0.5%, and 1.0% v/v of original detergent). Serum sodium and potassium were measured using direct ISE (Ortho_Vitros® 4600), while total protein and cholesterol were measured via colorimetric methods (BS 800M). All analytes were tested in a single run to avoid inter-run variability. Statistical significance was assessed via Pearson correlation and comparison against 95% confidence intervals derived from quality control data.

Results: Serum sodium and potassium showed a concentration-dependent decline with increasing 'Teepol'. At 1.0%, sodium decreased by ~12% and potassium by ~43% compared to control, with values falling outside the 95% confidence intervals, confirming significant interference. Total protein and cholesterol measurements remained within expected analytical variation. Strong negative correlations were observed for sodium ($R=-0.966$) and potassium ($R=-0.989$) with 'Teepol' concentration ($p< 0.05$).

Conclusion: Residual 'Teepol' ≥0.5% v/v significantly interferes with serum sodium and potassium measurements using direct ISE. These findings highlight the importance of strict tube-washing protocols or the use of disposable tubes for critical assays. Inconsistent cleaning practices in low-resource laboratories may allow such interference, posing a risk to result accuracy and clinical decision-making.

Introduction

Laboratory testing errors can occur at the pre-analytical, analytical, or post-analytical phases, with pre-analytical errors accounting for the majority (up to ~70%) of all lab errors [1]. Among pre-analytical factors, the quality of specimen collection tubes is critical to produce accurate results. Best practices recommend using new, disposable tubes for single use to avoid contamination. However, in low-resource settings such as Sri Lanka, economic constraints have led to the reuse of sample collection tubes after cleaning, in public sector hospitals. In the aftermath of a severe economic crisis, most hospitals implemented protocols to wash and reuse tubes instead of purchasing new ones. These washed tubes are cleaned with detergents like 'Teepol'(an anionic surfactant detergent), 'Lysol' (disinfectant solution composed of cresols and alcohol), or hypochlorite solutions provided by the health sector [2]. Unfortunately, the cleaning process is not standardized, and variations in detergent concentration and rinsing can leave residual detergent in the tubes.

Clinicians at some public sector hospitals in Sri Lanka had observed cases of unexplained hyponatremia in samples collected in reused tubes, which normalized when the tests were repeated using fresh tubes. 'Teepol' was identified as the agent used to clean these tubes in almost all these situations. This raised suspicion that residual 'Teepol' might be interfering with certain assays in the reused tubes. 'Teepol' is a multi-purpose laboratory detergent composed primarily of anionic surfactants: sodium dodecylbenzene sulphonate, sodium ether sulphate, and an alcohol ethoxylate [3]. Such surfactants could plausibly affect biochemical measurements by either binding ions or interacting with assay reagents [4]. Indeed, interference is defined as the effect of a substance (identified or not) that causes the measured concentration of an analyte to deviate from its true value [5]. Sodium measurements are generally robust, and true interferents are uncommon; one known example is heparin (an anticoagulant), which can artifactually lower sodium readings by chelating sodium ions [6–8]. Other factors known to affect sodium results include the type of ion-selective electrode used, sample handling (e.g. dilution in indirect ISE methods leading to pseudohyponatremia), extreme pH or bicarbonate levels, and very high glucose or lipid concentrations [8–14]. We hypothesized that residual 'Teepol' in reused tubes could similarly interfere with direct ISE measurements of electrolytes by either binding sodium/potassium or altering the ISE membrane environment.

Limited prior literature addresses detergent residue interference in clinical chemistry. One recent study in Sri Lanka evaluated reusing tubes washed with 'Teepol', 'Lysol', or bleach according to WHO guidelines [2]; it found no significant effect of 'Teepol' residues on electrolytes (Na+, K+) or other analytes under proper washing protocols [2]. In contrast, that study noted 'Lysol' (a phenolic disinfectant) residues were associated with significantly lower sodium results, likely due to inadequate rinsing and residual contamination. However, the 'Teepol' concentration and washing conditions in that study were not fully detailed, leaving open the possibility that higher residual levels of 'Teepol' could indeed cause interference. Moreover, most government hospital laboratories in Sri Lanka do not consistently adhere to standardized washing and drying protocols due to resource limitations and reliance on manual cleaning processes. This raises concern that clinically relevant residual 'Teepol' concentrations may be more common than previously reported.

Older reports have also suggested that certain detergents can alter biochemical measurements or enzyme activities [15]. Given the ongoing practice of tube reuse in lower resource settings and the inconsistent observations in different settings, we aimed to investigate how residual 'Teepol' affects the measurement of common serum analytes quantitatively.

