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Accuracy and Precision of HDL Measurement With the Precipitation Method and LDL Measurement With the Friedewald Method Compared to the Enzymatic Method Using a Chemistry Analyzer

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ABSTRACT
HDL (High Density Lipoprotein) is a lipoprotein with high density that transports LDL (Low Density Lipoprotein) from peripheral blood vessels to the liver. LDL, a cholesterol-rich lipoprotein, can cause health issues if levels are too high. HDL levels can be measured by the precipitation method, while LDL levels are measured by the Friedewald method. To ensure these methods' feasibility, their accuracy and precision must be known. Accuracy refers to how closely examination results match the actual value, while precision refers to the consistency of repeated results. This study aimed to assess the accuracy and precision of HDL measurement by the precipitation method and LDL measurement by the Friedewald method compared to the enzymatic method using a chemistry analyzer. A descriptive analytic approach was used, with research conducted from December 2023 to May 2024 at RSUD Haji Provinsi Jawa Timur, East Java Province, and Farmalab Laboratory. The study involved 35 samples for accuracy and 2 for precision, analyzed using Microsoft Excel. Results showed that the HDL precipitation method had an accuracy of 107%, while the Friedewald method for LDL had an accuracy of 95%. Precision (CV) for HDL was 4% for both samples, and for LDL, it was 1% and 2% in the first and second samples, respectively. Both methods' accuracy and precision fall within acceptable limits.

Keywords: Accuracy; Precision; HDL; LDL; Friedewald

BACKGROUND
According to Permenkes RI No. 411/Menkes/Per/III/2010, a clinical laboratory provides health services by examining clinical specimens to support disease diagnosis and health restoration. These laboratories conduct microbiological, hematological, clinical chemistry, and serological tests(Bailey, Ledeboer, and Burnham 2019). Cardiovascular disease is a major cause of global morbidity and mortality, with 17.8 million deaths reported in 2021 by WHO (Wardhani 2023). This figure is projected to rise to 23.6 million annually by 2030 (Talebi et al. 2020). Dyslipidemia, characterized by high levels of total cholesterol, LDL, and triglycerides, along with low HDL, is strongly linked to cardiovascular disease and atherosclerosis (Liana 2014)

HDL, a high-density lipoprotein, helps remove LDL from blood vessel tissues and transport it to the liver for excretion, keeping blood vessels free of atheroma. Normal HDL levels are >60 mg/dL (Maulida, Mayasari, and Rahmayani 2018). LDL, the primary cholesterol transporter, has smaller particles that pose a higher cardiovascular risk due to their susceptibility to oxidation and arterial wall permeability. Optimal LDL levels are <100

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mg/dL (Sanhia, Pangemanan, and Engka 2015; Talebi et al. 2020).

Precipitation involves adding chemicals to reduce or eliminate suspended solids in a liquid (Nugroho 2020). In HDL testing, reagents like phosphotungstic acid and magnesium ions bind to low-density lipoproteins such as chylomicrons, VLDL, and LDL, causing them to precipitate at the bottom of the tube after centrifugation (Bishop, Fody, and Schoeff 2013). The Friedewald method estimates LDL levels using known triglycerides, total cholesterol, and HDL levels. It is commonly used in practice due to its simplicity and cost-effectiveness (Rosmala, Asmara, and Widiastuti 2018). However, it is not suitable for patients with triglyceride levels >400 mg/dL, as this can distort the VLDL to triglyceride ratio, leading to overestimated VLDL cholesterol and underestimated LDL levels (Krishnaveni and Gowda 2015; Radhasiwi 2016).

A chemistry analyzer is a clinical laboratory tool used to measure blood chemical levels. It can process multiple samples automatically with high accuracy, reducing human error (Yulianti et al. 2021). The device operates on a photometer principle, where light absorbed by the sample at specific wavelengths is measured (Akhzami, Rizki, and Setyorini 2017). However, its high cost and maintenance needs limit its use in some health laboratories, leading many to rely on manual methods (Damayanti 2016).

A laboratory's quality is reflected in the accuracy and precision of its examination results (Kusmiati, Nurpalah, and Restaviani 2022). High-quality results are crucial for accurate HDL and LDL testing, which guide treatment and risk assessment. Accuracy refers to how close the results are to the true value (Mowoka, Waworundeng, and Kumayas 2023), while precision indicates the consistency of results across multiple tests (Ulfiati et al. 2017).

