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Impact of Storage Temperature on Stability and Quantitative Determination of Human Serum Albumin Measured by Bromocresol Green Method

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ABSTRACT

Precise determination of serum albumin level is vital for detecting and managing liver, kidney, and inflammatory conditions. The present study aimed to evaluate the effect of different storage temperatures and durations on serum albumin levels. An analytical cross-sectional study was conducted involving twenty-six healthy participants between 22 and 29 years of age. Four milliliters of venous blood were collected from each participant, and serum was separated. Each serum specimen was divided into four portions and stored as given below and the albumin concentrations were measured using the Bromocresol Green method on the BS240 Clinical Chemistry Analyzer. Mean albumin values (±SD) obtained for (i) serum obtained immediately after separation (ii) serum stored for 6 hours at room temperature (iii) serum stored for 24 hours at +4°C and (iv) serum stored for 48 hours at −20°C were 4.74±0.20, 4.69±0.16, 4.74±0.21, and 4.45±0.31 g/dL, respectively. The results demonstrated a statistically significant reduction in albumin levels in serum stored at −20°C for 48 hours compared to serum analyzed immediately after separation ($p<0.001$). In the comparison between serum analyzed immediately after separation and serum stored at ambient temperature for 6 hours ($p=0.512$) or at +4°C for 24 hours ($p=0.997$), no statistically significant difference in albumin levels was observed. Among these conditions, serum stored at +4°C for 24 hours showed the closest agreement with the immediately analyzed serum. Serum albumin level should ideally be measured immediately after blood collection. If immediate analysis is not feasible, serum specimen should be stored at +4°C for up to 24 hours, as this condition showed a better stability than storage at ambient temperature for 6 hours.

KEYWORDS: *Albumin, Serum, Temperature, Storage*

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1 INTRODUCTION

Albumin makes up half of the total protein concentration of plasma in a healthy human body and it is a multifunctional protein. Since albumin is synthesized in the liver, serum albumin level is used to detect the functional state of the liver (Moman et al., 2022). In healthy individuals, the normal serum albumin concentration ranges from 3.9 to 5.5 g/dL (Pupim et al., 2013). Serum Albumin levels may deviate from the reference range due to various conditions, including liver and kidney diseases, malabsorption, malnutrition, and inflammatory disorders.

In liver diseases such as cirrhosis and hepatic failure, impaired hepatic synthesis results in decreased albumin production, leading to hypoalbuminemia. Renal loss of albumin, particularly in conditions such as nephrotic syndrome, can also reduce serum albumin levels (McPherson & Pincus, 2021). Furthermore, inflammatory conditions, burns, and sepsis are recognized causes of hypoalbuminemia (Gounden et al., 2023). Hypoalbuminemia may additionally occur in patients with cardiac failure. Hemodilution following intravenous fluid administration can falsely lower measured albumin concentrations (McPherson & Pincus, 2021). In contrast, hyperalbuminemia is most commonly associated with dehydration and may also result from prolonged tourniquet application during venipuncture (McPherson & Pincus, 2021).

Laboratory evaluation of albumin plays a crucial role in diagnosing and monitoring many diseases, as serum albumin is a key marker of liver function, nutritional status, and systemic health. Therefore, the accuracy of serum albumin test results is critical. Erroneous measurements may lead to misinterpretation of a patient's clinical condition. Falsely elevated or decreased serum albumin levels can result in inappropriate medical interventions or failure to detect underlying disease. Precise and reliable measurement is necessary to ensure accurate diagnosis, guide appropriate treatment, and minimize potential risks associated with incorrect clinical decisions.

In an optimally conducted serum albumin assay, blood specimens should be processed promptly after collection to ensure accurate results. However, immediate processing is not always feasible due to heavy laboratory workload or delay in specimen transportation. This challenge is particularly common in medical laboratory settings in developing countries.

Therefore, this study aimed to assess the effect of different storage temperatures and storage durations on human serum albumin levels and to determine whether statistically significant changes occur during storage. The findings of this study are expected to provide valuable guidance for medical laboratories in reporting reliable serum albumin test results, especially in situations where immediate blood specimen processing is not possible.

2 MATERIALS AND METHODS

The study was carried out after obtaining ethical approval from the Ethical Review Committee, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka (Ref. No: ERC/MLS/01.)

2.1 Study design

This was an analytical observational cross-sectional study with laboratory investigations.

2.2 Sample size calculation

The sample size calculation was based on the ability to detect a clinically significant change in serum albumin concentration during storage. The sample size for this study was determined using a paired t-test power analysis performed with Minitab statistical software. A paired study design was selected because serum albumin levels were measured repeatedly from the same blood specimen under different storage conditions and time intervals. Therefore, each specimen served as its own control, making the paired t-test the most appropriate statistical method. The calculated sample size was 26.

