COMPARISON OF TOTAL CHOLESTEROL LEVELS IN SAMPLES SERUM TO BE CHECKED IMMEDIATELY AND SERUM SAMPLE WHICH WAS POSTPONED FOR 7 DAYS IN THE REFRIGERATOR WITH A TEMPERATURE OF 2-8°C AT BHAYANGKARA HOSPITAL

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ABSTRACT

Total cholesterol measurement is an essential laboratory examination for assessing cardiovascular risk and monitoring lipid metabolism. However, immediate analysis is not always feasible in routine clinical practice, making refrigerated storage a common pre-analytical procedure. This study aimed to compare total cholesterol levels between serum samples analyzed immediately after collection and serum samples stored at 2–8°C for seven days. A quantitative analytical observational study with a repeated-measurement comparative design was conducted at Bhayangkara Hospital, Gorontalo, Indonesia, from June to July 2025. Fifteen paired serum samples were selected using accidental sampling. Total cholesterol concentrations were measured using the BioSystem BA200 automated chemistry analyzer. Differences between immediate and delayed analyses were evaluated using the paired-samples t-test after confirmation of data normality. Of the 15 samples, seven (47%) had normal cholesterol levels and eight (53%) had abnormal levels. The mean total cholesterol concentration increased slightly from 203 mg/dL to 206 mg/dL after seven days of refrigerated storage. However, the paired-samples t-test showed no statistically significant difference between the two conditions ($p = 0.052$). These findings indicate that serum storage at 2–8°C for up to seven days maintains total cholesterol stability and can be considered an acceptable pre-analytical practice when immediate laboratory analysis is not feasible.

Keywords: total cholesterol; serum storage; pre-analytical phase; refrigerated storage; laboratory analysis.

INTRODUCTION

Cardiovascular diseases (CVDs) remain the leading cause of morbidity and mortality worldwide, making the accurate assessment of lipid profiles an essential component of preventive and clinical healthcare. Among lipid biomarkers, total cholesterol is widely used for cardiovascular risk assessment, diagnosis of dyslipidemia, therapeutic monitoring, and evaluation of metabolic disorders such as diabetes mellitus and obesity. Reliable cholesterol measurements are therefore fundamental to evidence-based clinical decision-making and the development of appropriate treatment strategies for reducing cardiovascular risk [1][2][3].

The accuracy of laboratory results, however, depends not only on analytical performance but also on the quality of the pre-analytical process. Previous studies have consistently shown that pre-analytical errors account for the majority of laboratory inaccuracies and frequently arise from

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improper specimen collection, transportation, storage, or delayed analysis. These factors may compromise the stability of biochemical analytes, potentially resulting in inaccurate laboratory findings, unnecessary repeat testing, increased healthcare costs, and inappropriate clinical decisions [4][5][6]. Consequently, maintaining sample integrity throughout storage and handling has become a critical component of laboratory quality assurance.

In routine clinical laboratory practice, immediate analysis of serum specimens is not always feasible because of logistical constraints, high testing workloads, transportation from satellite collection centers, equipment maintenance, or the need for repeat verification. Under these circumstances, serum samples are commonly refrigerated at temperatures between 2°C and 8°C to preserve analyte stability until analysis can be performed. Although refrigeration is widely recommended to minimize biochemical degradation, concerns remain regarding whether prolonged storage may alter total cholesterol concentrations and consequently influence laboratory interpretation [7].

Several studies have investigated the stability of cholesterol and other biochemical analytes under different storage conditions. Previous research has primarily focused on long-term frozen storage at −20°C or −80°C, delayed serum separation, or extended preservation of serum samples [8][9][10]. While these studies generally reported acceptable analyte stability under controlled laboratory conditions, their findings cannot be directly generalized to routine hospital laboratories, where short-term refrigeration at 2–8°C is the most frequently applied storage method. Furthermore, differences in storage duration, laboratory protocols, analytical instruments, and sample characteristics have produced inconsistent findings, limiting the development of standardized recommendations for delayed cholesterol testing [6][10].

