

Effect of Clotting Time Before Centrifugation on Total Cholesterol: A Cross-Sectional Study

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ABSTRACT

Introduction: Pre-analytical variables are a major source of laboratory testing error, with clotting time before centrifugation representing a potentially critical factor in serum-based biochemical analysis. Although total cholesterol is considered a relatively stable analyte, minor operational variations in clotting time may affect measurement accuracy. This study aimed to determine whether differences in clotting time (30 minutes versus 60 minutes) before centrifugation affect total cholesterol results under controlled laboratory conditions.

Research Methodology: A quantitative analytic study with a paired cross-sectional design was conducted in a clinical chemistry laboratory from June to August 2025. Eighteen adult participants were recruited using purposive sampling. For each participant, venous blood samples were divided into two conditions: clotting for 30 minutes and 60 minutes before centrifugation. Total serum cholesterol was measured using the enzymatic CHOD-PAP method. Data were analyzed using descriptive statistics and a Paired Sample T-Test with $\alpha = 0.05$.

Results: The mean total cholesterol level after 30 minutes of clotting was 141.92 ± 18.45 mg/dL, compared to 146.33 ± 19.12 mg/dL after 60 minutes. The mean difference was 4.41 mg/dL (3.10%). Statistical analysis showed no significant difference in clotting duration between the two groups ($p = 0.179$).

Conclusion: Extending clotting time from 30 to 60 minutes before centrifugation does not significantly affect total cholesterol measurement. These findings support analytical stability within this interval and may inform evidence-based refinement of pre-analytical laboratory procedures.

Keywords: Blood Specimen Collection; Cholesterol; Clinical Laboratory Techniques; Preanalytical Phase; Serum.



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INTRODUCTION

Total cholesterol measurement remains one of the most frequently requested biochemical tests in clinical laboratories due to its central role in cardiovascular risk assessment [1]. Accurate quantification of serum cholesterol is essential for diagnosis, therapeutic monitoring, and epidemiological screening of dyslipidemia [2]. However, the reliability of laboratory results is not determined solely by analytical performance. Evidence consistently demonstrates that the pre-analytical phase accounts for the majority of laboratory errors, contributing up to 60–70% of total testing inaccuracies [3]. Among pre-analytical variables, clotting time before centrifugation represents a critical yet often underestimated factor in serum-based biochemical assays. In routine practice, delays in sample processing may occur due to high workload, transportation constraints, or equipment availability, potentially affecting serum quality and analyte stability [4].

Theoretical and experimental literature suggests that improper clotting duration may influence serum composition. Insufficient clotting time can result in incomplete fibrin formation, residual fibrin strands, or microclots that interfere with automated photometric analysis. Conversely, excessive clotting time may promote evaporation, blood cell metabolism, or subtle biochemical alterations [5]. Previous studies investigating pre-centrifugation delays have primarily focused on extended processing times, often ranging from several hours to eight hours post-collection. These studies generally report that total cholesterol and lipoprotein fractions are relatively stable compared to more labile analytes such as glucose or enzymes [6]. However, many of these investigations evaluate long delays rather than short, clinically relevant clotting intervals commonly encountered in laboratory workflows [7].

Moreover, heterogeneity in methodological design limits direct extrapolation of existing findings. Some studies employ advanced analytical platforms such as nuclear magnetic resonance (NMR), while others assess broad chemistry panels without isolating clotting time as an independent variable [8]. In several reports, clotting duration is embedded within the overall processing delay, making it difficult to distinguish the specific impact of the clotting interval before centrifugation. Additionally, variations in temperature control, tube type, centrifugation parameters, and analytical method (e.g., enzymatic CHOD-PAP) introduce further variability [9]. As a result, although cholesterol is considered relatively stable, the evidence base remains fragmented regarding short clotting intervals within the standard 30–60 minute window recommended by clinical laboratory guidelines [10].

An explicit research gap therefore emerges: there is limited empirical evidence directly comparing total cholesterol results between two short, operationally relevant clotting durations, 30 minutes and 60 minutes, under controlled centrifugation conditions [11]. Most available literature addresses prolonged pre-analytical delay or alternative tube technologies, rather than evaluating whether extending clotting time from the commonly recommended minimum (30 minutes) to a longer yet practical interval (60 minutes) produces measurable analytical variation [12]. Given that laboratory standard operating procedures (SOPs) require evidence-based justification, clarification of this interval is necessary to support quality assurance and reproducibility.

