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**ORIGINAL RESEARCH**

# Effect of serum clot contact time –A preanalytical variation on serum electrolytes

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## ABSTRACT

**Background:** The preanalytical phase is critical in laboratory testing, accounting for 46-68.2% of errors. One key factor affecting serum electrolyte levels is the serum clot contact time, which is the time between blood collection and separation of serum. This study evaluates the effect of delayed centrifugation and analysis on sodium, potassium, and chloride stability **Objectives:** To evaluate time lag between centrifugation and sample analysis on stability of sodium, potassium and chloride in serum. **Methods:** Fifty serum samples from healthy individuals were collected and analysed at the Infosys Central Laboratory. One set of samples was processed after 30minutes, while the other was kept at room temperature for 3 hours before processing. Sodium, potassium, and chloride concentrations were measured using ion-selective electrodes. **Statistical analysis:** The quantitative variables were expressed as mean and standard deviation. The difference in the mean values between the two groups was assessed using paired T -test and p -value of less than 0.05 was considered significant. **Results:** Results showed that delayed processing led to a significant reduction in sodium (p=0.001) and potassium (p=0.001) concentrations, while chloride levels remained relatively stable. Sodium levels decreased from 138±3.5mmol/L to 135.4±19mmol/L, and potassium levels dropped from 4.1±0.9mmol/L to 3.8±1.2mmol/L after 3 hours. **Conclusion:** The measurement of electrolytes is important for the treatment of critically ill patients. The serum clot contact time can affect the stability of electrolytes, like sodium and potassium showing a decreasing trend.

**Keywords:** Chloride, Electrolyte, potassium, preanalytical phase, Serum clot contact time



## INTRODUCTION

The preanalytical phase is an important step in the testing process. Most errors occur in the pre-analytic phase (46–68.2%). Preanalytical factors such as sample collection and handling, posture of patients, venous stasis, time of sampling, diet, exercise, and drugs can all impact a test result. To get accurate results, the preanalytical and analytical variations must be reduced to acceptable levels at which they cause no impact on the clinical interpretation of the results. The transition between the preanalytical and the intra-analytical phases is an important error-prone area. The period between collecting the sample and removing the serum from the clot should be sufficient for full clot formation, but not sufficiently long as to cause a notable difference in the test result due to contact between the serum and the clot. The minimum clotting time suggested is 20–30 min. Both the biological activity of the cells and transmembrane diffusion may affect the quantities of specific analytes in the serum over an extended period of contact between the clot and serum. Serum or plasma should be separated after natural clot formation. Many literatures recommend processing samples within 2 hours of collection. The WHO and CLSI's descriptions of analyte stability time are frequently challenging to implement in clinical settings. The time between blood collection and centrifugation affects the reliability of results. Serum clot contact time is the optimum interval between sample collection and separation of serum from clot. Serum electrolytes are commonly affected by serum clot contact time [1, 2, 3, 4].

Sodium is the major cation of the extracellular fluid compartment. It plays an important role in maintaining the normal distribution of water and osmotic pressure in extracellular fluid. It is important for nerve conduction and muscle contraction. The effect of hyponatremia and hypernatremia is most pronounced on nervous tissues. Hyponatremia can

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cause neurological manifestations. Hypernatremia can lead to brain hemorrhage and hematomas.

Potassium is an intracellular cation. Alterations in extracellular potassium levels are associated with an alteration in heart rhythm. Both hypokalaemia and hyperkalaemia are medical emergencies and should be managed immediately. [1,2,3]

Chloride is the major extracellular anion and is involved in the maintenance of water distribution, osmotic pressure, and anion-cation balance in the extracellular fluid. Chloride levels are affected in acid-base disorders. [1,2,3]

False elevations of these electrolytes not corresponding to the state of the patient are common findings in the laboratory now a days. But when these specimens are repeated and reanalysed, these values reach normal levels [5] Laboratory services make up 5% of a hospital budget but may amount to 60–70% of all critical decision-making, such as admission, discharge, and treatment of patients [6].

Many times the samples are processed late due to the large sample load, the breakdown of the analyser, and the nonavailability of a backup system. Information on serum clot contact time of serum electrolytes is often incomplete and contradictory. Hence, we conducted this study to evaluate whether the time lag between centrifugation and sample analysis has any effect on the stability of electrolytes in serum.

## OBJECTIVES

To evaluate time lag between centrifugation and sample analysis on stability of sodium, potassium and chloride in serum

## METHODS

The study was conducted in department of biochemistry, Infosys central Lab.50 samples from healthy subjects from out patient from Victoria hospital were drawn in replicates and was analysed in biochemistry lab between March 2023 to April 2023. The subjects were enrolled for the study after obtaining informed consent. Subjects not willing to give informed consent were excluded from the study.In this study we analysed the sodium, potassium and chloride of these subjects. Each blood sample was collected into red-topped vacutainer tubes and was centrifuged at 3200rpm for 15 minutes, serum was separated and analysed. One set of samples were processed immediately (after 30 minutes) and serum was separated for electrolyte analysis. The other samples were kept at room temperature(30 degree Celsius) for 3hours and later centrifuged and serum separated and analysed.

All the samples are analysed by ion selective electrodes in Cobas 6000 integrated analyser. Sodium was analysed by ISE indirect method. Potassium and chloride analysed by ISE direct method.

Hemolyzed samples were excluded from the study.