This limitation represents an important research gap because evidence regarding the stability of total cholesterol after seven days of storage at 2–8°C remains limited, despite this condition being commonly encountered in everyday clinical laboratory practice. Establishing whether delayed analysis under routine refrigeration conditions affects total cholesterol measurements is essential for strengthening laboratory quality management, optimizing specimen handling protocols, minimizing unnecessary repeat blood collection, and ensuring accurate clinical interpretation.

Therefore, this study aims to compare total cholesterol levels between serum samples analyzed immediately after collection and serum samples stored for seven days at 2–8°C. The findings are expected to provide evidence-based recommendations for optimal serum storage practices and contribute to improving the reliability of pre-analytical procedures in clinical laboratory services.

Literature Review

Cholesterol Metabolism and Clinical Significance

Cholesterol is an essential lipid molecule involved in maintaining cell membrane integrity, synthesizing steroid

hormones, producing bile acids, and facilitating vitamin D metabolism. Cholesterol homeostasis is regulated through a dynamic balance between endogenous synthesis, intestinal absorption, transport by lipoproteins, and excretion. Disruption of these regulatory mechanisms may result in hypercholesterolemia, a major risk factor for cardiovascular diseases. Consequently, accurate determination of serum total cholesterol has become an indispensable component of clinical diagnosis, cardiovascular risk assessment, and therapeutic monitoring [11].

Serum Characteristics for Cholesterol Measurement

Serum is the preferred biological specimen for routine biochemical analysis because it contains dissolved proteins, lipids, enzymes, electrolytes, and metabolites without clotting factors that may interfere with analytical procedures. Compared with plasma, serum generally provides greater analytical consistency for lipid measurements due to the absence of anticoagulants that may influence assay performance. Nevertheless, serum quality remains susceptible to several pre-analytical factors, including specimen collection procedures, centrifugation, storage conditions, transportation, and the interval between blood collection and laboratory analysis [12]. Therefore, maintaining serum integrity throughout the pre-analytical phase is essential to ensure accurate laboratory results.

Pre-Analytical Variables Affecting Cholesterol Testing

The pre-analytical phase represents one of the most critical stages of laboratory testing because it contributes substantially to overall laboratory errors. Previous studies have demonstrated that inappropriate patient preparation, prolonged sample handling, improper storage conditions, delayed processing, and environmental exposure may significantly influence biochemical measurements [13][14][15]. Variations in temperature, storage duration, repeated freeze–thaw cycles, and specimen transportation may alter analyte concentrations through biochemical degradation, evaporation, oxidation, or cellular metabolic activity. Consequently, minimizing pre-analytical variability has become an important strategy for improving laboratory quality assurance and ensuring reliable clinical interpretation.

Stability of Serum Cholesterol During Storage

Among the various pre-analytical variables, storage temperature and storage duration are particularly important determinants of serum analyte stability. Refrigerated storage between 2°C and 8°C is widely recommended for temporary preservation of serum samples because low temperatures reduce enzymatic activity and delay biochemical degradation. Flores et al. [16], reported that most routine biochemical analytes, including lipid parameters, remain relatively stable under refrigerated conditions for several days, although stability varies depending on the analyte, storage duration, and analytical methodology. Similarly, Pawlik-Sobecka et al. [17], demonstrated that prolonged storage and repeated handling may

gradually affect several biochemical parameters associated with oxidative processes, emphasizing the importance of standardized storage protocols.

Previous Studies and Research Gap

Several studies have specifically investigated cholesterol stability under different storage conditions. Adhikari et al. [18], demonstrated that serum total cholesterol remained relatively stable during short-term refrigerated storage, supporting the feasibility of delayed laboratory analysis under controlled conditions. Likewise, Muzakova et al. [9], found that lipid biomarkers exhibited remarkable long-term stability during frozen storage, although gradual degradation could occur after prolonged preservation. França et al. [19], and Gislefoss et al. [20], also reported acceptable stability of serum lipid profiles during extended storage when appropriate preservation protocols were implemented. These findings collectively indicate that cholesterol is generally more stable than many other biochemical analytes; however, stability remains dependent on storage temperature, storage duration, and laboratory procedures.