The novelty of the present study lies in its focused evaluation of short-term variation in clotting interval as an isolated pre-analytical factor affecting total cholesterol measurement using a standardized enzymatic photometric method. By applying a paired cross-sectional design, this research controls inter-individual biological variability and directly assesses within-sample analytical differences. This targeted approach contributes context-specific evidence

relevant to routine clinical laboratory practice, particularly in settings where minor delays before centrifugation are unavoidable. Accordingly, the objective of this study was to compare total cholesterol examination results in blood samples allowed to clot for 30 minutes and 60 minutes before centrifugation, and to determine whether the difference between these intervals is statistically and clinically significant within standard laboratory conditions.

MATERIALS AND METHODS

Study Design

This study employed a quantitative analytic design with a paired cross-sectional approach. The paired design was selected to minimize inter-individual biological variability by comparing two clotting time conditions (30 minutes and 60 minutes) within the same participant. This design is appropriate for evaluating pre-analytical procedural effects on biochemical measurement under controlled laboratory conditions.

Setting and Participants

The study was conducted at the Clinical Chemistry Laboratory of Politeknik Sandi Karsa Makassar, Indonesia, from June to August 2025. The study population consisted of adult volunteers who met the eligibility criteria. Inclusion criteria were: (1) age ≥ 18 years, (2) willing to participate and sign informed consent, and (3) no visible hemolysis in collected specimens. Exclusion criteria included: (1) insufficient sample volume, (2) hemolyzed, lipemic, or icteric serum, and (3) history of acute illness at the time of blood collection. A purposive sampling technique was used. The final sample comprised 18 participants. Sample size determination was based on feasibility and a paired comparison design, consistent with minimal sample recommendations for parametric paired statistical testing in laboratory-based analytic studies.

Variables and Measurement

The independent variable was clotting time before centrifugation, operationally defined as the duration (30 minutes and 60 minutes) during which whole blood was allowed to clot at room temperature (20–25°C) before centrifugation. The dependent variable was total serum cholesterol concentration (mg/dL), measured using the enzymatic photometric CHOD-PAP (Cholesterol Oxidase–Peroxidase Aminophenazone) method. Blood samples (± 3 mL) were collected via venipuncture into plain tubes without anticoagulant. Each specimen was divided into two treatment conditions: clotting for 30 minutes or 60 minutes, followed by centrifugation at 3500 rpm for 10 minutes. Instrument calibration was performed before analysis, according to the manufacturer's guidelines. Internal quality control (IQC) materials were analyzed daily to ensure analytical accuracy and precision. Instrument validity was ensured through routine calibration and compliance with the laboratory's standard operating procedures (SOPs). Duplicate quality control measurements and stable control values within acceptable ranges supported reliability. Primary data were obtained directly from laboratory measurement results. No secondary data sources were used.

Data Collection Procedures

Venous blood was collected under aseptic conditions by trained laboratory personnel. After collection, samples were allowed to clot at room temperature. The first aliquot was centrifuged after 30 minutes, and the second aliquot after 60 minutes. Serum was separated carefully to avoid cellular contamination. Total cholesterol measurement was performed using a calibrated photometer. All procedures followed standardized laboratory protocols. Quality control measures included temperature monitoring, equipment maintenance verification, adherence to centrifugation parameters, and rejection of unsuitable specimens.

Data Analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 26. Descriptive statistics were presented as mean and standard deviation (SD). Normality of data distribution was assessed using the Shapiro–Wilk test. Since the data were normally distributed ($p > 0.05$), inferential analysis was conducted using a Paired-Samples T-Test to compare mean total cholesterol levels between the 30-minute and 60-minute clotting conditions. Statistical significance was set at $\alpha = 0.05$.

Ethical Approval

Ethical approval for this study was obtained from the Health Research Ethics Committee of Politeknik Sandi Karsa Makassar (Approval No.: 045/KEPK-PSK/V/2025). All participants received a full explanation regarding the study objectives and procedures. Written informed consent was obtained before blood collection. Confidentiality of participant identity and laboratory results was strictly maintained throughout the study.

RESULTS

A total of 18 participants were included in the analysis. All collected specimens met the pre-analytical quality criteria (no hemolysis, adequate volume, and clear serum after centrifugation).

Table 1. Baseline Characteristics of Participants (n = 18)

Variable	Mean $\pm$ SD / n (%)
Age (years)	22.8 $\pm$ 2.1
Male	8 (44.4%)
Female	10 (55.6%)
Baseline Total Cholesterol (30 min clotting) (mg/dL)	141.92 $\pm$ 18.45
Total Cholesterol (60 min clotting) (mg/dL)	146.33 $\pm$ 19.12

The participants were predominantly young adults with a relatively balanced sex distribution. The mean total cholesterol level after 30 minutes of clotting was 141.92 mg/dL, and after 60 minutes, 146.33 mg/dL, indicating a mean difference of 4.41 mg/dL (3.10%).