Jabbar et al. (2006) found that the mean standard deviations for HDL cholesterol using the precipitation and automatic methods were 43.12±8.97 mg/dL and 43.86±10.34 mg/dL, respectively (p-value = 0.301). For LDL cholesterol, the mean standard deviations were 111.76±25.57 mg/dL and 111.8±28.41 mg/dL (p-value = 0.981). The calculated "t" and "F" values for HDL-C were 0.0172 and 0.75, and for LDL-C, they were 0.047 and 0.809. The manual method took 45 minutes, while automation took 20 minutes. Both methods provided accurate results with no significant difference.

Liazarti and Valzon (2021) found that various formulas for calculating indirect LDL levels, including Friedewald, Chen, and others, showed significant discrepancies, with the Friedewald formula deviating notably from the enzymatic method. Similarly, Damayanti (2016) reported a significant difference in LDL cholesterol levels between the precipitation and Friedewald methods, with the precipitation method yielding higher levels ($t = 8.352$, $p = 0.000$). Based on the background, it is necessary to conduct research to analyze the accuracy and precision of HDL levels in the precipitation method and LDL levels in the Friedewald method against enzymatic methods using a chemistry analyser.

RESEARCH METHODS

This study utilized a descriptive analytic research design to assess the accuracy and precision of the HDL precipitation method and the LDL Friedewald method in comparison to the enzymatic method, using a chemistry analyzer with a cross-sectional approach. The study population included serum samples from inpatients and outpatients at RSUD Haji Provinsi Jawa Timur in East Java Province.

Samples were selected through purposive sampling, adhering to specific inclusion and exclusion criteria. Inclusion

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criteria required triglyceride levels to be less than 400 mg/dL, and the samples to be non-lipemic and non-lytic. Exclusion criteria were triglyceride levels greater than 400 mg/dL, and samples that were lipemic or lytic. A total of 35 samples were used for accuracy analysis, based on the monthly control requirements outlined by Siregar et al. (2018). Precision was evaluated using two samples, each tested ten times.

Accuracy is expressed as inaccuracy/biased value in percent. The accuracy value of the precipitation method HDL examination was calculated with the enzymatic method HDL level as the true value. Accuracy can also be presented in the form of recovery, namely conducting an examination of a mixture of sample material and pure analytes, then the results are calculated against the expected results. Recovery is expressed as the ratio between the result obtained and the actual result. Precision is presented in the form of impression expressed in the size of the coefficient of variation (CV). The CV value is inversely proportional to the precision of a system/method.

The research was conducted from December 2023 to May 2024 at RSUD Haji Provinsi Jawa Timur and Farmalab Laboratory. Primary data collection involved measuring HDL levels using the precipitation method with a photometer, and LDL levels using the Friedewald method. Additionally, triglyceride and total cholesterol levels for LDL calculation were measured with a photometer. Reference HDL and LDL values were obtained from the clinical pathology laboratory at the RSUD Haji Provinsi Jawa Timur. The collected data were subsequently analyzed to determine accuracy and precision using Excel software.

RESULTS AND DISCUSSION

Based on the results of the study of accuracy and precision of HDL levels of precipitation method and LDL friedewald method against enzymatic method using chemistry analyzer conducted at RSUD Haji Provinsi Jawa Timur with serum obtained by purposive sampling technique, the following results were obtained.

Table 1. Accuracy of HDL Measurement with the Precipitation Method and LDL Measurement with the Friedewald Method Compared to the Enzymatic Method Using a Chemistry Analyzer

	HDL		LDL	
	Precipitation	Enzymatic	Friedewald	Enzymatic
Mean	43 mg/dl	40 mg/dl	116 mg/dl	123 mg/dl
Inaccuracy	7%		-5%	
Recovery	107%		95%	

According to Table 1, the mean HDL levels measured using the precipitation method across 35 samples were 43 mg/dL, compared to 40 mg/dL using the enzymatic method, indicating a difference of 3 mg/dL between the two methods. The inaccuracy of HDL measurements with the precipitation method relative to the enzymatic method was 7%, with a recovery value of 107%. For LDL levels, the Friedewald method yielded a mean of 116 mg/dL from 35 samples, which is 7 mg/dL lower than the 123 mg/dL mean obtained using the enzymatic method. The average inaccuracy of LDL measurements with the Friedewald method compared to the enzymatic method was -5%, with a recovery value of 95%.

