



# Impact of Time Delay in the Analysis of Serum Ionized Calcium, Sodium, and Potassium

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## Abstract

**Introduction** Delay in the analysis of serum electrolytes along with clot contact time can lead to difference in results significant enough to affect clinical decisions. This study was undertaken to evaluate the effect of time lag between centrifugation and analysis on levels of serum sodium, potassium, and ionized calcium in a tertiary level health care set up.

**Materials and Methods** In this cross-sectional study, 70 serum samples were analyzed for ionized calcium, sodium, and potassium under different conditions with respect to time lag and clot contact time. The analysis of ionized calcium was done on Eschweiler Combiline 2, a direct ion-selective electrode (ISE) analyzer. Serum sodium and potassium were analyzed on fully automated chemistry analyzer, which is an indirect ISE analyzer. The statistical analysis was done in IBM SPSS software version 21.

**Results** The results for intergroup comparison with different time lag and clot contact time between all the four groups for sodium, potassium, and ionized calcium were statistically significant, as obtained by application of Kruskal–Wallis test. There was consistent decrease in the concentration of sodium and ionized calcium, and an increase in serum potassium with increased delay in analysis and clot contact time.

**Conclusion** The accurate measurement of electrolytes is of paramount importance for the treatment and better prognosis of critically ill patients. This can be accomplished by better management of the preanalytical phase of analysis by maintaining a standard protocol in the laboratory and sample transportation.

## Keywords

- ▶ time delay
- ▶ clot contact
- ▶ ionized calcium
- ▶ sodium
- ▶ potassium

## Introduction

The analysis of electrolytes (sodium, potassium, chloride, and ionized calcium) forms a major decisive step in the diagnosis and management of the critically ill. Electrolyte abnormalities can precipitate life-threatening events by derangements in its metabolic process or as a consequence

of an underlying disease. It is a general recommendation to complete the analysis of electrolytes as soon as possible, failing which the results can be inaccurate and unreliable.

Testing in a laboratory is a complex interrelated process, involving preanalytical, analytical, and postanalytical phase and each phase is prone to error, thus affecting the final

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result.<sup>1</sup> The preanalytical phase is most prone to error mounting up to 46 to 68.2% which is almost two-thirds of the whole process. The errors in the other phases have been minimized by automation and laboratory information systems.<sup>2</sup>

Sodium and potassium estimation forms an important part of the investigation of diagnoses like diarrhea, kidney failure, Addison's disease, brain injury, diabetic coma, cardiac arrhythmia, muscular weakness, and hepatic encephalopathy.<sup>1</sup> Estimation of sodium and potassium is very sensitive to change in temperature, delayed centrifugation, and period of contact with clot.<sup>3</sup>

Ionized calcium which is the free form of calcium, plays a major role in the body and is very finely regulated between a range of 1.1 and 1.35 mmol/L, given its potentially severe toxicity outside this physiological range. Routinely it was the total calcium that was estimated but recent evidence has emerged in the favor of estimation of ionized calcium. The measurement of ionized calcium requires strict sample handling and a watchful preanalytical phase.<sup>4</sup>

Several studies have been conducted to study the stability of biochemical analytes in varying conditions of temperature, time, and period of clot contact time.<sup>1,5-7</sup>

There is still no standard defined for the stability limits of analytes and the criteria for rejection before processing them due to the lack of standard experimental designs and wide variability in maximum permissible instability specifications.<sup>3</sup>

In a tropical country like India where the ambient temperature can go very high, maintaining the stability of a sample if not processed immediately can be a big deterrent in the accuracy of the result. Also, situations like instrument breakdown, power failure, shortage of manpower, lack of awareness about the sample stability, and casual behavior of the technical staff, some tests being performed later can make the situation even worse. In such situations, the samples are left unprocessed with the sera being unseparated from the clot or they are stored in the refrigerator within a temperature range of 2 to 8°C. The measurement of electrolytes at this point may not be accurate then, leading to a spurious rise or fall.

With this background, this study was conducted to evaluate the impact of time delay and clot contact time of serum in the analysis of serum sodium, potassium, and ionized calcium.

## Materials and Methods

This cross-sectional study was undertaken in the Clinical Biochemistry Laboratory, All India Institute of Medical Sciences (AIIMS), Bhubaneswar, Odisha between October and December 2018.