## STATISTICAL ANALYSIS

Statistical methods have been used to analyse the collected data for the present study. The collected data was entered in Microsoft excel 2021 and analyzed by vasserStats software. The quantitative variables were expressed as mean and standard deviation. The difference in the mean values between the two groups was assessed using paired T -test and p -value of less than 0.05 was considered significant.

## RESULTS

- The mean± SD values of sodium, potassium, chloride concentration were 138±3.5mmol/L, 4.1±0.9mmol/L and 97.2±21mmol/L($T_0$) at 30minutes.
- The mean Sodium, potassium and chloride concentration after 3hours ($T_3$) of serum clot contact time were 135.4±19mmol/L, 3.8±1.2 mmol/L and 96±20mmol/L. [Table 1] shows mean± SD values, and p-values of sodium, potassium and chloride after zero hour and 3hours.
- Sodium and potassium shows a decreasing trend with delay in processing. Chloride levels donot show much significant variation after 3hours.

**Table 1: Change in mean values of serum electrolytes at zero hour and after three hours**

| | $T_0$ Mean ±SD | $T_3$ Mean ±SD | p- value |
|---|---|---|---|
| Sodium | 138±3.5mmol/L | 135.4±19mmol/L | 0.001* |
| Potassium | 4.1±0.9mmol/L | 3.8±1.2 mmol/L | 0.001* |
| Chloride | 97.2±21mmol/L | 96±20mmol/L | Not significant |
| Values are expressed in Mean and SD.<br>p-value is from unpaired t test.<br>p-value <0.05 is considered significant.<br>*significant | | | |

## DISCUSSION

Serum electrolytes are the most common electrolytes requested in biochemistry laboratory. During a prolonged serum clot contact time both biological activity and transmembrane diffusion can change the concentration of analytes.In this study, we tried to find the effect of serum clot contact time on electrolytes. Sodium, potassium showed decreasing trend with serum clot contact time. Glycolysis was dominant initially, sopotassium from serum went inside cell lowering the potassium values. The mild decrease in chloride level was postulated due to the

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chloride-bicarbonate shift and subsequent action of bicarbonate buffer.The decrease in sodium levels may be due to the effect of passive diffusion into cells.Many studies in past have been conducted to demonstrate the stability of many analytes in both serum and plasma.

The study by Baruah A et al done in New Delhi, showed that stability of sodium, potassium as well as chloride is altered, if there is a delay in analysis. Sodium and chloride results increased after 3hours and potassium results increased even earlier after1 hour. This gross difference was due to improper temperature maintenance in laboratory leading to significant evaporation from sample cups. Climatic conditions as well as uncovered sample cups left under the fan for hours are responsible for this evaporation and falsely high serum electrolyte values. These findings was completely different from our findings[5].

Ono et al studied the serum clot contact time duration and effect of temperature of different analytes. His study revealed that electrolytes like sodium and potassium changed significantly after prolonged exposure at specific temperatures. These findings underline the critical importance of controlling temperature and storage conditions in order to give accurate report. Our study was done only at room temperature[7].

Boyanton et al did a study onplasma and serum specimens which was kept in prolonged contact with cells in uncentrifuged tubes at room temperature (25°C) in the dark. These specimens and additional double-spun aliquots were analysed at various intervals up to 56 hours. Sodium showed no significant change, while potassium was stable for 24 hours and the change was more pronounced in plasma.Our study assessed the clot contact time only for 3 hours. More studies are required to assess the change in electrolyte[8].

Donnelly et al , in his study analysed the stability of 25 analytes in serum from healthy donors at room temperature, 4°C, and -20°C over 48 hours, 14 days, and 4 months. It was found that electrolytes (sodium, potassium, chloride) were stable across all temperatures and durations[9].

According to study by Dash P et al. at the Clinical Biochemistry Laboratory, All India Institute of Medical Sciences (AIIMS), Bhubaneswar, samples that are left without serum separation at room temperature are subjected to evaporation. The sodium potassium pump failure , which causes potassium to leak from the cells is more pronounced at low temperatures at around 4°C. They 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. However, at 24 hours the combination of both clot contact time and the low temperature raises the level significant[10].

Study done by Kachhawa K et al. investigated the stability of 17 biochemical analytes in the serum of ten patients after storage at −20°C for 7, 15, and 30 days. Baseline measurements from fresh samples were compared to those taken after storage to assess any changes. Results showed that most analytes, including sodium, potassium, remained stable under these conditions[11].These findings are almost similar to study.

In study by Selvkumar etal. serum sodium levels were significantly decreased in lysed samples ,whereas the serum potassium levels were increased in lysed samples compared with normal samples[12].Our study was done in normal samples and lysed sample was an exclusion criteria.

**Limitations**

Because of the practical and economic constraints only small number of patient specimens could be tested. Further studies with large sample size required. More studies with different time intervals and storage temperatures are required to conclude about the stability of electrolytes.

Chloride showed a steady decrease which was attributable to the chloride-bicarbonate shiftwith subsequent buffering action of bicarbonate.

## CONCLUSION

According to our study there were decrease in both sodium and potassium levels, while the change in chloride levels is not significant.

The stability of electrolytes is sensitive to prolonged contact time. Among the electrolytes potassium is the least stable. The stability of both analytes (sodium and potassium )might be sensitive to temperature.Thus it is essential to process the electrolytes immediately to prevent the preanalytical variation.

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**Conflict of Interest:** None

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