COMPARISON OF TRIGLYCERIDE LEVELS BETWEEN SERUM STORED AT -20°C AND SERUM AT 25°C FOR 2 DAYS AT TOTO KABILA REGIONAL GENERAL HOSPITAL

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ABSTRACT

The pre-analytical phase carries a relatively high risk of error, accounting for 50%-75% of total laboratory errors, including those related to sample storage. In triglyceride testing, storage conditions are crucial as they can affect serum stability . This study compares triglyceride levels between serum samples stored at -20°C and those stored at 25°C for two days at Toto Kabila Regional General Hospital. The study employed a quantitative approach with an analytical observational design, using 16 serum samples from hospital patients. Data were analyzed using the Independent Sample T-test. The results showed that the average triglyceride level in serum stored at -20°C was 131.83 mg/dL, while in serum stored at 25°C it was 188.48 mg/dL. The Independent Sample T-test yielded a significance value of 0.023 (<0.05), indicating a significant difference between the two storage temperatures. These findings confirm that storage temperature affects triglyceride stability, therefore, maintaining the validity and reliability of low-temperature storage is recommended to maintain the laboratory test results.

Keywords: Triglycerides, Serum, -20°C 25°C

INTRODUCTION

Analysis of triglyceride levels in the blood is one of the types of tests commonly performed in clinical laboratories. Triglycerides are a type of fat formed through an esterification process involving one molecule of glycerol and three molecules of fatty acids. This test aims to determine the concentration of triglycerides in a person's blood, whether high or low. Physiologically, triglycerides play an important role as the main source of energy for the heart and skeletal muscles, as well as functioning as an energy reserve capable of producing a large amount of adenosine triphosphate (ATP)[1] .

The pre-analytical stage is the most critical component in the entire laboratory testing process, as it is estimated to contribute around 60–70% of all potential errors that may occur. Errors related to patient preparation, sample collection methods, anticoagulant selection, temperature control, storage, and specimen transportation are examples of errors at this stage that can lead to inaccurate test results. These pre-analytical errors have direct implications for the accuracy of the diagnostic process and can influence clinical decision-making. Therefore, the pre-analytical stage requires meticulous attention and supervision[2] .

Triglycerides themselves are fat molecules circulating in the bloodstream. High triglyceride levels have been shown to have a significant association with an

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increased risk of cardiovascular disease. Several factors that can affect triglyceride levels include smoking, a diet low in fruits and vegetables, coffee consumption, an inactive lifestyle, excessive alcohol consumption, obesity, and gender differences[3] .

Triglyceride levels that exceed the normal threshold can be an early indication of hyper triglyceridemia, a condition closely related to metabolic syndrome and obesity. This condition is often accompanied by insulin resistance, which, if it continues, can develop into type 2 diabetes mellitus. Thus, maintaining triglyceride levels within the normal range is an important step in maintaining metabolic health[4]. It is crucial to inform the public about the significance of maintaining a balanced diet and exercising regularly as preventive measures in regulating triglyceride levels [5] .

In laboratory testing practices, serum or plasma specimens should be analyzed immediately after sample collection. Delays between collection and testing can affect enzyme stability and the concentration of dissolved substances in the sample. However, implementing this procedure is not always easy due to external factors, such as the length of the transportation process, improper sample storage, or storage at inappropriate temperatures, which can cause inaccurate test results[6] .

Delays in testing or storing specimens at inappropriate temperatures can potentially reduce the accuracy of the analysis results. Such errors can have an impact on patient management, for example, in making therapeutic decisions based on triglyceride levels that do not reflect the actual condition. Therefore, serum samples for triglyceride testing must be stored at the appropriate temperature—usually cold or frozen—and analyzed within the recommended time frame to prevent degradation or changes in lipid levels[7] .

