

Electrolytes Levels (Na, K, Cl) in Serum Stored at 4°C Temperature

Amalia Nurul Fauziah¹, M. Atik Martsiningsih², Budi Setiawan^{3,4}

¹Pratama Hospital Yogyakarta,
Yogyakarta City, Yogyakarta,
Indonesia

²D-III of Medical Laboratory
Technology, Poltekkes Kemenkes
Yogyakarta, Yogyakarta, Indonesia

³Bachelor's Degrees in Medical
Laboratory Technology, Poltekkes
Kemenkes Yogyakarta,
Yogyakarta, Indonesia

⁴Pusat Unggulan IPTEK Inovasi
Teknologi Bidang Kesehatan
Masyarakat (PUI-Novakesmas),
Poltekkes Kemenkes Yogyakarta,
Yogyakarta, Indonesia

Correspondence:

Amalia Nurul Fauziah, Jl
Srandakan Km 01, Pandak, Bantul,
Yogyakarta, Indonesia
Zip Code: 55761
Email: amalianurul112@gmail.com

Received: January 2, 2021

Revised: March 5, 2021

Accepted: June 6, 2021

Published: October 30, 2021

DOI: 10.33086/ijmlst.v3i2.1870



Abstract

The samples used for serum electrolyte measurement should be analyzed immediately after being received in the laboratory within 1-2 hours to avoid an increase in the error of the results. Serum should be stored at 4°C for a period to prevent damage. The analyst should consider maximum delay time in the examination to maintain the serum's quality. This study compared the 2-hour and 3-hour delays in sodium (Na), potassium (K), and chlorine (Cl) tests. The method used in this study is an observational analysis with a cross-sectional study design. The samples in this study used 35 patient serum residues. The study was conducted in November 2020 with a continuous sampling technique. Electrolyte levels in the sample were measured by AVL 9180 Electrolyte Analyzer using Ion-Selective Electrode (ISE) method. The differences in electrolyte (Na, K, Cl) levels were analyzed by the Kruskal-Wallis Statistical test at a 95% confidence level. The results showed that the content of sodium, potassium, and chlorine were 0.719; 0.976; and 0.772. This study showed that there was no significant difference in the electrolyte content of sodium (Na), potassium (K), and chlorine (Cl) in the serum directly detected from the serum stored at 4°C for 2 hours and 3 hours. In conclusion, it is acceptable to postpone the serum test for 3 hours with various considerations.

Keywords

Chloride, Delay Time, Potassium, Serum, Sodium.



This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. ©2021 by author.

INTRODUCTION

Fluid and electrolyte balance disorders are common in clinical practice, especially in hospitals (1). The fluid amount and electrolyte concentration changes can cause a variety of problems if not appropriately managed. The electrolyte tests are usually performed as regular check-ups for specific medical conditions, such as decreased renal function, heart failure, and diarrhea (2–4).

Sodium (Na^+), Potassium (K^+), and Chloride (Cl^-) are the primary electrolytes (ions) in the body. Changes in electrolyte concentration and or ratio of anions and cations will cause changes in cell activity that can endanger life (5). Na^+ is the primary electrolyte of extracellular fluid, which in the case of hyponatremia or hypernatremia, the Na^+ concentration is regulated by the kidneys. K^+ is the central intracellular cation and plays a vital role in cell metabolism. Changes in plasma potassium levels (hyperkalemia or hypokalemia) can affect neuromuscular and heart function (6). Clinical features of potassium disorder can be the most life-threatening disorder compared to others (7). Besides, chloride is the primary extracellular anion in humans. It is essential to maintain serum neutrality, acid-base balance, homeostasis of body fluids, osmotic pressure, production of hydrochloric acid (HCl) in the gastrointestinal tract, kidney function, and electrical activity in muscular activity (8). Someone should give immediate

treatment when their body electrolytes are imbalanced (9). If not managed properly, changes in fluid and electrolyte concentration can cause serious problems.

Laboratory error leads to unnecessary delays and additional costs by obligatory repeated samples and causes unnecessary suffering to patients. Currently, pre-analytical errors account for up to 70% of all errors in laboratory diagnosis, most of which arise from problems in patient preparation, sample collection, transportation, and preparation for analysis and storage (10,11).

The samples used to measure serum electrolytes should be analyzed as soon as possible, preferably within 1-2 hours after being received in the laboratory. The sodium, potassium, and chlorine stability will change after a few hours of centrifugation (12). Proper specimen storage is an option to avoid repeated sampling in patients. If the serum should be stored for a moment, it should be closed and stored in the refrigerator before analyzing the serum at room temperature (13).