2.3 Study population

The study population consisted of apparently healthy males and females aged 22–29 years, with females and males comprising 69% (n=18) and 31% (n=8) of the participants, respectively. Patients with liver failure, cirrhosis, renal disease, cardiac failure, sepsis, malabsorption disorders, myeloma, neurological disorders, burns, alcohol consumption/alcoholism, medication use, or a history of surgery, as well as pregnant and lactating women, were excluded from the study. Health status of the participants was assessed through self-reported health information and review of their medical history.

2.4 Selection of the study population

A self-administered questionnaire was utilized to gather information and to screen eligible healthy participants for the study. The questionnaire was developed based on the study objectives. It was reviewed by subject experts to ensure content validity, and necessary modifications were made according to their recommendations. A pilot study was conducted prior to data collection to assess the clarity, relevance, and suitability of the questionnaire.

2.5 Processing of blood specimens

2.5.1 Blood specimen collection and serum separation

Four milliliters of whole blood were collected from each participant by venipuncture performed by a qualified phlebotomist. Each blood specimen was put into separate plain tubes with red cap which were free from any anticoagulants. All specimens were protected from direct sunlight and artificial light using appropriate handling protocols. The blood specimens were then allowed to clot at room temperature. After completing clot formation, the specimens were centrifuged at 3000 rpm for 5 minutes, and the supernatant was carefully separated and collected as serum.

2.5.2 Storage conditions of serum specimens

Each serum specimen was separated into four aliquots. The first aliquot was analyzed immediately after separation to serve as the

baseline. The second aliquot was kept at room temperature (31°C) for 6 hours before analysis. The third aliquot was stored at +4°C for 24 hours prior to testing, while the fourth aliquot was stored at −20°C for 48 hours before being analyzed.

2.5.3 Determination of serum albumin

2.5.3.1 Analyzer

Serum albumin concentration was measured using the Mindray BS-240 Clinical Chemistry Analyzer (manufactured by Mindray, China) with the Bromocresol Green (BIOLABO, France) method.

2.5.3.2 Calibration and quality control of analyzer

Before analyzing the serum specimens, the BS-240 analyzer was calibrated for serum albumin using a human multi-calibrator (Mindray) following the manufacturer's guidelines. After calibration, two quality control (QC) materials; human assayed controls from Mindray were tested to ensure proper measurement performance: one representing normal serum albumin levels and the other representing pathological levels. Only after obtaining satisfactory QC results were the serum specimens analyzed.

2.6 Data analysis

The sample size for this study was calculated using Minitab. All statistical analyses were conducted using SPSS, version 29. The dataset was first assessed for normality and was found to be normally distributed. Since there was only one dependent variable, univariate analysis was conducted. Tukey's HSD test was used to identify which group's mean serum albumin levels differed significantly from each other. This was performed following ANOVA, which indicated that there were overall differences in mean serum albumin levels among the groups.

3 RESULTS AND DISCUSSION

3.1 Serum albumin concentrations under different storage conditions

The serum albumin concentrations measured immediately after separation were within the reference range, with values ranging from 4.43 g/dL to 5.17 g/dL. This finding is consistent with the fact that all participants were healthy adults. The variation in the serum albumin concentrations under the different storage conditions investigated in this study is presented in Table 1.

Table 1. Serum albumin concentrations under different storage condition

Group	Storage condition of serum	Mean±SD (g/dL)
01	Serum specimen obtained immediately after the blood collection	4.74±0.20
02	6 hours at ambient temperature (31°C)	4.69±0.16
03	24 hours at 4 °C	4.74±0.21
04	48 hours at −20 °C	4.45±0.31

SD: Standard Deviation

3.2 Comparison of serum albumin levels across different storage conditions

Group 4 (serum stored at −20°C for 48 hours) formed a distinct subset, demonstrating a significantly lower mean serum albumin level (4.45 g/dL) compared to the other three groups. In contrast, Groups 1, 2, and 3 clustered together, as their mean serum albumin levels (4.74 ± 0.20, 4.69 ± 0.16, and 4.74 ± 0.21 g/dL, respectively) did not differ significantly. Compared with Group 1 (analyzed immediately after separation), Group 4 (stored 48 hours at −20ºC) showed a significant reduction of 0.29 g/dL in serum albumin level ($p < 0.001$). Meanwhile, Group 3 (stored for 24 hours at +4°C) demonstrated only a minimal negative deviation of 0.01 g/dL relative to Group 1 ($p = 0.997$). Group 2 (stored for 6 hours at an ambient temperature of 31°C) showed a slightly greater negative deviation of 0.05 g/dL compared with Group 1; however, this difference was not statistically significant ($p = 0.512$).