Methodology

This study was designed as an interference experiment to assess the impact of increasing concentrations of 'Teepol' residue on the measured levels of serum electrolytes (sodium and potassium, via direct ISE), as well as on total protein and total cholesterol (via standard colorimetric methods). By simulating worst-case residual detergent levels in vitro, we sought to determine the threshold at which 'Teepol' contamination produces statistically and clinically significant biases in results. A controlled experimental interference study was conducted in August 2023 at the Chemical Pathology Laboratory of Colombo North Teaching Hospital (Ragama, Sri Lanka). The study followed guidelines for interference testing, including the CLSI EP^{7}-A^{2} protocol [16] and Westgard rules for interference and recovery experiments [17]. No human subjects were directly involved. A pooled serum sample was prepared using anonymized, leftover specimens from routine clinical testing, which were otherwise destined for disposal.

Reagents and instruments

Concentrated 'Teepol' detergent was obtained from hospital supplies. A working 'Teepol' solution was prepared by diluting the original 'Teepol' 1:10 (v/v) with distilled water, to mimic typical cleaning dilutions. A fresh serum pool was prepared by combining leftover serum samples from healthy individuals' routine biochemistry tests, ensuring a homogeneous serum matrix. Laboratory measurements were carried out on two automated analysers: serum Na+ and K+ were measured on the Ortho Vitros® 4600 Chemistry Analyzer using direct ISE potentiometry (dry slide technology), and total protein and total cholesterol were measured on the BS 800M automated analyser (chemistry analyser) using colorimetric methods (biuret method for protein, cholesterol oxidase–peroxidase (CHOD-POD) for cholesterol). Both analysers were calibrated and quality-controlled on the day of testing, as per manufacturer guidelines.

Sample preparation

To simulate potential residual contamination when cleaning protocols are not properly followed, four test conditions were created. Aliquots of 900 µL of the pooled serum were dispensed into four new, detergent-free non-vacuum clot activator tubes. These tubes had polypropylene bodies and low-density polyethylene caps (Ceylon MediTech Instruments, Product Code 02). The working 'Teepol' solution (10% v/v 'Teepol') was then added in volumes of 0, 20, 50, and 100 µL to the four tubes, respectively. Distilled water was added as needed to each tube to bring the total volume to 1000 µL (1.0 mL). This protocol ensured each tube had a consistent 9:1 ratio of serum to added liquids, maintaining matrix consistency across all test conditions. The composition of each test tube is detailed in Table 1. Tube 1 (0 µL 'Teepol' added) served as the control (no detergent), while tubes 2–4 had progressively higher concentrations of 'Teepol'. The corresponding final fraction of original 'Teepol' (i.e., undiluted detergent) in each 1 mL sample was 0%, ~0.2%, ~0.5%, and ~1.0% for tubes 1 through 4, respectively. These concentrations were chosen to simulate worst-case residual contamination scenarios, which may occur in settings where cleaning protocols are inconsistently followed. All samples were prepared by an experienced laboratory medical officer to ensure precise pipetting and handling. After preparation, samples were analysed in a single run per analyte to avoid inter-run variability.

Table 1: Composition of the different test tubes used for the experiment.

Tube	Pooled sera (µL)	'Teepol' 10% added v/v (µL)	Distilled water (µL)	Total Volume (µL)	Original 'Teepol' in Sample (%)
Tube 1	900	0	100	1000	0%
Tube 2	900	20	80	1000	~0.2%
Tube 3	900	50	50	1000	~0.5%
Tube 4	900	100	0	1000	~1.0%

Preparation of 1 mL serum samples with increasing volumes of 10% v/v 'Teepol' working solution added. "Original 'Teepol' in sample" is the approximate percentage of undiluted detergent in the final mixture (e.g., 0.5% = 1:200 dilution of original 'Teepol'). All tubes had a 9:1 ratio of serum to added solution, maintaining matrix consistency.

Analyte measurement

The four prepared samples were first analysed for electrolytes (Na+ and K+) on direct ISE system, alongside routine patient samples. Immediately thereafter, the same samples were analysed for total protein and total cholesterol. Internal quality control (QC) data for both instruments were reviewed to ensure analytical precision. Table 2 summarizes the performance of each assay at the relevant QC level (level 1 controls approximating physiological concentrations). The coefficients of variation (CV%) were all under 4% for these analytes, indicating good analytical precision. This QC data was later used to evaluate whether any observed changes fell beyond normal analytical variation.