The limitation of sample analysis in some laboratories is the delay in sample analysis after the centrifugation process. This condition is due to follow-up requests from clinicians, large sample loads, tool damage, and unavailability of backup equipment in the laboratory. According to preliminary research, the average time required to refer a serum sample to the laboratory is 2 to 3 hours.

The study by Baruah *et al.* (12) also showed that the validity of the sodium and chlorine results was affected 3 hours after centrifugation. In contrast, the validity of the potassium results was affected 1 hour after centrifugation. Therefore, it is essential to find out the differences in the electrolyte levels of sodium (Na), potassium (K), and chloride (Cl) in the serum after being stored for 2 hours and 3 hours at 4°C.

MATERIALS AND METHODS

This study used an analytical observational study with a cross-sectional study design. The study was conducted in November 2020. A total of 35 serum samples residue from patients who performed chemical examinations in the laboratory of one hospital in Yogyakarta City, Indonesia was collected by consecutive sampling.

Inclusion criteria in this study were the remaining serum from patients examined in the laboratory with a volume of ≥ 1 mL. The samples used were visually clear, not hemolysis, icteric, and lipemic. The research procedure was carried out by inserting a blood sample into the vacutainer tube of the clot separator gel. The blood was allowed to clot at room temperature. Then the sample serum is grouped into two treatments. We directly examined the first sample group, and the second sample group was stored in a refrigerator. The serum was stored in a fridge at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 2 hours and 3 hours. The

serum was allowed to stand for 30 minutes at room temperature before the test (13).

The samples obtained were centrifuged at 3,000 rpm for 10 minutes. The serum was separated into Eppendorf tubes. Thirty-five serum samples were analyzed for Na^+ , K^+ , and Cl^- levels were measured by AVL 9180 *Electrolyte Analyzer* using the Ion-Selective Electrode (ISE) method.

The differences in sodium, potassium and chloride levels in samples between direct-used and cold-stored (2 hours and 3 hours at 4°C) samples were analyzed by a statistical test. The data obtained were primary data and scaled ratio data. The statistical analysis was performed by *SPSS 24.0 for Windows*. The data was analyzed with a *One-Sample Kolmogorov-Smirnov Test*. Abnormal result resulting caused by data dissemination. Therefore, we were analyzed further by statistical tests, namely non-parametric statistical tests used *K-Independent Samples (Kruskal-Wallis H)*. The data is presented as tables and statistical tests of non-parametric *Kruskal-Wallis* with a confidence level of 95% using statistical programs.

The Ethics Commission has approved this research for Health Research of Politeknik Kesehatan Kementerian Kesehatan (Poltekkes Kemenkes Yogyakarta) under the Ethics Feasibility Letter No. e-KEPK/POLKESYO /0629/X/2020 dated October 20, 2020.

RESULTS

The average time required to send a serum sample to the laboratory was 2-3 hours (after time for collection). The results of

serum electrolyte testing for several treatments (direct-tested and stored for 2 hours and 3 hours at 4°C) are shown in Table 1.

Table 1. Serum electrolyte level for several treatments (direct-tested and stored for 2 hours and 3 hours at 4° C)

Electrolyte Test	n	Mean (mmol/L)	Min (mmol/L)	Max (mmol/L)	SD	P-Value
Na						
Directly tested	35	138.7	133	144	2.64	0.719
2 h	35	139.2	134	146	2.70	
3 h	35	139.0	134	145	2.82	
K						
Directly tested	35	4.31	3.2	6.1	0.65	0.976
2 h	35	4.32	3.3	6.0	0.63	
3 h	35	4.31	3.3	6.0	0.63	
Cl						
Directly tested	35	103	96	110	3.38	0.772
2 h	35	104	97	110	3.24	
3 h	35	104	97	110	3.21	

Source: Primary Data, 2020

Table 1 present the sodium levels of 2 hours-stored-serum (139.2 mmol/L) is 0.5 mmol/L higher than directly-tested-serum (138.7 mmol/L). While 3 hour-stored-serum sodium (139.0 mmol/L) is 0.3 mmol/L higher than directly-tested-serum (Table 1). Potassium levels of serum stored for 2 hours (4.32 mmol/L) is 0.01 mmol/L higher than 3-hours-stored and direct-tested one (4.31 mmol/L) (Table 1). Chloride levels of both serums stored for 2 hours and 3 hours (104 mmol/L) is 1 mmol/L higher than direct-tested one (103 mmol/L) (Table 1). In this statistically analysis, a p-value higher than 0.05 (> 0.05). It means that no difference was observed.