Normality testing using the Shapiro–Wilk test demonstrated that both data sets were normally distributed ($p > 0.05$). Therefore, the Paired Sample T-Test was applied to compare the mean cholesterol levels between the two clotting durations.

Table 2. Comparison of Total Cholesterol Levels Between 30 and 60 Minutes Clotting Time

Variable	Mean $\pm$ SD (mg/dL)	Mean Difference (mg/dL)	95% CI	p-value
30-minute clotting	141.92 $\pm$ 18.45			
60-minute clotting	146.33 $\pm$ 19.12	4.41	-2.14 to 10.96	0.179

The Paired Sample T-Test showed no statistically significant difference in total cholesterol levels between samples allowed to clot for 30 minutes and 60 minutes ($p = 0.179$; $\alpha = 0.05$). Although the 60-minute clotting time yielded slightly higher mean values, the difference was small (3.10%) and remained within clinically acceptable variation limits. These findings indicate that extending clotting time from 30 to 60 minutes before centrifugation does not significantly affect total cholesterol measurement under controlled laboratory conditions.

This study demonstrated that total cholesterol levels measured after 30 minutes and 60 minutes of clotting time before centrifugation showed minimal variation. The mean difference between the two conditions was 4.41 mg/dL (3.10%), with slightly higher values observed in the 60-minute group. However, the Paired Sample T-Test indicated that this difference was not significant ($p = 0.179$; $\alpha = 0.05$). These findings suggest that extending clotting time from 30 to 60 minutes does not produce a statistically or clinically meaningful change in total

cholesterol measurement under controlled laboratory conditions. Therefore, both clotting durations remain analytically acceptable within routine clinical laboratory practice, provided that other pre-analytical procedures are standardized and consistently implemented.

DISCUSSION

This study evaluated whether extending blood clotting time from 30 minutes to 60 minutes before centrifugation influences total cholesterol measurement under controlled laboratory conditions. The findings demonstrated a small mean increase of 4.41 mg/dL (3.10%) in the 60-minute clotting condition compared to the 30-minute condition. However, this difference was not statistically significant ($p = 0.179$) and remained within clinically acceptable analytical variation limits. These results indicate that clotting durations of 30–60 minutes do not produce meaningful alterations in total cholesterol values when centrifugation and analytical procedures are standardized. Beyond the statistical outcome, the study provides evidence that short, operationally realistic variations in clotting time may be tolerable within routine clinical laboratory workflows. This has implications for pre-analytical standardization, particularly in settings where minor delays before centrifugation are unavoidable [13].

Pre-analytical variables represent the most vulnerable phase in laboratory testing, accounting for the majority of total testing errors. Clotting time before centrifugation is biologically relevant because serum formation depends on complete fibrin network stabilization [14]. Inadequate clotting may result in residual fibrin strands, microclots, or incomplete cellular separation, potentially interfering with photometric detection methods. Conversely, prolonged clotting time theoretically permits ongoing cellular metabolism, subtle diffusion of intracellular components, or evaporative concentration changes [15].

Total cholesterol, however, is biochemically categorized as a relatively stable analyte. Unlike glucose or certain enzymes, which are rapidly influenced by glycolysis or cellular leakage, cholesterol is structurally incorporated into lipoprotein particles and is less susceptible to short-term metabolic shifts [16]. The enzymatic CHOD-PAP method further enhances analytical specificity by relying on cholesterol oxidase and peroxidase reactions that selectively quantify cholesterol-derived hydrogen peroxide [17]. Therefore, small temporal differences in clot stabilization may not substantially alter cholesterol concentration within a 30–60 minute window. From a kinetic perspective, once coagulation is completed and serum is separated, the lipid fraction remains largely unchanged, provided that temperature is controlled and hemolysis is avoided [18]. The absence of a significant difference in this study supports the theoretical expectation that cholesterol stability is maintained during short clotting intervals at room temperature [19].

International literature generally reports that total cholesterol is relatively stable compared to labile metabolites [20]. Several studies assessing delayed centrifugation for extended durations ranging from hours to several hours have found minimal clinically relevant changes in lipid profiles [21]. Investigations using advanced platforms, such as nuclear magnetic resonance (NMR), have similarly shown that lipoprotein fractions remain stable during short-term processing delays [22]. Regional studies in Southeast Asia evaluating pre-analytical variables have focused primarily on temperature variation, transport delays, and long processing intervals, rather than specifically isolating clotting duration within the first hour post-collection. Some studies report statistically detectable changes after prolonged delay; however, those differences often remain clinically negligible [23]. The present study contributes by isolating clotting time as an independent pre-analytical factor within a narrowly defined operational range (30–60 minutes), thereby addressing a practical laboratory scenario that is underrepresented in prior research [24].