Table 2: Analyzer performance for relevant analytes (quality control level 1).

Analyte	QC level	Mean	Coefficient of variation (CV, %)
Serum sodium	1	125 mmol/L	3.4
Serum potassium	1	3.94 mmol/L	3.8
Serum total protein	1	7.19 g/dL	3.03
Serum total cholesterol	1	104 mg/dL	3.25

Instrument quality control data (mean and CV%) at normal concentration (level 1) for each analyte. These values were used to calculate the 95% confidence intervals of each measurement. The CV% values were derived from internal quality control (IQC) data obtained over the preceding 30 days as part of routine analyzer performance monitoring.

Statistical Analysis

Data from the four test tubes were analysed to determine the relationship between 'Teepol' concentration and analyte levels. We plotted the measured concentration of each analyte against the fraction of 'Teepol' in the sample. A linear regression analysis was performed for each analyte; Pearson correlation coefficients (R) were calculated to assess the strength and significance of association between increasing 'Teepol' and the analyte result. Given the small number of points (n=4), a Pearson's correlation test was used to identify statistically significant trends (with $p<0.05$ was considered significant). Additionally, to evaluate whether changes in results exceeded normal analytical variability, we calculated the 95% confidence interval (95% CI) around the control tube's result for each analyte based on the instrument's precision. Specifically, using the CV% from Table 2, we estimated one standard deviation (SD) at the concentration of Tube 1, then multiplied by 1.96 to get the 95% CI range (mean ±1.96 SD). Any measured

value in the 'Teepol'-added tubes falling outside this range would indicate a change greater than expected from analytical uncertainty alone (i.e., a likely true interference effect).
For example, for sodium, Tube 1's result (128 mmol/L) with CV 3.4% yields approximately ±8.5 mmol/L as the 95% confidence limits; results below ~119.5 mmol/L or above ~136.5 mmol/L would thus be considered significantly different at ~95% confidence. This approach was applied similarly to potassium, total protein and cholesterol. The percentage interference (bias) for each analyte was calculated using the following equation.
Interference (bias) = (([analyte in test sample] - [analyte in control sample])/[analyte in control sample]) x 100%
In addition to internal quality control-based confidence intervals, Analytical Performance Specifications (APS) from the Royal College of Pathologists of Australasia (RCPA QAP) were also reviewed for sodium and potassium to assess whether observed differences exceeded clinically acceptable performance limits.

Results

The measured results for each analyte in the four test conditions are presented in Table 3. As the volume of 'Teepol' in the sample increased, serum Na+ and K+ concentrations showed a progressive decline. The control sample (Tube 1, no 'Teepol') had a sodium concentration of 128 mmol/L and potassium concentration of 4.4 mmol/L. At the highest 'Teepol' level (Tube 4, 1% original 'Teepol'), sodium dropped to 112 mmol/L and potassium to 2.5 mmol/L. In contrast, total protein concentrations remained relatively stable (around ~6.0 g/dL in all tubes, with no clear trend). Total cholesterol showed a slight decreasing trend (from 162 mg/dL in Tube 1 down to approximately 157 mg/dL in Tube 4), but the magnitude of change was small (approximately 3% decrease across the full 'Teepol' range).

To determine if these changes are significant, we compared the values against the calculated 95% CIs based on analytical precision (Table 4). For sodium, the result in Tube 1 (128 mmol/L) had a 95% CI of approximately 119.5 – 136.5

Table 3: Measured serum analyte levels in samples with increasing Teepol contamination.

Analyte	Method	Test tube 1 (0% 'Teepol')	Test tube 2 (0.2% 'Teepol')	Test tube 3 (0.5% 'Teepol')	Test tube 4 (1.0% 'Teepol')
Serum sodium (mmol/L)	Direct ISE	128	128	118	112
Serum potassium (mmol/L)	Direct ISE	4.4	4.2	3.7	2.5
Total protein (g/dL)	Biuret	5.98	6.02	6.07	5.91
Total cholesterol (mg/dL)	CHOD-POD	162	161	160	156.9