DISCUSSION

Fluid and electrolyte balance is crucial for understanding the maintenance of homeostasis and the successful treatment of many metabolic disorders. There are many regulatory mechanisms for electrolyte balance in organisms. Disturbances of these mechanisms lead to electrolyte imbalance, which may be a life-threatening clinical condition. Electrolyte imbalance is a common manifestation of many diseases. All electrolyte imbalances should be comprehensively considered. The examination is essential to clarify the clinical scenario for effective and successful treatment. Most electrolyte imbalances are

low and high sodium, potassium, calcium, and magnesium (14).

Potassium maintains the cardiac rhythm and contributes to neuromuscular conduction. K level imbalance, indicated by hyperkalemia or hypokalemia, will cause cardiac arrhythmias and neuromuscular weakness. Chloride (Cl) helps to maintain electrical neutrality with Na. Cl also maintains acid-base balance by buffering H⁺ equal hydrogen changes and Cl changes to maintain electrical neutrality through the movement of bicarbonate ions (HCO₃⁻). Kidney and endocrine disorders are usually characterized by plasma electrolyte imbalance. Changes in electrolyte levels are associated with pathological consequences and increased mortality (15).

Analysts use laboratory diagnostic for diagnosis, monitoring, and prognosis in patients. Laboratory medicine has the potential to improve patient safety since it crosses many pathways and organizational boundaries. Clinicians can implement proactive interventions to highlight high-risk situations, such as pre-assessment and drug therapy monitoring. Laboratories have a considerable role to play in diagnostic and therapeutic decision-making and monitoring safety. There are many such examples highlighted in this paper, but increasing the knowledge of the use and abuse of testing, and studying the outcomes, should be part of the value-added function of laboratory

services. This condition will improve the quality of care and affect the results (16). Distinguishing between the normal and abnormal fluid balance in the patient can be challenging. The diagnosis of fluid balance abnormalities requires the informed and reasonable interpretation of clinical and laboratory data (17).

Several pre-analytical variables affect electrolyte results, including the type of anticoagulant, storage conditions, and hemolysis. Hemolysis of blood causes a false increase in plasma K results by releasing intracellular K. However, grossly hemolyzed specimens will affect the analyses of Na and Cl levels due to a dilutional effect. The presence of excess anticoagulants when small volumes of blood are collected will similarly cause a dilutional effect and falsely decreased plasma levels of Na and Cl. Refrigeration of unseparated whole blood may enhance the intracellular release of K from erythrocytes (15).

Various studies on the stability of chemical metabolites, especially sodium, potassium, and chloride in serums, give mixed results. Hedayati *et al.* (18) revealed that after the first cycle (24 h) at 2 to 8°C, changes in all the analytes (sodium, potassium, and chloride) were less than 10%. After serum stored in refrigeration in three cycles (72 h). At this moment, serum chloride was changes as 0.4% and statistically significant at the 5% level. Donnelly *et*

al. (19) compared sodium, potassium, and chloride levels in serums stored at room temperature (4°C) and -20°C for 48 hours, 14 days, and four months. While O'Keane *et al.* (20) showed that all metabolites, including sodium, potassium, and chloride, have the stability of up to 48 hours if stored at a temperature of 4°C. Some researchers discover that sodium and chloride levels in the serum can be stable for up to 12 hours with serum separation. After rapid centrifugation and storage at 4°C, the electrolytes can be stable for 48 hours (21). These data indicate that changes in these analytes are not consistent across all cycles and storage temperatures tested (18).

In the cooling process, glycolysis is inhibited. Thereby Na-K ATPase-depending power cannot maintain its gradient. Consequently, intracellular potassium will exit the erythrocytes, leading to an increase in potassium levels in plasma (22). Furthermore, storing of erythrocytes showed increased potassium leakage, and all these effects increased with increasing storage time (23). Since sodium density is lower than the chloride (24), there is only one-tenth of sodium in erythrocytes, so serum testing delays do not cause sodium leakage into the serum (13).

Various electrolyte results depended on the temperature conditions of each country. Research conducted in tropical countries will provide higher temperatures so that the

stability of sodium, potassium, and chloride can change after a few hours of centrifugation. Na and Cl results are affected at 3 hours, but K results are affected at 1 hour. Climatic conditions and uncovered sample cups left under the fan for a few hours are responsible for this evaporation and falsely high serum electrolyte values (12). Research conducted in tropical Indonesia gave similar results. The effect of long delays in serum examination for 0, 3, 5, and 7 hours was observed by other researchers that performed a serum examination of sodium and chloride levels (25). Other researchers discovered that delayed sample-processing of over 2 (two) hours did not affect the serum sodium and chloride level. On the other hand, delayed sample-processing of more than 2 (two) hours can affect potassium levels (13).