Inaccuracies in HDL cholesterol measurements can arise from several factors, including variability in precipitation techniques, impurity of reagents, or errors in sample handling. Proper sample storage is crucial for maintaining accuracy, with delayed testing requiring adherence to appropriate storage temperatures (Holzer et al. 2017). In this study, samples collected from the RSUD Haji Provinsi Jawa Timur were stored at -20°C, in accordance with established

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sample storage standards. However, during the transfer to Pharmalab Laboratory in Bangkalan, potential temperature mismatches could have led to inaccuracies, including possible elevation of HDL levels. Soderi (2021) demonstrated that delays and deviations from recommended storage temperatures can indeed result in increased HDL levels, highlighting the need for stringent storage and handling protocols to ensure accurate test results.

Additionally, this research aligns with the findings of Jabbar et al. (2006), which compared HDL measurement using the precipitation method with the enzymatic method. Both methods were found to be reliable, precise, and accurate, with no significant statistical differences. The acceptable accuracy range for HDL measurements is 90-110% (Happy and Aryani 2020). Although the precipitation method showed a 7% discrepancy compared to the enzymatic method in this study, this inaccuracy remains within the acceptable range.

The accuracy of the Friedewald method for LDL cholesterol measurement is evaluated against the gold standard reference values provided by enzymatic assays conducted using a chemistry analyzer. According to research findings, the Friedewald method demonstrated an inaccuracy of -5% and a recovery rate of 95% (Table 1). This negative inaccuracy indicates that the measured LDL levels were lower than the reference values (Siregar et al. 2018). Since the Friedewald method relies on a calculation-based approach, an increase in HDL levels can lead to a corresponding decrease in calculated LDL levels. As noted in Section 6.1, deviations such as delays and improper storage temperatures can result in elevated HDL levels, which in turn affects LDL calculations (Soderi 2021).

These findings are consistent with the study by Damayanti (2016), which also reported lower LDL levels with the Friedewald method compared to the reference enzymatic method. Despite the -5% inaccuracy observed with the Friedewald method, this deviation falls within the acceptable accuracy range of 90-110% for LDL measurements (Happy and Aryani 2020).

Table 2. Precision of HDL Measurement with the Precipitation Method and LDL Measurement with the Friedewald Method

	HDL		LDL	
	ST	SI	ST	SI
Mean	48.2 mg/dl	43.9 mg/dl	136.16 mg/dl	109.24 mg/dl
SD	2.04	1.66	1.04	1.72
CV	4%	4%	1%	2%

Based on the data presented in Table 2, the mean HDL cholesterol levels, measured after ten replicates, were 48.2 mg/dL for samples coded (ST) and 43.9 mg/dL for samples coded (SI). The precision of HDL measurements using the precipitation method at Farmalab Laboratory yielded a SD of 2.04 and a CV of 4% for sample (ST). For sample (SI), the SD was 1.66, resulting in a CV of 4%. In contrast, the mean LDL cholesterol levels after ten replicates were 136.16 mg/dL for sample (ST) and 109.04 mg/dL for sample (SI). The precision of LDL measurements using the Friedewald method, also conducted at Farmalab Laboratory, produced an SD of 1.04 and a CV of 1% for sample (ST). For sample (SI), the SD was 1.72, corresponding to a CV of 2%.

The precision of the HDL cholesterol measurement using the precipitation method was assessed by performing ten replicate tests on each sample. According to the findings, the CV for both tested samples was 4% (Table 5.7). This CV value is within the acceptable limit for HDL cholesterol measurements, which is set at a maximum of 4%.

Furthermore, the replication results fell within the ±2SD range on the Levey-Jennings graph, indicating precision. For

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the sample coded ST, with a mean HDL value of 48.2 mg/dL, the ±2SD range was 44.1 mg/dL to 52.3 mg/dL. For the sample coded SI, with a mean HDL value of 43.9 mg/dL, the ±2SD range was 40.6 mg/dL to 47.2 mg/dL. The Levey-Jennings graphs for samples ST and SI are illustrated in the following pictures.

