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Impact of Delayed Analysis on Hemoglobin Levels in EDTA Blood: Implications for Cancer Management

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ABSTRACT

Cancer remains a leading global health challenge, with anemia being one of the most common complications affecting patient prognosis and treatment outcomes. Hemoglobin (Hb) plays a critical role as a biomarker for transfusion decisions, therapeutic monitoring, and survival prediction. Accurate Hb measurement, however, is highly dependent on the preanalytical phase, particularly the time between blood collection and analysis. Although several studies have explored hematological stability during long-term storage, evidence regarding the short-term impact of delayed analysis on Hb measurement remains limited. This study aimed to evaluate differences in Hb levels of EDTA-anticoagulated blood samples subjected to immediate analysis, 2-hour delay, and 6-hour delay. Thirty-five venous blood samples were analyzed using the cyanmethemoglobin method with spectrophotometric reading at 546 nm. Descriptive statistics showed a progressive decline in Hb values with increasing delay: mean Hb was 14.3 g/dL (0 h), 13.3 g/dL (2 h), and 12.4 g/dL (6 h). Data distribution met normality and homogeneity assumptions. One-Way ANOVA demonstrated a significant difference among groups ($F = 11.139$, $p < 0.001$). At the same time, Post Hoc LSD tests confirmed significant pairwise differences between immediate and delayed measurements, as well as between 2-hour and 6-hour delays ($p < 0.05$). These findings indicate that even short-term delays significantly reduce measured Hb concentration, with potential clinical implications for oncology practice where Hb values inform transfusion thresholds and prognosis. Laboratories should ensure that hemoglobin is measured within two hours of blood collection to minimize preanalytical errors and optimize cancer patient management.

Keywords: Hemoglobin; Pre analytical phase; Cancer management

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INTRODUCTION

Cancer remains one of the most significant global health challenges in the 21st century. In 2022, the International Agency for Research on Cancer (IARC) reported more than 20 million new cases and 9.7 million deaths worldwide, with low- and middle-income countries (LMICs) carrying a disproportionately high mortality burden due to limited healthcare infrastructure and delayed access to treatment (Bray et al., 2024; IARC, 2024). Beyond its health impact, cancer also imposes substantial economic costs. Global projections estimate that the cumulative burden of 29 cancer types will exceed USD 25 trillion by 2050, reflecting not only direct medical expenditures but also productivity losses and premature mortality (S. Chen et al., 2023). These figures underscore the urgency of developing sustainable, evidence-based cancer management strategies in which reliable laboratory services play a critical role.

Among cancer-related complications, anemia is one of the most prevalent and clinically significant. Cancer-related anemia (CRA) affects 30–90% of patients, depending on tumor type, disease stage, and therapeutic regimen (Tałasiewicz & Kapała, 2023). Its etiology is multifactorial, including bone marrow infiltration, chronic bleeding, nutritional deficiencies, systemic inflammation, and cytotoxic effects of chemotherapy or radiotherapy (Su et al., 2024). Clinically, CRA contributes to fatigue, dyspnea, impaired cognitive function, and reduced quality of life. More importantly, anemia is consistently associated with poor treatment outcomes, reduced tolerance to therapy, and shorter survival (Bolkun & Kloczko, 2021).

A key mechanism linking anemia to cancer prognosis is tumor hypoxia. Reduced hemoglobin concentration limits oxygen delivery, creating a hypoxic tumor microenvironment that promotes angiogenesis, invasion, metastasis, and resistance to radiotherapy, chemotherapy, and novel therapies such as photodynamic treatment (Wang et al., 2025). These biological processes explain why patients with low hemoglobin levels typically have inferior survival outcomes, underscoring the clinical value of accurate Hb measurement.

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Hemoglobin is therefore more than a routine hematological parameter; it is a critical biomarker in oncology. Hb levels guide transfusion decisions, inform using erythropoiesis-stimulating agents, and serve as an independent prognostic factor (Álvarez et al., 2021). For instance, in non-small cell lung cancer (NSCLC), low baseline Hb is strongly associated with decreased survival independent of tumor stage or clinical status (C. Chen et al., 2021; Wei et al., 2022). Similarly, a hospital-based study in Sudan revealed that more than half of cancer patients were anemic, with Hb values differing across tumor types (Abuidris et al., 2023).