Serum analyte results in each test tube (Tube 1: control with no 'Teepol'; Tubes 2–4: increasing 'Teepol' contamination as per Table 1). CHOD-POD method: Cholesterol oxidase peroxidase method, ISE: Ion selective electrode

mmol/L. The sodium concentrations in Tube 3 (118 mmol/L) and Tube 4 (112 mmol/L) fall below the lower confidence limit, indicating that these decreases were greater than expected from random instrument error alone. Similarly, for potassium, Tube 1's value of 4.4 mmol/L had a 95% CI of approximately 4.1 – 4.7 mmol/L. The potassium concentrations in Tube 3 (3.7 mmol/L) and Tube 4 (2.5 mmol/L) were well below the 95% lower limit, confirming a true significant drop. In contrast, the slight variations in total protein (95% CI proximately 5.6 – 6.3 g/dL) and total cholesterol (95% CI approximately 151.7 – 172.3 mg/dL) remained within the expected range for all tubes. Thus, 'Teepol' contamination at concentrations of 0.5% or higher resulted in statistically significant decreases in measured Na+ and K+, while changes in protein and cholesterol were not statistically significant.
In addition to comparing results against 95% confidence intervals derived from internal quality control data, we also considered published Analytical Performance Specifications (APS) from external Quality Assurance Programs such as the Royal College of Pathologists of Australasia (RCPA QAP). For serum sodium (≤ 150 mmol/L), the allowable total error is ±3 mmol/L, and for potassium (≤ 4.0 mmol/L), the allowable imprecision is ±0.2 mmol/L [18]. In our study, the observed declines in sodium (up to 16 mmol/L) and potassium (up to 1.9 mmol/L) with increasing 'Teepol' concentration substantially exceeded these APS thresholds. This supports the conclusion that the observed changes are not only statistically significant but also clinically unacceptable, reinforcing the potential impact of detergent residue on critical electrolyte measurements.

Table 4: Baseline values and 95% confidence intervals (CI) for each analyte.

	Tube 1 (control) value	95% CI range (Analytical)	Presence of a significant change
Serum Sodium (Na+)	128 mmol/L	119.5 – 136.5 mmol/L	Yes – Tubes 3 & 4 below 119.5
Serum potassium (K+)	4.4 mmol/L	4.1 – 4.7 mmol/L	Yes – Tubes 3 & 4 below 4.1
Total protein	5.98 g/dL	5.6 – 6.3 g/dL	No (all within range)
Total cholesterol	162 mg/dL	151.7 – 172.3 mg/dL	No (all within range)

Baseline = Tube 1 (no 'Teepol') result. 95% CI represents the expected range of variation due to analytical uncertainty (calculated from CV in Table 2). "Significant change observed" indicates whether any 'Teepol'-added sample fell outside the 95% CI, suggesting a true interference effect.

Statistical analysis confirmed these observations. Pearson correlation analysis showed a strong negative correlation between 'Teepol' concentration and the levels of Na+, K+, and cholesterol. The correlation coefficients were R = –0.966 for sodium, –0.989 for potassium, and –0.992 for cholesterol (all with $p<0.05$), indicating that as 'Teepol' percentage increased, these analyte values consistently decreased in a nearly linear fashion. In contrast, the correlation between 'Teepol' and total protein was weak and not statistically significant (R = –0.482, p=0.52), reflecting essentially no meaningful trend in protein values with added detergent. The strong linear relationship for sodium and potassium is illustrated in the figures below (Figure 1).

Figure 1: changes of analyte concentration with increasing 'Teepol' concentration; 1a; serum sodium vs 'Teepol', 1b; serum potassium vs 'Teepol', 1c; total protein vs 'Teepol', 1d; total cholesterol vs 'Teepol'. CI: confidence interval.