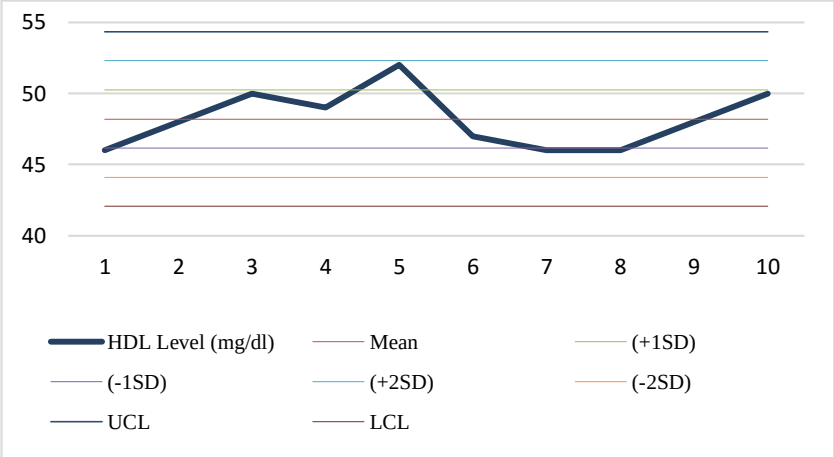

Figure 1. Levey-Jennings graphs for HDL precipitaion sample ST

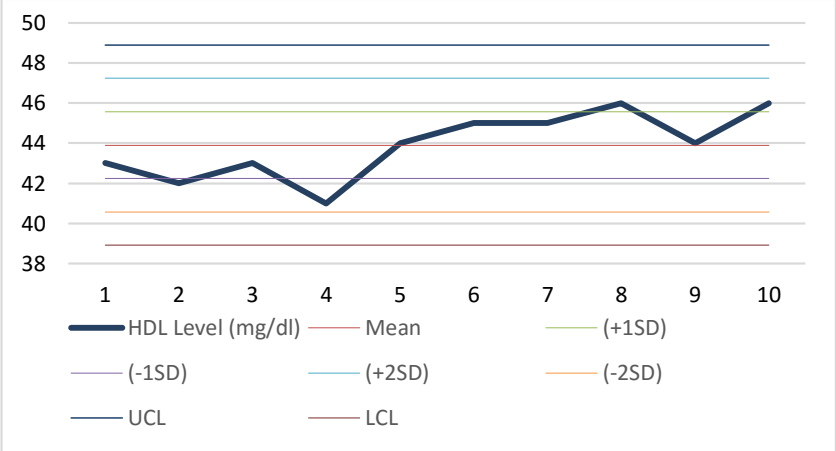

Figure 2. Levey-Jennings graphs HDL precipitaion sample SI

These results are consistent with Huda's (2021) study on the performance metrics of the enzymatic method for total cholesterol, HDL, and triglycerides. Huda's research demonstrated that the CV values for these parameters were well within acceptable limits set by the Ministry of Health and the Clinical Laboratory Improvement Amendments (CLIA).

The precision of the Friedewald method for LDL cholesterol measurement was assessed by performing ten replicate tests for HDL, triglycerides, and total cholesterol, followed by the calculation of LDL levels. The results revealed that the coefficient of variation (CV) for the LDL measurements was 1% for the sample coded (ST) and 2% for the sample coded

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(SI). Both CV values are well within the acceptable range for LDL measurements, which is set at a maximum of 4%.

Additionally, the replication results fell within the ±2 standard deviation (SD) range on the Levey-Jennings graph, indicating good precision. For the sample coded (ST), with a mean LDL value of 136.2 mg/dL, the ±2SD range was 134.1 mg/dL to 138.3 mg/dL. For the sample coded (SI), with a mean LDL value of 109.2 mg/dL, the ±2SD range was 105.8 mg/dL to 112.7 mg/dL. The Levey-Jennings graphs for samples ST and SI are provided in the following pictures.