The accuracy of Hb measurement, however, depends heavily on laboratory quality. Errors in the preanalytical phase, including patient identification, specimen collection, handling, storage, and transport, account for more than 60% of total laboratory errors (Giavarina & Lippi, 2017; Lippi et al., 2012). Such errors can bias results, leading to inappropriate transfusion, misinterpretation of therapy tolerance, or inaccurate prognostic assessment.

While several studies have examined hematological stability under prolonged storage (24–72 hours) and varying temperatures (Kangwende et al., 2024; Ozmen & Ozarda, 2021), few have addressed hemoglobin stability. Even fewer have evaluated the effect of short-term delays, such as two to six hours, common in everyday laboratory practice due to heavy workloads, staff shortages, or transport barriers. Despite the clinical significance of Hb in oncology, empirical evidence on its stability under such conditions is limited.

The clinical consequences of even small inaccuracies are considerable. In oncology, transfusion thresholds are typically set within a narrow Hb range (7–9 g/dL) (Carson et al., 2016). A measurement error due to processing delays may shift patients across these thresholds, leading to unnecessary transfusion or withholding needed therapy (Bolkun & Kloczko, 2021; Carson et al., 2016). Beyond individual treatment decisions, inaccurate Hb values may bias clinical trial outcomes, distort patient risk stratification, and weaken the evidence base for healthcare policies (Giavarina & Lippi, 2017). In Indonesia,

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where the Ministry of Health prioritizes strengthening laboratory services as part of healthcare reform (Kemenkes RI, 2024), addressing vulnerabilities in the preanalytical phase is essential for ensuring clinical reliability and system-level credibility.

Despite growing awareness of preanalytical errors, there remains a paucity of research specifically evaluating the impact of short-term delays on Hb levels in EDTA blood samples. Most prior studies have focused on long-term storage stability or examined anemia's prevalence and prognostic significance in cancer patients, without linking these issues to laboratory accuracy.

Therefore, this study aims to evaluate, for the first time, the effect of short-term preanalytical delays (two and six hours) on hemoglobin concentration in EDTA-anticoagulated blood samples compared with immediate analysis. This novelty addresses a frequently overlooked aspect of hematology, with direct implications for laboratory quality assurance and oncology practice. We hypothesized that delayed analysis would significantly reduce Hb levels compared with immediate testing.

METHOD

Study Design and Setting

This study employed an experimental posttest-only control group design. It was conducted between March and April 2025 at the Hematology Laboratory, Poltekkes Kemenkes Denpasar, Bali, Indonesia.

Participants and Sampling

Venous blood samples were collected from 35 healthy volunteers who were active students of the Applied Bachelor Program in Medical Laboratory Technology at Poltekkes Kemenkes Denpasar. Sampling was conducted using a cluster random sampling technique to ensure representativeness. The minimum required sample size was calculated using the population proportion formula, with assumptions of 50% estimated proportion, a 95% confidence level (Z = 1.96), and a 15% margin of error, yielding a sample size of 35. In addition, a power analysis for one-way ANOVA with three groups indicated that a minimum of 30 participants would be

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sufficient to detect a medium effect size (f = 0.25) at 80% power and a 5% significance level. Therefore, including 35 samples satisfied population-based estimation and statistical power requirements, ensuring the reliability of the study results.

Data Collection and Instruments

Hemoglobin levels were measured under three conditions: (1) immediate analysis (0 hours), (2) analysis after a 2-hour delay, and (3) analysis after a 6-hour delay. Hb concentration was determined using the cyanmethemoglobin method with a Microlab 300 spectrophotometer at 546 nm and a conversion factor 36.77. Essential equipment included Vacutainer needles, EDTA tubes, micropipettes, and tube racks, while the reagents used were Drabkin's solution and distilled water.

Experimental Procedure

For each measurement, 5 mL of Drabkin's reagent was pipetted into a clean test tube, followed by 20 µL of EDTA-anticoagulated blood. Excess blood was removed before dispensing into the reagent. The mixture was gently homogenized and incubated at room temperature for five minutes. Hemoglobin concentration was then measured spectrophotometrically. All procedures were performed at controlled room temperature (24–26 °C) to minimize variability due to thermal effects on erythrocyte stability.