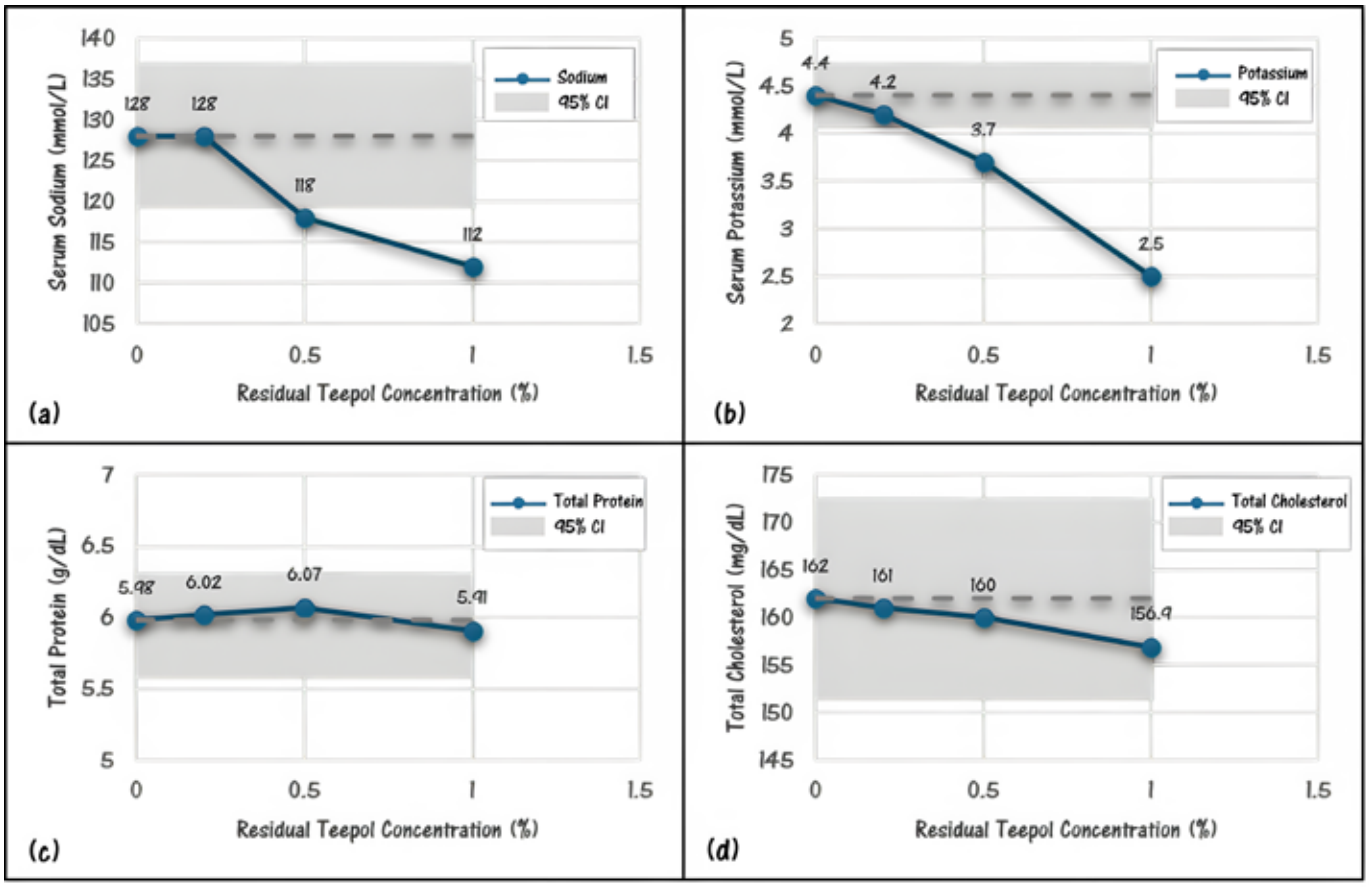

Figure 1a: Relationship between residual 'Teepol' in sample and serum sodium concentration. Each point represents one test tube ('Teepol' fraction 0%, 0.2%, 0.5%, 1.0%). Sodium concentrations remained stable up to ~0.2% 'Teepol', then showed a marked decline at higher 'Teepol' levels. The dashed grey line indicates the control value; values falling below the shaded area (analytical 95% CI) signify a significant decrease beyond normal instrument variation (seen at ≥0.5% 'Teepol'). This shows that high 'Teepol' residue leads to spuriously low sodium readings.

Figure 1b: Serum potassium vs residual 'Teepol' concentration. A strong inverse linear trend is evident, with K+ dropping progressively as 'Teepol' increases. Even a small 'Teepol' residue (~0.2%) caused a slight K+ decrease, and larger amounts (0.5–1.0%) led to clinically large decreases (from 4.4 to 2.5 mmol/L at 1.0% 'Teepol'). The potassium in tubes with ≥0.5% 'Teepol' was significantly below the control 95% CI, confirming a true interference effect on K+ measurement.

Figure 1c: Total protein vs residual 'Teepol' concentration. In contrast to electrolytes, total protein results did not show a meaningful change with added 'Teepol'. The slight fluctuations observed (5.98 to 6.07 to 5.91 g/dL) were within the analytical error range. There was no significant correlation between 'Teepol' level and protein concentration, suggesting 'Teepol' did not interfere with the biuret protein assay up to 1.0% contamination.

Figure 1d: Total cholesterol vs residual 'Teepol' concentration. Cholesterol values showed a minor downward trend with higher 'Teepol' (162 → 157 mg/dL over the range), but all values remained within the expected variability range. The correlation with 'Teepol', while mathematically high, reflects a very small absolute change. Thus, no significant interference of 'Teepol, on the cholesterol CHOD-POD assay was evident up to 1.0% contamination.