The findings converge with prior evidence suggesting that lipid parameters are more stable than glucose or enzymatic markers under short pre-analytical delays. The absence of a significant difference reinforces the classification of total cholesterol as a robust analyte in the early post-collection phase [25]. However, some studies have reported subtle increases in

analyte concentration with prolonged clot contact due to evaporative or concentration effects. The slight mean increase observed in the 60-minute condition in this study aligns directionally with this phenomenon, although the magnitude was small and statistically non-significant. This suggests that while theoretical mechanisms may permit minor drift, such variation remains within analytical tolerance limits under controlled conditions. Any divergence between this study and those reporting significant changes may be attributable to differences in environmental temperature, tube type, centrifugation delay exceeding one hour, or analytical platform variability. Therefore, context-specific procedural control remains critical.

Conceptually, this study strengthens the evidence base surrounding the pre-analytical robustness of total cholesterol measurement. It supports a quality management perspective in which certain short-duration deviations from minimum clotting recommendations may not compromise analytical validity. This contributes to laboratory governance by distinguishing between critical and non-critical pre-analytical variables. From a systems perspective, these findings may inform laboratory standard operating procedures (SOPs). In high-throughput laboratories or resource-limited settings where immediate centrifugation is not always feasible, allowing flexibility within a 30–60 minute clotting window could enhance workflow efficiency without sacrificing result reliability. This has implications for accreditation standards, internal quality assurance, and risk-based laboratory management. Moreover, understanding analyte-specific stability supports a differentiated pre-analytical strategy. Rather than applying uniform urgency across all tests, laboratories may prioritize rapid processing for labile analytes while recognizing acceptable tolerance for stable markers such as cholesterol. This risk-stratified approach aligns with modern laboratory quality frameworks emphasizing evidence-based decision-making.

Strengths and Limitations

A major strength of this study lies in its paired design, which minimizes inter-individual biological variability by comparing two clotting conditions within the same participant. This enhances internal validity and isolates clotting time as the primary experimental variable. Standardized centrifugation parameters, controlled room temperature, and use of internal quality control further strengthen methodological reliability. However, several limitations should be acknowledged. First, the sample size was relatively small, which may have limited the statistical power to detect very small differences. Second, the study population consisted primarily of young adults, potentially limiting generalizability to older or dyslipidemic populations with extreme lipid concentrations. Third, only one analytical method (CHOD-PAP) was used; alternative platforms might exhibit different sensitivity to pre-analytical variation. Fourth, environmental factors were controlled within a laboratory setting and may not reflect field conditions with variable temperature exposure. Future research should increase sample size, include a broader range of demographics, and examine additional lipid fractions, such as LDL and HDL, under similar pre-analytical conditions. Multicenter studies could provide stronger external validity and inform guideline development. In conclusion, this study contributes conceptually and operationally to laboratory medicine by demonstrating that total cholesterol measurement remains analytically stable over a 30–60-minute clotting interval before centrifugation. The findings support evidence-based refinement of pre-analytical standards and promote risk-informed laboratory practice.

CONCLUSIONS

This study aimed to determine whether differences in clotting time before centrifugation (30 minutes versus 60 minutes) affect total cholesterol measurement results. The findings demonstrate that extending clotting time from 30 to 60 minutes does not produce a statistically significant or clinically meaningful difference in total cholesterol levels under controlled laboratory conditions. Although a slight numerical increase was observed at 60 minutes, the variation remained within acceptable analytical limits. These results indicate that total cholesterol is analytically stable within a 30–60-minute clotting interval when standard centrifugation and quality control procedures are properly implemented. Therefore, minor

delays within this timeframe are unlikely to compromise the validity. From a policy perspective, laboratory standard operating procedures may incorporate evidence-based flexibility in clotting duration for stable analytes such as total cholesterol. However, continued adherence to quality control protocols and further multicenter studies are recommended to strengthen generalizability and support guideline refinement in clinical laboratory practice.

Ethical Considerations

This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Ethical approval was obtained from the Health Research Ethics Committee of Politeknik Sandi Karsa Makassar (Approval No.: 045/KEPK-PSK/V/2025). All participants received a detailed explanation of the study objectives, procedures, potential risks, and benefits before enrollment. Written informed consent was obtained before blood sample collection. Participant confidentiality was ensured by anonymizing all data and restricting access to research records to the study investigators only.

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Conflict of Interest

The authors declare that there are no conflicts of interest related to this study.

Authors' Contributions

Fontianus Nong Melky: Conceptualization, Methodology, Investigation, Data Curation, Formal Analysis, Writing – Original Draft.

Nuril Sofiantin: Supervision, Validation, Writing – Review & Editing, Project Administration.

Suprpto: Methodology Support, Resources, Data Verification.

Marisca Jenice Sanakya: Investigation Support, Data Curation, Writing – Review.

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