Data Analysis

Data were analyzed using descriptive and inferential statistics. Normality was tested with the Shapiro–Wilk test, and homogeneity of variances was assessed with Levene's test. Differences in mean hemoglobin levels across the three groups were evaluated by One-Way Analysis of Variance (ANOVA). Post Hoc Least Significant Difference (LSD) tests were applied for pairwise comparisons. A significance threshold of $p < 0.05$ was used. All statistical analyses were performed using IBM SPSS Statistics Version 25.

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RESULTS

Descriptive Statistics of Hemoglobin Levels

Hemoglobin (Hb) concentrations were measured in 35 EDTA-anticoagulated venous blood samples analyzed immediately after collection, after a 2-hour delay, and after a 6-hour delay. The descriptive results are presented in Table 1.

Table 1. Hemoglobin levels according to testing delay

Testing Time	Minimum (g/dL)	Maximum (g/dL)	Mean (g/dL)	SD
Immediate (0 h)	11.6	18.2	14.3	1.81
2-hour delay	9.9	16.7	13.3	1.55
6-hour delay	8.1	15.6	12.4	1.53

A progressive decline in mean Hb levels was observed with increasing delay. Immediate testing produced the highest mean (14.3 g/dL), while approximately 1.0 g/dL and 1.9 g/dL reductions were seen after 2-hour and 6-hour delays, respectively. These findings indicate that even short delays in analysis may affect the accuracy of Hb measurement. The mean Hb levels for each group are illustrated in Figure 1.

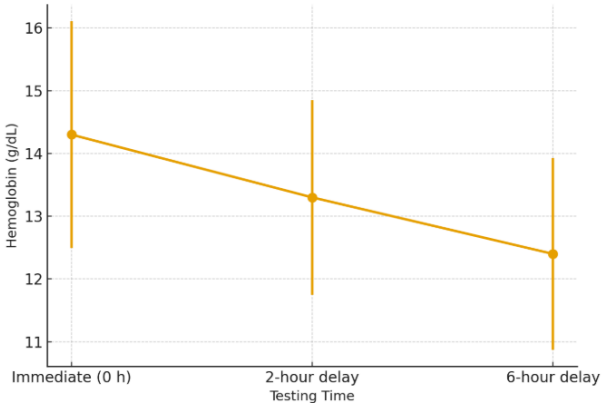

Figure 1. Mean hemoglobin concentration across testing times

The figure illustrates a progressive decline in hemoglobin (Hb) concentration as the interval between blood collection and analysis increases. Immediate testing (0 h) yielded the highest mean Hb value (14.3 g/dL), which decreased to 13.3 g/dL after a 2-hour delay and further to 12.4 g/dL after a 6-hour delay. The error bars indicate the variability within each group, showing that the decline was consistent across samples. This downward trend highlights that even short-term preanalytical delays can systematically underestimate Hb levels, underscoring the importance of timely analysis for ensuring accurate laboratory results in clinical oncology practice.

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Normality and Homogeneity Tests

Preliminary analysis confirmed that the data met assumptions for parametric testing. Shapiro–Wilk tests indicated normal distribution for all groups ($p = 0.094$, 0.532, and 0.329 for immediate, 2-hour, and 6-hour groups, respectively; all $p > 0.05$). Levene's test confirmed homogeneity of variances ($p = 0.508$). Fulfilling normality and homogeneity assumptions validates applying parametric statistical analysis (One-Way ANOVA) for group comparisons.

One-Way ANOVA

A One-Way ANOVA was conducted to evaluate differences in mean Hb across the three testing times. The results are shown in Table 2.

Table 2. One-Way ANOVA of hemoglobin levels

Hemoglobin levels					
Source of Variation	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Between groups	59.618	2	29.809	11.139	.000

The ANOVA revealed a statistically significant difference in mean Hb concentrations among the three testing times ($F = 11.139$, $p < 0.001$). This indicates that preanalytical delays exert a measurable and statistically robust effect on Hb measurement.

Post Hoc Pairwise Comparisons

To further explore these differences, Post Hoc Least Significant Difference (LSD) tests were performed (Table 3).