These experimental results demonstrate a clear interference effect of 'Teepol' on electrolyte measurements. When the residual original 'Teepol' content exceeded roughly 0.5% of the sample volume (≥ 50 µL of 10% 'Teepol' in 1 mL serum), the measured sodium and potassium values were significantly depressed beyond normal error limits. At the highest tested contamination (1% 'Teepol'), sodium was lowered by ~12% and potassium by ~43% relative to the control, which would be clinically significant reductions. Meanwhile, total protein and cholesterol tests were essentially unaffected by 'Teepol' at these levels, suggesting that the interference is specific to certain types of assays (particularly the direct ISE method for ions).

Discussion

Our investigation confirms that detergent residues can markedly interfere with electrolyte results obtained by direct ISE. Specifically, when residual 'Teepol' (an anionic surfactant detergent) in the sample tube exceeded about 50 µL of a 10% solution (equivalent to 0.5% v/v of the original detergent in the sample), we observed significant artefactual reductions in measured serum Na+ and K+ levels. This finding has important implications for clinical laboratories that practice tube reuse. Accurate Na+ and K+ measurements are critical for patient care, as even moderate errors can alter clinical management decisions. If unrecognized, falsely low electrolyte results due to detergent interference could lead to misdiagnosis or unnecessary interventions, adversely impacting patient safety.

Mechanism of Interference

The results indicate a negative bias introduced by 'Teepol' on the ISE measurements of Na+ and K+. There are several possible mechanisms by which a surfactant like 'Teepol' could cause this effect. ISEs generate a potential in response to the activity of specific ions (Na+, K+) in the sample via a selective membrane, as described by the Nernst equation [19]. Surfactants such as the components of 'Teepol' (e.g. sodium dodecylbenzene sulfonate, analogous to sodium dodecyl sulphate) could interfere by binding free cations or altering their activity [20]. Another hypothesis is that the detergent's anionic molecules chelate or sequester sodium and potassium ions, reducing the "free" ion activity that the electrode senses [21]. This is analogous to the effect of excess heparin (another polyanion) on sodium measurement, where sodium ions are bound by heparin, causing spuriously low readings. In our study, 'Teepol' may similarly be binding some fraction of Na+ and K+ in complexes or micelles, effectively lowering the ion activity in the plasma water phase.

Another contributing factor could be physical interference at the electrode membrane. Surfactants are known to adsorb to surfaces [22,23]. 'Teepol' residue in the sample might coat the ISE membrane or alter its surface charge, disrupting the normal ion-selective potential [11,24]. The Ortho Vitros® 4600 analyser uses a dry-slide ISE technology where the sample contacts selective dry reagents – residual detergent could disrupt this interaction [25]. Surfactant adsorption can lead to an electrode response drift or a reduced slope (sensitivity), manifesting as lower reported concentrations [24,26]. In essence, the electrode may become less responsive to Na+ and K+ in the presence of detergent. Some research in electrochemistry has addressed surfactant interference in potentiometric measurements, and even special "surfactant-resistant" electrodes have been devised to mitigate this issue [27,28], underscoring that surfactants are recognized interferents in electrochemical ion detection. It is also notable that 'Teepol' introduces its own sodium ions (being a sodium salt detergent); however, in our results we did not see any increase in sodium. Instead a decrease occurred, supporting the notion that the dominant effect is not additive sodium from the detergent but rather an interference lowering the measured activity.

Specificity to ISE vs Colorimetric Assays

An important observation is that total protein and cholesterol were not significantly affected by 'Teepol' residue in our experiment. Both of these analytes were measured by colorimetric methods (biuret for protein, enzymatic CHOD-POD for cholesterol) which involve dilution of the sample with reagent and end-point spectrophotometric detection. There are a few hypothetical reasons why these assays proved resistant to 'Teepol' interference. First, the effective concentration of 'Teepol' in the reaction mixtures for protein and cholesterol was likely very low – automated analysers typically dilute sample or include reagents that could further neutralize or dilute contaminants [29]. Any minor surfactant present might have been rendered negligible by the assay reagents.

Second, the chemistry of these assays may not be impacted by surfactant at these levels; for instance, the biuret reaction for protein (binding of Cu^{2+} to peptide bonds) and the enzymatic oxidation of cholesterol might tolerate a small amount of detergent without change in absorbance.

