

## Overview of Hemoglobin Levels Using the Cyanmethemoglobin Method in Lipidemia Specimens at the East Kalimantan Provincial Health Laboratory

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### ABSTRACT

**Background:** Hemoglobin level measurement using the cyanmethemoglobin method is affected by lipemic samples. Lipemic serum, caused by lipoprotein particles such as chylomicrons, VLDL, and triglycerides, creates chromophoric interference in photometric analysis, affecting wavelength readings and causing light scattering due to lipid particles.

**Methods:** An observational analytic study using a total sampling technique was conducted at the East Kalimantan Provincial Health Laboratory during August–September 2024. A total of 30 lipemic specimens were included, collected from patients undergoing routine blood examination with cholesterol levels >200 mg/dL. Data were obtained from secondary medical records.

**Results:** Of 30 lipemic samples, 28 samples (93%) showed hemoglobin levels within the normal range and 2 samples (7%) were below normal. The minimum hemoglobin value was 12.0 g/dL, maximum was 15.7 g/dL, and the mean was 13.7 g/dL. Regarding cholesterol levels, 22 samples (73%) had cholesterol of 201–250 mg/dL and 8 samples (27%) had cholesterol of 251–300 mg/dL.

**Conclusion:** Hemoglobin measurement in lipemic serum using the cyanmethemoglobin method can serve as an accurate reference. The average results showed no values outside the normal range. The two samples below normal may be attributable to unknown confounding variables such as dietary habits, smoking behavior, and age.

### ABSTRAK

**Latar Belakang:** Pemeriksaan kadar hemoglobin metode sianmethemoglobin dipengaruhi sampel lipemik. Serum lipemik disebabkan partikel lipoprotein seperti cylumicrons, VLDL maupun trigliserida yang menyebabkan gangguan kromoforik dalam analisis fotometri, gangguan pada panjang gelombang dan hamburan cahaya akibat adanya partikel lipid.

**Metode:** Penelitian observasional analitik menggunakan teknik total sampling dilakukan di Laboratorium Kesehatan Provinsi Kalimantan Timur pada Agustus–September 2024. Sebanyak 30 spesimen lipemik dari pasien pemeriksaan darah rutin dengan kadar kolesterol >200 mg/dL diikutsertakan. Data diperoleh dari rekam medis sekunder.

**Hasil:** Dari 30 sampel lipemik, diperoleh kadar hemoglobin pada 28 sampel (93%) dalam batas normal dan 2 sampel (7%) di bawah nilai normal. Nilai minimum Hb adalah 12,0 g/dL, maksimum 15,7 g/dL, dengan rata-rata 13,7 g/dL. Kadar kolesterol 22 sampel (73%) berada pada rentang 201–250 mg/dL dan 8 sampel (27%) pada rentang 251–300 mg/dL.

**Kesimpulan:** Pemeriksaan kadar hemoglobin pada serum lipemik dengan metode sianmethemoglobin dapat dijadikan referensi yang akurat. Rata-rata hasil tidak menunjukkan nilai di luar batas normal. Dua sampel yang di bawah normal kemungkinan disebabkan variabel lain seperti pola makan, kebiasaan merokok, dan usia.

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## INTRODUCTION

The World Health Organization's (WHO) Global Health Observatory (GHO) shows that the prevalence of dyslipidemia in 2008 was 37% in the male population and 40% in the female population, and is thought to be responsible for 2.6 million deaths and causes 29.7 million people to experience helplessness each year (Health & Observatory, 2018). In Indonesia, Riskesdas data in 2013 shows that 35.9% of the population aged >15 years have abnormal cholesterol levels (>200 mg/dL), with a higher prevalence in women and urban dwellers. Riskesdas also recorded 15.9% of the population with very high LDL proportions ( $\geq 190$  mg/dL), 22.9% with HDL levels <40 mg/dL, and 11.9% with very high triglyceride levels (>500 mg/dL) (RISKESDAS, 2013).