According to the guidelines of the Indonesian Ministry of Health (2013), specimens that cannot be examined immediately must be stored according to the type of test. Storage can be done at room temperature, refrigerator temperature (2–8°C), or frozen (-20°C to -120°C) provided that they are not refrozen. The ideal storage for triglyceride testing is in serum form.

Serum for triglyceride testing can remain stable for five to seven days at temperatures between 2 and 8 degrees Celsius, as stated by Nugraha and Badrawi (2018). On the other hand, research by Hedayati et al. (2020) and Maulidiyanti et al. (2021) shows that serum stored at the same temperature remains stable for one to two weeks[6] .

Based on these circumstances, the researchers decided to conduct a study entitled "Comparison of Triglyceride Levels between Serum Stored at –20 °C and 25 °C for 2 Days at Toto Kabila Regional General Hospital."

Observations at Toto Kabila Regional General Hospital indicate that blood serum samples are generally stored in refrigerators. In a study conducted by Maulidiyanti and colleagues (2019), triglyceride levels in serum stored in a freezer showed stability, consistent with the results found during storage periods of 1 to 2 weeks at temperatures of 2 to 8 degrees Celsius, exposure to sunlight, microorganism

contamination, inappropriate storage temperatures, and blood cell metabolic activity[8].

Both whole blood and serum can be stored as blood samples. Serum stored at 2–8°C usually remains stable for 5–7 days, while whole blood remains stable for only 24 hours at the same temperature. The fundamental difference in this study compared to previous studies lies in its focus on directly comparing triglyceride levels between serum stored at -20°C and 25°C. The purpose of this study is to investigate the effect of storage temperature variations on triglyceride level stability, thereby contributing to scientific knowledge regarding the optimal storage conditions for serum in triglyceride level testing.

RESEARCH METHODS

This study used a quantitative approach with an analytical observational type to compare triglyceride levels in serum stored at −20°C and 25°C for two days at Toto Kabila Regional General Hospital. A total of 16 serum samples were used, each divided into two storage temperature groups. Primary data were obtained from laboratory test results using the enzymatic colorimetry method (GPO–PAP) with an analyzer, while secondary data were obtained from supporting literature. Data analysis was performed using SPSS through an Independent Sample T-Test with a significance level of 0.05 to determine the difference in triglyceride levels between the two storage conditions.

RESEARCH RESULT

From the results of the research conducted for 26 days starting from July 20, 2025 to August 14, 2025, based on the examination of erythrocyte index in pulmonary tuberculosis patients at Mokoyurli Buol Regional General Hospital with the following results details:

1. Univariate Analysis

Table 1. Sample Distribution Based on Storage

Storage	Frequency (f)	Percentage (%)
Temperature -20º C	8	50
Temperature 25ºC	8	50.0
Total	**16**	**100.0**

(*Source*: Primary Research Data, 2025).

Based on Table 4.1, the results of triglyceride level distribution based on storage conditions show that 8 samples (50.0%) were stored at −20°C and 8 samples (50.0%) were stored at
25°C, bringing the total number of samples analyzed to 16 samples (100%).

a. Description of Triglyceride Level Test Results Stored at -20º C and Stored at 25º C for 2 Days

Table 2. Categories of triglyceride level test results at −20°C and at 25°C

No	Triglyceride Level Category	Triglyceride Level			
		-20°C		25ºC	
	Triglyceride	F	%	F	%
1	Normal	8	100	2	25
2	Abnormal	0	0	6	75
	Number	8	100	8	100

(*Source*: Primary Research Data, 2025).

Based on Table 4.2, it is known that of the 8 serum samples stored at −20 °C, all (100%) showed normal results. Meanwhile,

of the 8 serum samples stored at 25 °C, only 2 samples (25%) were in the normal category, while 6 samples (75%) showed abnormal results.

b. Description of Average Triglyceride Levels Stored at -20°C and Stored at 25°C for 2 Days.