Seventy serum samples from the inpatient department were analyzed for sodium, potassium, and ionized calcium. They were received in the red top vacutainers with clot activator (BD Vacutainer, SST II Advance tubes). The samples were drawn by trained nursing staff from the antecubital vein under all aseptic precautions after obtaining informed consent from all the patients. The tubes were filled up to the

desired mark as labeled and allowed to stand for 30 minutes for proper clotting and then transported to the Clinical Biochemistry Laboratory. The samples were centrifuged at 3,500 revolutions per second for 10 minutes on reception. Samples with any grade of hemolysis (using the visual scale of hemolysis) were excluded from the study.<sup>8</sup>

The analysis of sodium, potassium, and ionized calcium was done in the following manner:

Group 1: samples were processed immediately and analyzed within 1 hour-T1.

Group 2: analysis done at 4 hours, samples kept at room temperature (24–26°C), uncovered, and without sera separation from clot-T2.

Group 3: analysis done at 24 hours, samples kept in the same vacutainer as received, covered, and without sera separation from clot at 2 to 8°C-T3.

Group 4: analysis done at 24 hours, sera separated from the clot in microcentrifuge tubes and kept at 2 to 8°C-T4.

The idea behind such a type of allocation of groups was to simulate conditions that can occur in a laboratory with a large sample load. The samples are usually analyzed immediately or kept after centrifugation at room temperature due to some other technical reasons like instrument failure or power outage, absence of a backup analyzer, error in sample handling by technical staff, tests being missed due to bar code error, etc.

Serum sodium and potassium were analyzed on Beckman Coulter AU 5800 chemistry analyzer, which is an indirect ion-selective electrode (ISE) analyzer. Quality control was maintained by using Bio-Rad internal quality control samples daily and external quality control by monthly samples from the Association of Clinical Biochemists of India prepared by Christian Medical College (CMC), Vellore, Tamil Nadu, India.

The analysis of ionized calcium (IC) was done on Eschweiler Combiline 2, a direct ISE analyzer, which performs an autocalibration every 90 minutes, and quality control was maintained using the controls provided by the manufacturer and Bio-Rad internal quality control samples daily.

During the study period, the % coefficient of variation of the parameters serum sodium, potassium, and IC was within the target and satisfactory.

The study was approved by the Institutional Ethics Committee, AIIMS, Bhubaneswar, Odisha, India.

## Statistical Analysis

It was done on IBM SPSS software version 21.

All data were subjected to Shapiro–Wilk test to assess the distribution of data. They were accordingly presented as either mean or standard deviation in the case of parametric data or median and interquartile range (IQR) in the case of nonparametric data.

Kruskal–Wallis test and Tukey's honestly significant difference post hoc test were used as required for analysis. The level of significance was expressed as a *p*-value, and a *p*-value less than 0.05 was considered as significant.

**Table 1** Intergroup comparison of sodium, potassium, and ionized calcium

Sl. No.	Parameter (mmol/L)	T1 Median (IQR)	T2 Median (IQR)	T3 Median (IQR)	T4 Median (IQR)	Kruskal–Wallis test
1	Sodium	139.00 (136–142)	138.00 (135–141)	132.00 (126–138)	134.00 (129–139)	0.000
2	Potassium	4.20 (4.15–4.25)	4.35 (4.30–4.40)	9.15 (6.60–11.7)	4.5 (4.0–5.0)	0.000
3	Ionized calcium	1.03 (0.91–1.15)	0.975 (0.855–1.095)	0.925 (0.875–0.975)	0.935 (0.885–0.985)	0.000

Abbreviation: IQR, interquartile range.

## Results

The data obtained after statistical analysis for sodium, potassium, and IC was not normally distributed (Shapiro–Wilk  $p$ -value < 0.001), thus it was represented as median and IQR.

The results for intergroup comparison between all the four groups for sodium, potassium, and IC were statistically significant, as obtained by application of the Kruskal–Wallis test (► **Table 1**). There was a consistent decrease in the concentration of sodium, IC, and an increase in serum potassium with time.

On further analysis, it was observed that the concentration of sodium decreases with time. The difference was not significant at 4 hours but it was statistically significant beyond that (► **Table 2**).

The increase in the concentration of potassium was not significant at 4 hours, but at 24 hours it was highly significant in the samples stored as such in the vacutainer, signifying the effect of clot contact. The difference in the concentration between T2 and T4 was also not significant, directing toward the importance of proper storage of samples if not analyzed immediately (► **Table 3**).

The decrease in IC was statistically significant when T1 was compared with T2, T3, and T4, but the decrease in concentration when compared with other test groups was not significant (► **Table 4**).