Despite these advances, existing evidence has predominantly focused on frozen storage at −20°C or −80°C, long-term biobanking, or delayed serum separation rather than routine refrigerated storage in hospital laboratories. Moreover, previous investigations have reported varying analytical protocols, storage periods, specimen types, and laboratory instruments, making direct comparison between studies difficult [16][7]. Consequently, evidence regarding the stability of serum total cholesterol after seven days of storage at 2–8°C under routine clinical laboratory conditions remains limited. This limitation creates uncertainty in laboratory practice, particularly in healthcare facilities where immediate analysis cannot always be performed because of logistical constraints, equipment availability, or high specimen workloads.

Therefore, further investigation is required to determine whether seven-day refrigerated storage significantly influences total cholesterol measurements under routine laboratory conditions. Establishing evidence regarding cholesterol stability during delayed analysis is expected to strengthen laboratory quality management, improve pre-analytical standardization, reduce unnecessary repeat testing, and provide evidence-based recommendations for serum specimen handling in clinical laboratories.

RESEARCH METHODS

Research Design

This study employed a quantitative approach using an analytical observational design to compare total cholesterol levels between serum samples analyzed immediately after collection and serum samples stored for seven days at a temperature of 2–8°C. The study adopted a comparative repeated-measurement design, in which each serum sample was analyzed under two different storage conditions. This approach enabled an objective evaluation of whether delayed analysis affected total cholesterol concentrations.

The research was conducted from June to July 2025 at Bhayangkara Hospital, Gorontalo, Indonesia.

Population and Sample

The study population consisted of all patients who underwent total cholesterol examinations at Bhayangkara Hospital during the study period. A total of 15 serum samples were selected using an accidental sampling technique. The sample size was determined using the Lemeshow formula, considering the minimum number of observations required for comparative quantitative analysis.

Data Collection

This study utilized quantitative data obtained through direct laboratory measurements of total cholesterol concentrations.

Primary data consisted of total cholesterol measurements obtained from:

serum samples analyzed immediately after collection; and serum samples stored in refrigerator at 2–8°C for seven days before analysis.

Secondary data were obtained from scientific literature, including textbooks, peer-reviewed journal articles, and other relevant publications supporting the theoretical framework of the study.

Laboratory Procedures

Venous blood samples were collected following standard laboratory procedures. After clot formation and serum separation, the first cholesterol measurement was performed immediately using the BioSystem BA200 automated chemistry analyzer. The remaining serum from the same sample was subsequently stored at 2–8°C in a laboratory refrigerator for seven days. After the storage period, total cholesterol was reanalyzed using the same instrument and analytical procedure to ensure measurement consistency.

Data Analysis

Data analysis consisted of descriptive and inferential statistical analyses.

Descriptive analysis was performed to summarize total cholesterol levels through frequency distributions and descriptive statistics. Cholesterol concentrations were further classified into normal and abnormal categories according to the applicable clinical reference values.

Inferential analysis was conducted to determine whether significant differences existed between immediately analyzed serum samples and serum samples stored for seven days.

Before hypothesis testing, the normality of the data distribution was assessed. If the data met the assumption of normality, differences between the two paired measurements were analyzed using the paired-samples t-test. If the normality assumption was violated, the Wilcoxon signed-rank test was employed as a non-parametric alternative. Statistical significance was determined at a 95% confidence level ($\alpha = 0.05$).

RESEARCH RESULT

Descriptive Analysis

Descriptive analysis was performed to summarize the distribution of total cholesterol levels among the serum samples included in this study. Based on the frequency distribution presented in Table 1, a total of 15 serum samples were analyzed. Among these samples, 7 (47%) were classified as having

normal total cholesterol levels, whereas 8 (53%) were categorized as abnormal.

Table 1. Distribution of Total Cholesterol Levels

Total Cholesterol Category	Frequency	Percentage (%)
Normal	7	47
Abnormal	8	53
Total	15	100

Source: Primary Data, 2025.

The results indicate that more than half of the participants presented total cholesterol concentrations above the normal reference range, suggesting that elevated cholesterol levels were slightly more prevalent within the study population.

To further describe the laboratory findings, total cholesterol concentrations were compared between serum samples analyzed immediately after collection and serum samples stored at 2–8°C for seven days, as presented in Table 2.