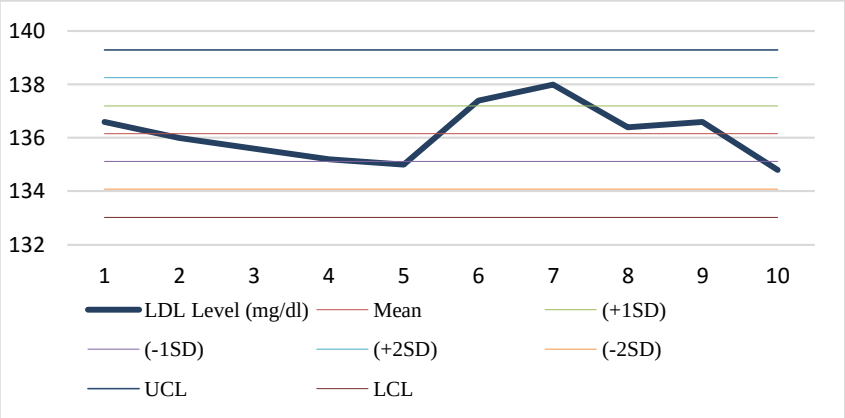

Figure 3. Levey-Jennings graphs for LDL friedewald sample ST

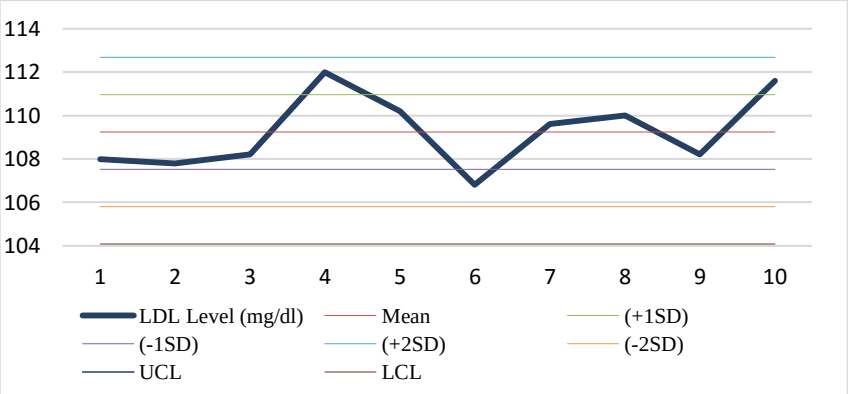

Figure 4. Levey-Jennings graphs for LDL friedewald sample SI

These findings contrast with the study by Liazarti and Valzon (2021), which reported that the Friedewald method showed significant discrepancies compared to the enzymatic method. Factors affecting the precision of HDL measurement using the precipitation method include patient fasting preparation, sample collection and handling, reagent stability, pipetting techniques, and instrument calibration (Kusliyana 2018). Conversely, the Friedewald method's accuracy can be compromised by high triglyceride levels, as it is not reliable for patients with

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triglyceride levels exceeding 400 mg/dL (Yani 2016).

Several factors impact the accuracy of HDL measurement using the precipitation method, including patient fasting protocols, sample collection and handling procedures, reagent stability, pipetting techniques, and the calibration and control of instruments (Kusliyana, 2018). Conversely, the Friedewald method for LDL measurement is influenced by the levels of total cholesterol, HDL, and triglycerides. A notable limitation of this method is its inapplicability for patients with triglyceride levels exceeding 400 mg/dL, as it produces unreliable results in such cases (Yani 2016).

This study is subject to several limitations, including a restricted sample size, the absence of observations regarding comorbidities or complications among research subjects, a lack of variability in participant profiles such as the categorization of normal versus abnormal HDL/LDL levels, and potential temperature instability during sample transportation.

CONCLUSION

The study evaluated the accuracy and precision of HDL and LDL measurement methods, finding notable results. The precipitation method for HDL yielded a mean level of 40 mg/dL, while the Friedewald method for LDL resulted in a mean of 116 mg/dL. The enzymatic method showed HDL and LDL levels of 43 mg/dL and 123 mg/dL, respectively. Accuracy analysis revealed that the HDL measurement with the precipitation method had a 7% inaccuracy and a recovery rate of 107%, both within the acceptable range of 90-110%. The Friedewald method for LDL showed a -5% inaccuracy with a 95% recovery rate, also within the acceptable range.

Regarding precision, the HDL measurement using the precipitation method had a coefficient of variation (CV) of 4% for both samples, which is acceptable. For LDL, the Friedewald method demonstrated a CV of 1% for samples coded (ST) and 2% for samples coded (SI), both within acceptable CV limits. Overall, the study found that both methods delivered accurate and precise results in line with established standards.

It is recommended that ATLMs ensure the proper temperature during sample transport to maintain stability. Future studies should expand sample sizes, consider comorbidities, and examine how variations in HDL/LDL levels affect test accuracy and precision, while also focusing on temperature control dwuring sample transport. Additionally, the public should consult with doctors to interpret results accurately and follow pre-examination instructions, such as fasting before blood sampling, to ensure more reliable outcomes

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