According to An *et al.* (26), Na, K, and Cl examination samples stored at room temperature will consistently increase over time, whereas low-temperature storage will prevent such changes. The evaporation of samples can cause an increase in such metabolites. Therefore, cold sample conditions and closed containers or tubes are highly recommended for long-term storage.

This research reveals that the average Na, K, and Cl levels in all treatments (directly examined and stored for 2 hours and 3 hours at a temperature of 4°C) are relatively comparable. There was no significant difference in sodium ($p = 0.719$), potassium

($p = 0.976$), or chloride ($p = 0.772$) levels in serums of all treatments, according to statistical tests. The findings are comparable to those of Donnelly *et al.* (19) and O'Keane *et al.* (20), who discovered that serum can be stored at 4 degrees Celsius for up to 48 hours. This discovery indicates that the storage of serum specimens for examination of sodium, potassium, and chloride should pay attention to the container, conditions, and storage temperature, making the serum's stability can last longer (27).

Serum storage in clinical laboratories is still widely used in Indonesia. As a result, each laboratory's guidelines should state the stability of each analyte's storage. The laboratory must follow standards of Operational Procedure for storage optimization.

The researcher should consider the type and stability of specimen, anticoagulants, preservatives, and containers when storing samples for electrolyte analysis. Serum samples for the study of Na, K and Cl can be stored for 14 days at a temperature of 20-25°C. Alternatively, if stored at 4°C, it can last for 14 days (27).

This study shows that the storage of serum for sodium, potassium, and chloride analysis can be done at a maximum of 3 hours at a temperature of 4°C. The limitation of this study is the determination of the storage length of serum samples, which is limited to only 2 hours and 3 hours. As a result, it is

unclear how long the maximum storage time will affect the serum sample's stability. In previous studies by Trisna *et al.* (13), sample-processing delays of over 2 hours do not affect the results of sodium and chloride examinations, while sample-processing delays of more than 2 hours can affect potassium results.

CONCLUSIONS

This study found no significant difference in Sodium (Na^+), Potassium (K^+), and Chloride (Cl^-) levels of the serum with all treatments (directly examined or stored for 2 hours and 3 hours at 4°C), indicating that postponing the serum examination for 3 hours with various considerations is still permissible. According to these findings, The analyst should keep serum specimens for electrolyte analysis (Na^+ , K^+ and Cl^-) at 4°C for a maximum of 3 hours. In previous studies, sodium and chloride were affected 3 hours after centrifugation at room temperature, while potassium results were affected 1 hour after centrifugation at room temperature. Further research is needed to determine the maximum storage time at 4°C, which can affect the stability of serum electrolytes.

AUTHOR CONTRIBUTIONS

Amalia Nurul Fauziah: term, conceptualization, methodology, formal analysis, investigation, resources, data

curation, writing-original draft, visualization, supervision, project administration, funding acquisition. M. Atik Martsiningsih: conceptualization, methodology, validation, writing - review & editing, visualization, editing or revision of the manuscript. Budi Setiawan: conceptualization, methodology, formal analysis, data curation, writing - review & editing, visualization, editing, or manuscript revision.