Our data showed only random small fluctuations within the analytical error for these tests. This contrast with the ISE results highlights that 'Teepol's' interference is method-dependent – it chiefly perturbs electrochemical detection of ions, whereas routine photometric assays for large molecules (protein, cholesterol) appear robust under the tested conditions. This is reassuring in that not all tests are compromised by tube residues, emphasizes that electrolyte tests are especially vulnerable.

Our findings align with and extend, those of Jayarathna et al. (2021) who examined detergent washing of tubes. In their study, 'Teepol'-washed tubes (when properly rinsed per WHO guidelines) did not significantly alter Na+ or K+ results [2], whereas Lysol (a phenolic detergent) did cause a drop in sodium. They did not report 'Teepol's' exact residual concentration; likely it was low due to good rinsing. Our study deliberately pushed the envelope by leaving higher residual levels, thereby defining the threshold where interference becomes significant. We demonstrate that at roughly >1:200 dilution of original 'Teepol' (0.5%), interference emerges, a scenario that could occur in practice if tubes are inadequately rinsed or if overly concentrated detergent is used for washing. Thus, while 'Teepol' is safe when properly rinsed, improper use can indeed cause clinically important errors.

While Jayarathna et al. followed a standardized WHO-recommended protocol that included overnight soaking, multiple rinses with tap and distilled water, and oven drying, such procedures are often not followed in Sri Lankan public sector hospitals due to limited resources, staff shortages, and absence of national guidelines. In many laboratories, specimen tubes are manually cleaned by minor staff with inconsistent rinsing and drying steps. These deviations increase the likelihood that residual Teepol concentrations approaching or exceeding 0.5% could remain in reused tubes. Our simulation of such worst-case conditions helps define the point at which interference becomes clinically meaningful. Additionally, anecdotal cases of unexplained hyponatraemia that normalized when samples were recollected in fresh tubes further support the plausibility of this interference in real-world practice.

In addition to analytical concerns, it is important to consider the practical and economic implications of tube reuse. Although washing and reusing glass tubes may appear cost saving, the hidden costs including labor, detergent, water usage, staff time, risk of injury from broken glass, and the potential for analytical errors due to residual contamination can outweigh the upfront cost of using single use plastic tubes. These indirect costs are rarely quantified in laboratory budgets. Therefore, formal cost benefit analyses comparing reused and disposable tubes are recommended to guide national laboratory procurement policies, particularly in resource limited settings.

Interestingly, earlier literature on detergent effects documented how even low concentrations (~0.1%) of certain detergents (like Triton X-100, a non-ionic surfactant) could inhibit enzymatic activities by affecting membrane proteins [30]. This concept of detergent perturbing biological or measurement systems is consistent with our findings on ISE interference. In a sense, the ISE membrane in our case can be thought of as analogous to a biological membrane whose function (ion selectivity) is disrupted by the surfactant. Moreover, a recent review on ion-selective electrodes emphasizes that while ISEs are highly selective, they are not free of interferences, and laboratories must be aware of substances that can skew results [11]. Our study identifies 'Teepol' residue as one such interferent in the context of reused collection tubes.

Clinical Implications

The magnitude of sodium and potassium depression observed at higher 'Teepol' contamination could have serious clinical ramifications. For example, in Tube 4 (simulating a badly rinsed tube), sodium was 112 mmol/L vs 128 mmol/L true value – these 16 mmol/L drops could classify a patient as moderately hyponatraemic when they are actually normonatraemic. Likewise, potassium dropping from 4.4 to 2.5 mmol/L could lead to a false diagnosis of severe hypokalaemia, potentially prompting unnecessary potassium supplementation or cardiac monitoring. Such pseudohyponatremia or pseudohypokalaemia due to tube contamination is particularly dangerous because clinicians might act on these spurious results. In settings where tube reuse is practiced, our findings stress the need for vigilance. If an unexpectedly low electrolyte result is obtained, one should consider the possibility of detergent interference (especially if other clinical or lab clues don't align) and perhaps repeat the test with a fresh tube sample before initiating any aggressive treatment. It is notable that in the anecdotal hospital reports, repeating the test in a new tube corrected the values, highlighting an approach to identify this issue in real time.