Table 2. Total Cholesterol Levels Before and After Seven-Day Storage

No.	Immediately Analyzed (mg/dL)	Stored for 7 Days (mg/dL)
1	179	176
2	189	181
3	216	214
4	175	177
5	121	121
6	181	186
7	155	156
8	239	247
9	217	219
10	353	366
11	209	212
12	74	79
13	287	292
14	232	234
15	219	229

Source: Primary Data, 2025.

The mean total cholesterol concentration in serum samples analyzed immediately after collection was 203 mg/dL, whereas the mean concentration in serum samples stored for seven days was 206 mg/dL. This represents a slight average increase of approximately 3 mg/dL following refrigerated storage. Although minor numerical differences were observed among several paired samples, the overall variation remained relatively small.

Comparative Analysis

Prior to hypothesis testing, the data were assessed for normality and met the assumptions required for parametric analysis. Therefore, differences in total cholesterol concentrations between the two storage conditions were evaluated using the paired-samples t-test.

Table 3. Paired-Samples t-Test Comparing Total Cholesterol Levels

Variables Compared	95% Confidence Interval	Sig. (2-tailed)
	Lower	Upper
Immediately analyzed vs. stored for 7 days	–0.5766	–0.033

Source: Primary Data, 2025.

The paired-samples t-test produced a mean difference of –2.867 mg/dL, indicating that total cholesterol concentrations measured after seven days of refrigerated storage were, on average, slightly higher than those obtained immediately after sample collection. However, the observed difference was not statistically significant ($p = 0.052$), as the significance value exceeded the predetermined alpha level of 0.05.

These findings indicate that storing serum samples at 2–8°C for seven days did not produce a statistically significant change in total cholesterol concentrations. Therefore, under the storage conditions evaluated in this study, delayed analysis for up to seven days maintained comparable total cholesterol measurements to those obtained immediately after sample collection.

DISCUSSION

The present study compared total cholesterol concentrations measured immediately after serum separation with those obtained after serum samples had been stored at 2–8°C for seven days. The findings demonstrated that the mean total cholesterol concentration increased slightly from 203 mg/dL to 206 mg/dL, representing an average increase of approximately 3 mg/dL. However, statistical analysis using the paired-samples t-test showed that this difference was not statistically significant ($p = 0.052$). These findings indicate that short-term refrigerated storage for seven days did not significantly alter total cholesterol concentrations, suggesting that serum samples maintained their analytical integrity under the storage conditions evaluated.

The absence of a statistically significant difference can be explained by the inherent biochemical characteristics of cholesterol. Cholesterol is a relatively stable lipid molecule that is less susceptible to rapid enzymatic degradation and oxidative reactions than many other biochemical analytes. Refrigerated storage at 2–8°C slows enzymatic activity and reduces oxidation, thereby preserving analyte stability during temporary storage. Consequently, only minimal fluctuations in cholesterol concentration are expected during short-term refrigeration, provided that serum separation and storage procedures are performed according to laboratory quality standards [9][21]. This biochemical stability explains why the small numerical increase observed in the present study did not translate into a statistically significant difference.

Although a slight increase in cholesterol concentration was observed after seven days of storage, the magnitude of change was relatively small and remained within acceptable analytical variation for routine clinical laboratory practice. Minor increases in concentration may result from physiological and analytical factors rather than true biochemical deterioration. For example, limited evaporation during storage, slight concentration changes due to water loss, analytical imprecision, or normal instrument variability may contribute to small differences between repeated measurements. Such variations are commonly encountered in clinical chemistry and generally do not affect clinical interpretation when laboratory quality control procedures are appropriately maintained [21].

The present findings are consistent with previous investigations demonstrating that total cholesterol remains stable during refrigerated storage. Muzakova et al. [9], reported that lipid biomarkers exhibit excellent stability even during long-term frozen storage, indicating that cholesterol possesses strong chemical stability compared with many other serum analytes. Similarly,

Razi et al. [22], found that cholesterol concentrations remained stable under appropriate storage conditions, supporting the reliability of delayed biochemical analysis in routine laboratory settings. Although these studies primarily evaluated frozen storage or different patient populations, they consistently demonstrated that cholesterol is among the more stable biochemical parameters.