However, the Bromocresol Green (BCG) method was selected for this study because it is a widely used and routinely established method for serum albumin determination in clinical laboratories. The objective of the present study was to evaluate the effect of storage temperature and duration on serum albumin measurements. Therefore, the use of a commonly employed and standardized laboratory method such as BCG was considered appropriate for assessing storage-related changes under routine laboratory conditions.

Our findings suggest that storing serum specimens at +4°C for up to 24 hours is preferable to storage at ambient temperature (31°C) for 6 hours, particularly when analysis is delayed. Contrary to our findings, an Iranian study investigating the effect of storage time and temperature reported a mean serum albumin value of 4.22±0.38 g/dL when the specimen was analyzed immediately after blood collection, compared to after storage at +4°C for 24 hours (4.41±0.42 g/dL). The authors did not provide an explanation for this unexpected increase in albumin concentration following storage (Marjani, 2008). Similarly, a study by Pawlik-Sobecka et al reported a statistically non-significant 0.2% increase in serum albumin levels when samples were stored at +4°C for 24 hours, compared to serum analyzed immediately after blood collection (Pawlik-Sobecka et al., 2020).

In the present study, serum specimens stored at −20°C for 48 hours showed a mean albumin level of 4.45±0.31 g/dL. This represented a statistically significant decrease compared to values obtained immediately after blood collection, making it the most negatively deviated group relative to specimens stored at ambient temperature (31°C) for 6 hours or at +4°C for 24 hours. Based on these findings, storing serum specimens at −20°C for 48 hours is not recommended for the analysis of serum albumin levels. Our findings are supported by a previous study on dairy cattle, which reported a negative deviation between serum analyzed immediately and after 24 hours of storage at

+4°C, although the difference was not statistically significant (Tothova et al., 2010).

The observed decrease in serum albumin levels after storage under different temperatures and durations is likely due to physical and biochemical factors affecting protein stability. Although serum albumin is generally a stable protein compared to others in the human body, its structure can still be influenced by environmental conditions. Temperature, in particular, may accelerate or slow degradation, induce denaturation, or compromise its structural integrity over time (Belinskaia et al., 2020; Wang et al., 2005).

At ambient temperature (31°C), the relatively higher thermal energy may enhance enzymatic activity and oxidation in serum, leading to gradual protein breakdown. Proteins can undergo conformational changes, destabilizing their three-dimensional structure and making them more prone to denaturation and degradation, ultimately reducing measurable protein levels. Although serum albumin is more heat-stable than many other proteins tolerating temperatures up to 60°C for 10 hours, it can still be slightly affected by 6 hours at 31°C (Belinskaia et al., 2020; Wang et al., 2005). This may explain the non-significant negative deviation observed in our study for specimens stored at ambient temperature for six hours.

When serum specimens are stored at +4°C, the lower temperature slows enzymatic and microbial activity, helping to preserve the structural integrity of albumin for a longer period. However, some protein instability or degradation may still occur at a much slower rate. Cold storage reduces metabolic reactions but does not completely prevent processes such as protein aggregation or oxidation (Pasella et al., 2013). This likely explains the minor, non-significant decline in albumin concentrations observed after 24 hours of storage at +4°C.

Although storage at freezing temperatures (−20°C) slows enzymatic degradation and microbial growth, the formation of ice crystals during freezing and thawing can disrupt protein structure, causing mechanical damage to albumin molecules (Gislefosse et al., 2017; Peiren et al., 2015). This can lead to irreversible denaturation, where albumin loses both functional and structural integrity, resulting in significantly lower measured levels. Additionally, frozen storage may cause proteins to precipitate out of solution, further contributing to measurement inaccuracies after thawing (Cuhadar et al., 2013).

Contrary to the idea that freezing may damage serum albumin, a study in which serum samples were stored for 25 years at −25°C reported albumin values that showed little change compared to the original measurements (Gislefoss et al., 2009). In contrast, another study by Hostmark et al found that prolonged storage of serum specimens for several years at −25°C led to a substantial increase in albumin concentrations (Hostmark et al., 2001), highlighting that long-term freezing can produce variable effects on serum albumin levels.

4 CONCLUSION AND RECOMMENDATIONS

Serum albumin levels should ideally be determined immediately after specimen collection to ensure accuracy. If immediate analysis is not possible, storing the serum at +4 °C for up to 24 hours is preferable rather than being left at room or ambient temperature for extended periods. Future studies are warranted to explore how a wider range of storage durations and temperature conditions impact serum albumin stability.

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