Table 3. Average triglyceride levels in serum stored at -20°C for 2 days

Respondents	Age (Years)	JK	Tg level (mg/dl)
			Temperature -20oC
Mrs. RM	28	P	135.09
Mrs. AI	25	P	120.56
Mr. ZD	30	L	134.38
Mr. IZ	29	M	138.63
Mrs. AT	26	P	129.89
Mrs. KA	21	P	120.86
Mr. RH	33	M	130.19
Mr. HD	29	M	145.11
Average			131.83

(*Source*: Primary Research Data, 2025).

Based on Table 4.3, the results show that triglyceride levels in serum stored at −20 °C ranged from 120.56 mg/dL to 145.11 mg/dL, with an average value of 131.83 mg/dL. All values were below the reference limit of 150 mg/dL.

Table 4. Average triglyceride levels in serum stored at 25°C for 2 days

Respondents	Age (Years)	JK	Triglyceride Level (mg/dL)
			Temperature 25oC
Mrs. RM	28	P	94.68
Mrs. AI	25	P	156.54
Mr. ZD	30	L	283.84
Mr. IZ	29	M	263.30
Mrs. AT	26	P	180.00
Mrs. KA	21	P	169.05
Mr. RH	33	L	211.26
Mr. HD	29	M	149.19
Average			188.48

(*Source*: Primary Research Data, 2025).

As shown in Table 4.3, over two days, the average triglyceride levels varied significantly. The average value at -20°C was 131.83 mg/dL and at 25°C was 188.48 mg/dL.

Table 5. Measures of Central Tendency for Numerical Data

Temperature	N	Mean ± SD	Min	Max
-20°C	8	131.83 ± 8.39	120.56	145.11
25º C	8	188.48 ± 6213	94.68	283.84

(*Source*: Primary Research Data, 2025).

2. Bivariate Analysis

a. Normality Test

Table 6. Normality Test Results

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Suhu Penyimpanan	Statistic	df	Sig.	Statistic	df	Sig.
Kadar Trigliserida	-20 Derajat Celcius	0.158	8	0.200*	0.947	8	0.684
	25 Derajat Celcius	0.179	8	0.200*	0.956	8	0.774

(*Source*: Primary Research Data, 2025).

In this study, the Shapiro-Wilk normality test was used. The purpose of this analysis was to evaluate the distribution of triglyceride levels in serum stored for two days at two temperature conditions, namely –20°C and 25°C, as shown in Table 4.6.

The results showed that both variables had significance values (p-values) greater than 0.05, indicating that the data followed a normal distribution. For serum stored at

−20°C, the Shapiro-Wilk statistic value was 0.947, with degrees of freedom (df) = 8 and p-value = 0.684. Meanwhile, for serum stored at 25°C, the Shapiro-Wilk statistic value was recorded at 0.956, with df = 8 and p-value = 0.774. These results indicate that both triglyceride level data variables can be analyzed using parametric tests, as the normality assumption is met.

Therefore, since the p-value is > 0.05 in each group, it can be concluded that the data on triglyceride levels at both storage temperatures are normally distributed. Therefore, the parametric test using the Independent Samples T-Test is appropriate.

b. Research Hypothesis Test

Table 7. Hypothesis Test Results (Independent Sample T-test)

Independent Samples Test

		Levene's Test for Equality of Variances				
		F	Sig.	t	df	Sig. (2-tailed)
Kadar Trigliserida	Equal variances assumed	11.408	0.005	-2.555	14	0.023
	Equal variances not assumed			-2.555	7.256	0.037

(*Source*: Primary Research Data, 2025).

Based on Table 4.7, the results of the Independent Sample T-Test were used to compare triglyceride levels in serum stored at two different temperatures, namely −20°C and 25°C.

A significance value (Sig. 2-tailed) of 0.023 was found in the Same Assumption Variation section, which is lower than 0.05. This indicates that the Null Hypothesis (H_0) is rejected and the Alternative Hypothesis (H_1) is accepted. Thus, it can be concluded that triglyceride levels in serum stored at temperatures of −20°C and 25°C differ significantly.