For laboratories, the clear message is that standardizing tube washing protocols is essential. 'Teepol' itself is an effective cleaner, but it must be thoroughly rinsed out. Standard Operating Procedures (SOPs) should dictate the proper dilution of 'Teepol' for washing (such as using a measured mild concentration), and a sufficient rinse with water (preferably multiple rinses) to ensure no significant residue remains. Drying of tubes after washing is also important since pooling of residual wash solution could carry detergent into the next sample [31]. We recommend that labs periodically test a batch of cleaned tubes by filling them with saline and measuring any residual effect on electrolyte readings as a form of quality assurance for the cleaning process. In critical care settings or for critical analytes like electrolytes, the safest practice remains to use new disposable tubes whenever possible [2]. Indeed, Jayarathna et al. concluded that while properly washed

reused tubes generally did not affect most tests, new tubes are still recommended for critical investigations such as serum electrolytes to avoid even the rare chance of interference.

Limitations

This study has some limitations. Firstly, the experiment was conducted using a single pooled serum sample, tested in singleton at each 'Teepol' concentration. While the observed changes were large enough to suggest meaningful interference, this approach limits statistical robustness and prevents evaluation of biological variability. We did not perform replicates due to resource constraints. Future studies should aim to include multiple independent serum pools and replicate measurements to better establish the reproducibility and generalizability of the interference effects.

Secondly, the sample testing was performed in a fixed sequence, from control to the highest 'Teepol' concentration. We did not randomize sample order, which may introduce potential bias. Future work should randomize the testing order and investigate whether order effects or matrix interactions influence results.

Thirdly, we only examined four discrete levels of 'Teepol'. The exact "breakpoint" of interference might lie somewhere between our tested concentrations; a finer concentration gradient (e.g. 0.1%, 0.2%, 0.5%, 1.0%) or testing the maximum tolerable residue without interference would allow better interpolation of the threshold and should be considered in future work.

Fourth, Pearson's correlation was used to explore the relationship between 'Teepol' concentration and analyte values. However, with only four data points, the reliability of these statistical estimates is limited. While the trends were visually compelling, we caution readers against over-interpreting the correlation coefficients and p-values. Future studies with repeated measurements and larger datasets should employ more robust statistical approaches, including non-parametric or regression-based models. Lastly, all samples were intentionally diluted by 10% (including the control) to maintain equal volume after adding interferent, following CLSI EP^{7}-A^{2} recommendations. This dilution lowered baseline sodium concentrations (e.g., 128 mmol/L in control), potentially affecting generalizability. However, since all samples were diluted equally, relative comparisons remain valid. The observed percentage declines in sodium and potassium remain clinically meaningful, though extrapolation to undiluted clinical samples should be approached with caution.

We also note that this study focused exclusively on 'Teepol'. Other cleaning agents, such as phenolic disinfectants may exhibit different interference profiles and thresholds, which were beyond our scope.

Conclusion

'Teepol' detergent residue in reused sample collection tubes can significantly interfere with serum Na+ and K+ measurements by direct ISE, particularly when the residual volume 10% 'Teepol' exceeds 50 µL per 1 mL of serum (≥0.5% of sample volume). This can lead to falsely low electrolyte results and potential misdiagnoses such as hyponatremia or hypokalaemia. By contrast, total protein or cholesterol measured by colorimetric methods remain unaffected, suggesting assay-specific interference. These findings highlight the need for standardized cleaning protocols, including well-defined maximum allowable 'Teepol' residues. Where possible, new tubes should be used for critical tests. Clinical laboratories must remain vigilant about pre-analytical factors like tube contamination to ensure accurate results and safeguard patient care.

Declarations

Conflict of interest

The authors declare no conflict of interests.

Author contributions

Kavindya Fernando: Conceptualization, Methodology, Writing- Original draft preparation, Creating, graphs and figures, Investigation. Dilini Jayasekara: Conceptualization, Methodology, Writing- Reviewing and Editing. Chiranthi Welhenge: Writing- Reviewing and Editing, Data curation. Mihilie Kulasinghe and Piumi Silva: Writing-Reviewing, BKTP Dayanath: Supervision.

Ethical approval

There was no involvement of any direct human or animal subjects in any experiments in the study.

Acknowledgements

The authors would like to express their sincere gratitude to the staff of the Chemical Pathology Laboratory at Colombo North Teaching Hospital (CNTH) for their invaluable support during the experimental work. We also extend our special thanks to Mr. P.G. Kalana Bandara for his assistance in editing and preparing the figures included in this manuscript.

Data availability statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request. All data were generated from anonymized pooled serum samples used solely for the purpose of this laboratory-based experimental study, with no direct patient identifiers involved.

Research Funding

None received.

Consent for Publication

Consent to submit has been received explicitly from all co-authors, as well as from the responsible authorities. Authors whose names appear on the submission have contributed sufficiently to the scientific work and therefore share collective responsibility and accountability for the results.

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