Comparable findings have also been reported by Shimizu and Ichihara [21], who investigated the stability profiles of common biochemical analytes across various storage temperatures. Their study showed that refrigerated storage effectively preserved the stability of numerous serum analytes by minimizing metabolic activity and slowing chemical degradation. These observations support the current study, in which storage at 2–8°C maintained total cholesterol concentrations with only negligible variation after seven days.

Conversely, previous studies have shown that delayed analysis may become problematic when serum specimens are stored under inappropriate conditions. Marashifard et al. [8], demonstrated that prolonged delays before serum separation may significantly affect several biochemical parameters because ongoing cellular metabolism continues while blood cells remain in contact with serum. Likewise, Chaudhry et al. [23], reported that delayed centrifugation increased analytical variability for several serum chemistry tests, particularly for analytes that are metabolically unstable. These findings highlight that the stability observed in the present study was likely attributable to proper serum separation prior to storage and maintenance of a controlled refrigeration temperature throughout the storage period.

From a clinical perspective, the findings have important implications for laboratory management. Immediate laboratory analysis is not always feasible because of transportation delays, instrument maintenance, high sample workloads, or limited laboratory personnel. Under such circumstances, refrigerated storage represents a practical alternative for preserving specimen quality until analysis can be performed. The present findings suggest that storing serum samples at 2–8°C for up to seven days does not significantly compromise total cholesterol measurements, thereby providing flexibility in specimen management while maintaining analytical reliability. This may reduce unnecessary repeat blood collection, improve laboratory workflow efficiency, and support consistent clinical decision-making.

Nevertheless, statistical interpretation should be performed carefully. Although the p-value obtained in this study (0.052) exceeded the conventional significance threshold of 0.05, it was very close to statistical significance. This finding suggests that the observed difference should not simply be interpreted as evidence of "no effect." Rather, the study indicates that no statistically significant difference was detected within the available sample size. The proximity of the p-value to the significance threshold raises the possibility that the study was underpowered to detect a small effect because only 15 paired serum

samples were included. Increasing the sample size may provide greater statistical power to determine whether the slight increase observed represents true biological variation or merely random measurement variability.

Several limitations should therefore be acknowledged. First, the relatively small sample size limits the generalizability of the findings and reduces statistical power. Second, the study was conducted at a single hospital laboratory using one analytical instrument, which may limit the applicability of the results to laboratories using different analytical platforms. Third, only one storage duration (seven days) and one storage temperature (2–8°C) were evaluated. Other storage periods, freeze–thaw cycles, or transportation conditions were not investigated and may produce different results.

Future studies should include larger multicenter populations, evaluate multiple storage durations and temperatures, and compare different analytical platforms to strengthen the external validity of the findings. In addition, complementary statistical approaches, such as Bland–Altman agreement analysis, Passing–Bablok regression, or total allowable error analysis, would provide a more comprehensive assessment of the analytical agreement between immediate and delayed cholesterol measurements. Such studies would contribute to developing evidence-based guidelines for serum storage in routine clinical laboratories.

Overall, the findings of this study indicate that serum samples stored at 2–8°C for seven days maintain total cholesterol concentrations comparable to those obtained from immediate analysis. Although a slight numerical increase was observed after storage, the difference was not statistically significant, supporting the use of short-term refrigerated storage as an acceptable pre-analytical practice for total cholesterol testing when immediate analysis cannot be performed.

CONCLUSION

This study demonstrated that serum samples stored at 2–8°C for seven days maintained total cholesterol concentrations comparable to those measured immediately after sample collection. Although the mean total cholesterol concentration increased slightly from 203 mg/dL to 206 mg/dL, the paired-samples t-test indicated that this difference was not statistically significant ($p = 0.052$). These findings suggest that short-term refrigerated storage does not substantially affect the analytical stability of total cholesterol and therefore represents an acceptable pre-analytical storage method in routine clinical laboratory practice.

The study contributes additional evidence supporting the stability of total cholesterol during refrigerated storage and reinforces the feasibility of delayed laboratory analysis when immediate testing cannot be performed because of logistical or operational constraints. Nevertheless, the relatively small sample size and single-center design limit the generalizability of the findings. Future studies should include larger multicenter populations, evaluate multiple storage durations and temperature conditions,

and compare different analytical platforms to establish more comprehensive evidence-based guidelines for serum specimen storage and cholesterol analysis.

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