ACKNOWLEDGEMENTS

Appreciation goes to the Management of Laboratory Unit, Pratama Hospital Yogyakarta city, which commits to carry out this research successfully.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Capasso G, Unwin R. Electrolytes and acid–base: common fluid and electrolyte disorders. *Medicine (Baltimore)* [Internet]. 2011 Jun;39(6):317–24. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S1357303911000636>
2. Rahmawati F. Aspect of chronic kidney disease. *J Ilm Kedokt Wijaya Kusuma*. 2018;
3. Makani M, Setyaningrum N. Patterns of furosemide use and electrolyte imbalance in heart failure patients at Hospital X Yogyakarta. *J Ilm Farm*. 2017;
4. Tyas RA, Damayanti W, Arguni E. Prevalence of serum electrolyte disorder in children under five with diarrhea and dehydration in Dr Sardjito Hospital on 2013-2016. *Sari Pediatr*. 2018;
5. Kusnanto. Modul learning modules meeting fluid and electrolyte needs. *Fakultas Keperawatan Universitas Airlangga*. 2016.
6. Yaswir R, Ferawati I. Physiology and balance disorders of sodium, potassium and chloride and laboratory examination. *J Kesehat Andalas*. 2012;
7. Horne M dan PS. Pocket guide to fluids, electrolytes, and acid-base balance. 2nd ed. Asih Y, editor. Jakarta: EGC; 2012.
8. Pfortmueller CA, Uehlinger D, von Haehling S, Schefold JC. Serum chloride levels in critical illness—the hidden story. *Intensive Care Med Exp*. 2018;
9. Salam SH. The Basics of fluid and electrolyte therapy. *Bahan Kuliah FK Unhas*. 2016;
10. Plebani M. Quality indicators to detect pre-analytical errors in laboratory testing. *Clinical Biochemist Reviews*. 2012.
11. Lippi G, Chance JJ, Church S, Dazzi P, Fontana R, Giavarina D, Grankvist K, Huisman W, Kouri T, Palicka V, Plebani M, Poro V, Salvagno GL, Sndberg S, Sikaris K, Watson I, Stankovic AK, Simundic AM. Preanalytical quality improvement: From dream to reality. *Clin Chem Lab Med*. 2011 Jul;49(7):1113-26.
12. Baruah A, Goyal P, Sinha A, Ramesh KL, Datta R. Delay in specimen processing-major source of preanalytical variation in serum electrolytes. *J Clin Diagnostic Res*. 2014;
13. Trisna YN, Mutmainnah M, DN Pakasi R, Hardjoeno H. Na, K, Cl concentration in time interval examination variations of serum. *Indones J Clin Pathol Med Lab*. 2018;
14. Balci AK, Koksall O, Kose A, Armagan E, Ozdemir F, Inal T, Oner N. General characteristics of patients with electrolyte imbalance admitted to emergency department. *World Journal of Emergency Medicine*. 2013;4(2):113-6
15. Arneson W. Electrolytes: The salts of the earth. *Laboratory Medicine*. 2014.
16. Barth JH. Clinical quality indicators in laboratory medicine. *Ann Clin Biochem*. 2012;
17. Roumelioti M-E, Glew RH, Khitan ZJ, Rondon-Berrios H, Argyropoulos CP, Malhotra D, et al. Fluid balance concepts in medicine: Principles and practice. *World J Nephrol*. 2018;
18. Hedayati M, Razavi SA, Boroomand S, Kheradmand Kia S. The impact of pre-analytical variations on biochemical analytes stability: A systematic review. *Journal of Clinical Laboratory Analysis*. 2020.
19. Donnelly JG, Soldin SJ, Nealon DA, Hicks JM. Stability of twenty-five analytes in human serum at 22 degrees C, 4 degrees C, and -20 degrees C.

- Pediatr Pathol Lab Med. 1995;
20. O'Keane MP, Cunningham SK. Evaluation of three different specimen types (serum, plasma lithium heparin and serum gel separator) for analysis of certain analytes: clinical significance of differences in results and efficiency in use. *Clin Chem Lab Med* [Internet]. 2006 Jan 1;44(5). Available from: <https://www.degruyter.com/view/j/cclm.2006.44.issue-5/cclm.2006.099/cclm.2006.099.xml>
 21. Dupuy AM, Cristol JP, Vincent B, Bargnoux AS, Mendes M, Philibert P, et al. Stability of routine biochemical analytes in whole blood and plasma/serum: focus on potassium stability from lithium heparin. *Clin Chem Lab Med* [Internet]. 2018 Feb 23;56(3):413–21. Available from: <https://www.degruyter.com/doi/10.1515/cclm-2017-0292>
 22. Scott MG, LeGrys VA, Hood JL. Electrolytes and blood gases. in: *tietz textbook of clinical chemistry and molecular diagnostics*. 2012.
 23. Burger P, Kostova E, Bloem E, Hilarius-Stokman P, Meijer AB, van den Berg TK, et al. Potassium leakage primes stored erythrocytes for phosphatidylserine exposure and shedding of pro-coagulant vesicles. *Br J Haematol*. 2013;
 24. Apriliani I, Santosa B, Sukeksi A. Difference in electrolyte levels (Na, K, Cl) in samples immediately and delayed by 150 minutes. *repository.unimus.ac.id*. 2018.
 25. Azizah N, Aliviameita A. Effect of delay of serum examination on electrolyte levels of sodium and chloride. *J Med Lab Sci Technol*. 2019;
 26. An B, Park C-E. Evaluation of stability of serum on different storage temperatures for routine chemistry analytes. *Korean J Clin Lab Sci*. 2014;
 27. Departemen Kesehatan RI. *Pedoman praktek laboratorium yang benar (Good laboratory practice)*. Jakarta; 2008.