## Discussion

This study shows that with an increased delay period in analysis and clot contact time, the concentration of serum

**Table 2** Tukey's HSD post hoc test matrix for intergroup comparison of sodium

Mean difference ( $p$ -value)	T1	T2	T3	T4
T1	–	2.143 (0.013)	8.100 (0.000)	4.086 (0.000)
T2	–2.143 (0.013)	–	5.957 (0.000)	1.943 (0.030)
T3	–8.100 (0.000)	–5.957 (0.000)	–	–4.014 (0.000)
T4	–4.086 (0.000)	–1.943 (0.030)	4.014 (0.000)	–

Abbreviation: HSD, honestly significant difference.

**Table 3** Tukey's HSD post hoc test matrix for intergroup comparison of potassium

Mean difference ( $p$ -value)	T1	T2	T3	T4
T1	–	–0.196 (0.792)	–5.652 (0.000)	–0.312 (0.460)
T2	0.196 (0.792)	–	–5.458 (0.000)	–0.115 (0.949)
T3	5.652 (0.000)	5.455 (0.000)	–	5.340 (0.000)
T4	0.312 (0.460)	0.115 (0.949)	–5.340 (0.000)	–

Abbreviation: HSD, honestly significant difference.

sodium and IC decreases whereas that of serum potassium increases.

It is a general recommendation that electrolytes should be analyzed as soon as possible. Samples that are left without serum separation at room temperature are subjected to evaporation and failure of maintenance of sodium potassium pump leading to potassium leak from the cells. This leak is more pronounced at low temperatures at around 4°C. A phenomenon called seasonal pseudohyperkalemia was documented where hyperkalemia was more frequent in winters when ambient transport temperature was lower than in summers.<sup>9</sup> We found that the difference in concentration of potassium is not significant after 4 hours, even when the sample is kept at room temperature without serum separation, but at 24 hours the effect of both clot contact time and the low temperature raises the level significantly. Baruah et al reported that potassium levels are altered even at 1 hour and sodium at 3 hours after separation of serum from clot.<sup>1</sup>

Zhang et al found that sodium is stable following different storage conditions and clot contact but for potassium, the serum should be separated within 3 hours following collection for stability.<sup>10</sup> In a study by Kachhawa et al serum potassium and potassium was stable up to 30 days, when sera are separated within 30 minutes after centrifugation.<sup>11</sup> We found that there is no significant change in sodium till 4 hours but it decreased significantly at 24 hours which is not consistent with the above studies.<sup>10,11</sup> Failure of sodium potassium pump can attribute to this change, as sodium finds a way intracellularly due to the concentration gradient.

**Table 4** Tukey's HSD post hoc test matrix for intergroup comparison of ionized calcium

Mean difference (p-value)	T1	T2	T3	T4
T1	–	0.081 (0.000)	0.118 (0.000)	0.0951 (0.000)
T2	–0.081 (0.000)	–	0.036 (0.225)	0.014 (0.890)
T3	–0.118 (0.000)	–0.037 (0.225)	–	–0.229 (0.630)
T4	–0.095 (0.000)	–0.014 (0.890)	0.023 (0.630)	–

Abbreviation: HSD, honestly significant difference.

In this study, the concentration of IC decreased significantly with increased clot contact time and delayed analysis. While the decrease is significant after 4 hours as the serum has not been separated which is not the case at 24 hours with serum separation and proper storage. After sample collection, there are various factors that influence the IC concentration like increase in pH due to loss of carbon dioxide during clotting, increase in lactate concentration due to continuous cell metabolism, and the concentration of protein. The increase in pH leads to increased binding of IC to anions and proteins thus decreasing the level.<sup>12–14</sup> Perović et al had reported similar results, where there was a significant decrease in IC when the analysis was performed 90 to 100 minutes postcentrifugation and without serum separation due to continued cell metabolism. They also found that delayed centrifugation of the samples leads to a significant decrease in IC.<sup>15</sup>

## Conclusion

The accurate measurement of electrolytes is of paramount importance for treatment and better prognosis of critically ill patients. This can be accomplished by better management of the preanalytical phase of analysis by maintaining a standard protocol in the laboratory and sample transportation.

### Ethical Approval

The study has been approved by the Institutional Ethics Committee, AIIMS, Bhubaneswar (T/IM-NF/Biochem/18/04).

### Authors' Contributions

P.D. and R.T. were responsible for substantial contributions to the conception and design of the work, case selection, sample collection, analysis, storage, and interpretation of data along with patient interaction and consent taking. S.N. was instrumental in statistical analysis of the data and their correct interpretation. M.M. was meticulously involved in the drafting of the work, revising it critically for important intellectual content, and final approval of the version to be published.

### Conflict of Interest

None declared.

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