Dyslipidemia examination using lipidic specimens can interfere with spectrophotometric readings, interact with analytes physically and chemically, and interfere with antigen-antibody reactions on immunoserological examinations. Detecting lipemia in complete blood specimens (DL) is more difficult than in serum. Serum lipemics can be seen at triglyceride concentrations >300 mg/dL, while new DL specimens can be seen at triglyceride levels >1000 mg/dL, so lipemia in DL specimens is often undetectable (Dian, 2018).

The DL specimen includes several examinations, one of which is hemoglobin. Hemoglobin examination using the cyanmethemoglobin method at the East Kalimantan Provincial Health Laboratory was carried out on Medical Check Up (MCU) patients. The cyanmethemoglobin method is a reference method for hemoglobin estimation because it can measure all types of hemoglobin except sulfhemoglobin, with an error factor of  $\pm 2\%$  (Norsiah, 2015). The principle of examination: heme (ferro) is oxidized by potassium ferrisianide into (ferri) methemoglobin, then reacts with cyanide ions to form brown cyanmethemoglobin whose absorbance is measured at 540 nm (Norsiah, 2015).

Hemoglobin levels are influenced by various factors, both body factors and laboratory factors. Laboratory factors are divided into three stages: pre-analytical (61%), analytical (25%), and post-analytical (14%) errors (Praptomo, 2018). Serum lipemic is one of the pre-analytical interferences that can increase absorbents and produce high false hemoglobin levels. Based on this background, this study aims to determine the description of hemoglobin levels using the cyanmethemoglobin method in lipidemia specimens at the East Kalimantan Provincial Health Laboratory.

## METHOD

### Types of Research

The type of research used is observational analysis because only observation and analysis of the relationship between variables are carried out without treatment or intervention. This research is quantitative descriptive.

### Research Location and Time

The research was conducted at the East Kalimantan Provincial Health Laboratory in August-September 2024.

### Population and Sample

The study population was all patients who had routine blood tests with cholesterol levels of >200 mg/dL at the East Kalimantan Provincial Health Laboratory. The sampling technique uses total sampling, where the entire population is used as a research sample. The number of samples was 30lympic specimens that met the inclusion criteria: patients with routine blood tests with cholesterol levels of >200 mg/dL.

### Data Collection

The study variable was hemoglobin levels in lipidemia specimens. Operational definition: examination of hemoglobin levels in lipidemia patients at the East Kalimantan Provincial Health Laboratory. Scale of Ratios. Normal Hb values for women:  $\geq 12.0$  g/dL; Male:  $\geq 13.0$  g/dL.

### Data Processing and Analysis

Hemoglobin examination of the cyanmethemoglobin method: (1) prepare 2 test tubes (1 blank tube, 2 test tube); (2) fill 1 tube with 2 mL of Drabkin reagent; (3) fill tube 2 with 5 mL of Drabkin; (4) sucking the sample with a sahli pipette to the 20  $\mu$ L mark; (5) put blood into the tube 2 and rinse the

pipette 3 times; (6) mix the solution slowly until homogeneous; (7) incubation at room temperature for 10 minutes; (8) read absorbants with a spectrophotometer at a wavelength of 546 nm using Drabkin solution as a blank (Gandasoebrata, 2010).

Data is processed computerized through editing, coding, processing, and cleaning stages. Univariate analysis was performed to describe the distribution of frequency and percentage of hemoglobin levels in lipidemia specimens. The percentage is calculated by the formula  $P = F/n \times 100\%$ , where P is the percentage, F is the frequency of the data, and n is the amount of data.

## RESEARCH RESULTS

**Table 1. Results of haemoglobin level examination in lipidemia specimens (n=30)**

No.	Cholesterol (mg/dL)	Rate Range Hb (g/dL)	n	%
1	201–250	12,30–15,72	22	73%
2	251–300	12,02–15,05	8	27%
<b>Total</b>			<b>30</b>	<b>100%</b>

Based on Table 1, of the 30 respondents who examined hemoglobin levels using the cyanmethemoglobin method in lipidemia specimens, there were 3 male and 27 female respondents. A total of 28 people (93%) had results within normal limits, and 2 men (7%) had results below normal values.