Thus, the results of this analysis indicate that differences in storage temperature affect triglyceride levels in serum, where temperature changes can affect the stability and activity of biochemical components in blood samples.

DISCUSSION

This study was conducted at Toto Kabila Regional General Hospital in Bone Bolango Regency, Gorontalo Province, using 8 blood samples taken directly from patients. Each blood sample was then centrifuged to obtain serum, resulting in a total of 16 serum cups after each sample was divided into two parts. For two days, one part was stored at −20°C and the other at 25°C. The purpose of this study was to compare triglyceride levels based on different storage conditions.

The results showed that all eight serum samples stored at −20°C were within the normal range. This finding is consistent with the study by Ellies et al. (2021), which stated that triglyceride levels in serum stored at −20°C are relatively stable because low temperatures help maintain enzyme activity balance. Enzymes remain inactive at low temperatures[9] .

Meanwhile, of the eight serum samples stored at 25°C, six samples were classified as abnormal. Research by Salsabillah et al. (2024) supports this, stating that storing serum at room temperature for several days can cause an increase in triglyceride levels. This occurs due to changes in lipoprotein concentration and lipoprotein electrophoresis mobility. Additionally, delayed testing can activate the lipoprotein lipase (LPL) enzyme, which is responsible for converting triglycerides into fatty acids

and glycerol, thereby increasing triglyceride levels. However, there were 2 serum samples that remained below 150 mg/dL. Faizah et al. (2017) explained that delays in testing can affect triglyceride levels, so samples must be analyzed as soon as possible to keep metabolites, enzymes, and electrolytes stable[10] . In these two samples, low initial levels and low enzyme activity likely kept triglyceride levels normal[11] .

After the data was obtained, it was processed using the SPSS application to analyze the difference in triglyceride levels between serum stored at −20°C and 25°C for 2 days. The analysis began with a frequency test to see the distribution of samples based on storage conditions. Next, a normality test (Explore Test) was performed because the data was normally distributed.

The results showed that the Sig value was 0.023, less than 0.05. Since H0 was rejected and H1 was accepted, serum stored for two days at −20°C and 25°C showed high triglyceride levels.

This difference is caused by storage conditions that affect the stability of serum biochemical components. At −20°C, enzymes remain inactive and the degradation process can be prevented, so triglyceride levels remain normal (Hedayati et al., 2020). Conversely, at 25°C, in addition to changes in lipoprotein concentration during storage, the activity of lipoprotein lipase, an enzyme that converts triglycerides into glycerol and fatty acids, causes triglyceride levels to increase[12].

Disintegration time and rapid dissolution rate are important indicators of the bioavailability of active substances.[13]

CONCLUSION

To analyze the difference in triglyceride levels between serum stored at −20°C and 25°C for 2 days.

1. Eight serum samples stored at −20°C showed triglyceride levels between 120.56 mg/dL and 145.11 mg/dL, with an average of 131.83 mg/dL. All samples were within the normal range (<150 mg/dL), indicating that storage at low temperatures was able to maintain the stability of triglyceride levels in serum.
2. Eight triglyceride serum samples stored at 25°C showed an average triglyceride level of 188.48 mg/dL, with a minimum level of 94.68 mg/dL and a maximum level of 283.84 mg/dL. Two samples were categorized as normal (less than 150 mg/dL) and six samples were categorized as high (more than 150 mg/dL). This indicates that changes in enzymatic activity during serum storage at room temperature can cause an increase in triglyceride levels.
3. The results of the T-free sample test showed that the Sig. (2-tailed) value = 0.023, which is less than 0.05. Therefore, H_0 is rejected and H_1 is accepted; both indicate significant serum triglyceride levels during two days of storage at 20 °C and 25 °C. Storage temperature was found to affect the stability of serum triglyceride levels.

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