**Table 2. Distribution of Cholesterol and Hemoglobin Levels in Lipidemia Specimens (n=30)**

No.	Kadar Hemoglobin		Jumlah (n)		Persentase (%)
	Male	Perempuan	L	P	
1	Normal (14–18 g/dL)	Normal (12–16 g/dL)	1	27	93%
2	Low (<14 g/dL)	Low (<12 g/dL)	2	0	7%
3	High (>18 g/dL)	Height (>16 g/dL)	0	0	0%
<b>Total</b>			<b>3</b>	<b>27</b>	<b>100%</b>

Based on Table 2, from 30 respondents, 22 samples (73%) with cholesterol levels of 201–250 mg/dL and 8 samples (27%) with cholesterol levels of 251–300 mg/dL were obtained. The lowest hemoglobin value was 12.02 g/dL and the highest was 15.72 g/dL with an average of 13.7 g/dL.

## DISCUSSION

The results showed that of the 30 lipidic samples examined at the East Kalimantan Provincial Health Laboratory, 28 samples (93%) had hemoglobin levels within normal limits and 2 samples (7%) below normal limits. The average value of hemoglobin is 13.7 g/dL with a minimum value of 12.0 g/dL and a maximum of 15.7 g/dL. This finding is in line with the research of Norsiah (2015) who stated that the cyanmethemoglobin method is an accurate reference method for estimating hemoglobin with an error factor of  $\pm 2\%$ .

Lipoprotein particles such as chylomicrons and VLDL can interfere with spectrophotometric readings through light scattering mechanisms. The turbidity in the lipid sample artificially increases the absorbent so that it can produce high false hemoglobin levels (Norsiah, 2015; Khotimah & Sun, 2022). However, in this study, the majority of the samples showed results within normal limits, which indicates that lipemia interference does not always result in results that significantly deviate from normal values.

Two male samples (7%) showed results below normal values were most likely not caused by lipemia interference, but by other factors not controlled in this study. These factors include a diet low in iron, smoking habits, age, and underlying clinical conditions such as iron deficiency anemia. Research by Rima Septiani (2022) found a significant relationship between the duration and frequency of smoking and hemoglobin levels. Adiwijayanti (2015) also reported that exposure to heavy metals such as lead can inhibit heme-forming enzymes and lower hemoglobin levels.

The distribution of cholesterol levels showed that 73% of the samples were in the range of 201–250 mg/dL and 27% in the range of 251–300 mg/dL. The highest cholesterol value is 297 mg/dL which is in the very high category according to PERKENI (2019). However, high cholesterol levels did not necessarily cause significant disruptions in hemoglobin levels in this study.

The limitations of this study include: (1) relatively small sample size (30 specimens); (2) the use of secondary data so that some confounding variables cannot be controlled; and (3) no lipemia correction was carried out before the hemoglobin examination. Further research is recommended to compare the hemoglobin results in lipemic samples before and after treatment (ultracentrifugation or precipitation) to assess the extent of lipemia interference more measurably.

## CONCLUSIONS AND SUGGESTIONS

Based on the results of the examination of hemoglobin levels in 30 lipidic samples at the East Kalimantan Provincial Health Laboratory, it can be concluded that: (1) the examination of hemoglobin levels in lipidic serum using the cyanmethemoglobin method can be used as an accurate reference in the measurement of hemoglobin; (2) the average hemoglobin level in the lipidic sample was 13.7 g/dL and none of the samples showed values above normal limits; (3) a total of 2 male samples (7%) showed results below the normal limit that were likely caused by other unknown variables such as diet, smoking habits, and age.

The recommendations of this study: (1) for laboratory officers, it is recommended to use a Hematology Analyzer for more maximum and efficient hemoglobin level examination results, and the cyanmethemoglobin method can be an option for measuring hemoglobin in lipemic serum; (2) for future researchers, it is recommended to pay attention to the criteria used for the sample and compare the results before and after the treatment of lipemic serum; (3) It is hoped that this study can add insight and knowledge about the influence of lipidemia on hemoglobin examination.

### Author's Contribution Statement:

**Muhammad Rio Kharismawan:** Conceptualization, Methodology, Investigation, Data Curation, Formal Analysis, Writing – Original Draft, Writing – Review & Editing.

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