Table 3. Post Hoc LSD pairwise comparisons of hemoglobin levels

Comparison	Sig.
Immediate vs. 2 h	.019
Immediate vs. 6 h	.000
2 h vs. 6 h	.021

All pairwise comparisons were statistically significant ($p < 0.05$). Immediate analysis yielded significantly higher Hb levels than delayed analyses, while the difference between 2-hour and 6-hour delays was also significant. These results highlight a progressive and time-dependent decline in Hb stability.

The findings demonstrate that Hb concentration decreases significantly with 2 to 6 hours of preanalytical delays. Even short-term delays result in clinically

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relevant reductions, which may affect decisions such as transfusion thresholds in oncology practice. This underscores the importance of minimizing processing delays to ensure reliable laboratory results for cancer patient management.

DISCUSSION

The present study demonstrates that hemoglobin (Hb) concentrations in EDTA-anticoagulated blood samples decline significantly when analysis is delayed for 2 and 6 hours compared with immediate testing. The mean Hb concentration decreased progressively with time, and all pairwise comparisons were statistically significant. These findings provide empirical evidence that even short-term preanalytical delays can compromise Hb stability, affecting laboratory results' accuracy.

Our results are consistent with previous research emphasizing the vulnerability of the preanalytical phase in laboratory medicine. More than 60% of laboratory errors occur before analysis, encompassing processes such as sample collection, handling, and storage (Giavarina & Lippi, 2017; Lippi et al., 2012). However, there is mixed evidence regarding hemoglobin stability. Some studies report that Hb values remain stable for 24–96 hours under controlled conditions and with certain analyzers (Doeleman et al., 2023; Ozmen & Ozarda, 2021), whereas other investigations show measurable changes after much shorter delays. For instance, (Kim & Kim, 2023) demonstrated significant Hb variations within 35–45 minutes depending on transfer handling, while local studies using the cyanmethemoglobin method documented clinically relevant declines after 2 and 4 hours (Hartini et al., 2024; Parwati et al., 2018). These findings suggest that analyzer model, storage temperature, and preanalytical handling practices strongly influence hemoglobin stability. Our study addresses an overlooked gap in preanalytical hematology research by examining Hb under short-term delays.

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The biological explanation for these findings likely involves changes in erythrocyte integrity and hemoglobin stability under less optimal handling conditions. Prior studies indicate that metabolic decline (including ATP depletion), membrane fragility, and oxidative stress during storage contribute to hemolysis and alteration of measurement accuracy (Anastasiadi et al., 2023; Ozmen & Ozarda, 2021). In addition, blood storage also induces alterations in red blood cells, leukocytes, and platelets (Yuniza et al., 2024), which may further affect sample stability and analytical accuracy during delayed analysis. Although direct evidence for these processes during very short delays (2–6 hours at room temperature) is limited, subtle hemolysis or inadequate mixing during early handling may similarly interfere with spectrophotometric readings, leading to underestimation of Hb values and variability across studies.

Clinically, these findings have important implications. Hemoglobin is a critical biomarker in oncology, informing transfusion decisions, guiding supportive care, and serving as an independent prognostic factor (Álvarez et al., 2021; C. Chen et al., 2021; Wei et al., 2022). Transfusion thresholds are often defined within a narrow Hb range of 7–9 g/dL. This study's mean reduction of approximately 1–2 g/dL caused by short-term delays is clinically meaningful, as it could shift patients across transfusion thresholds. For instance, an actual Hb value of 8.5 g/dL might be underestimated as 7.5 g/dL after a delay, leading to unnecessary transfusion, or conversely, a value near the lower limit may be overestimated, resulting in delayed intervention. Such misclassification may prompt unnecessary transfusion and its risks—alloimmunization, iron overload, and transfusion-related complications (Carson et al., 2016)— or delay needed transfusion, worsening hypoxia, and compromising treatment tolerance in cancer patients (Bolkun & Kloczko, 2021). Thus, accurate Hb measurement is essential for safe and effective oncology care.

From a laboratory quality assurance perspective, these findings reinforce the importance of minimizing preanalytical delays. Strict standard operating procedures should define acceptable timeframes for Hb

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testing, ideally within 2 hours of collection (Doeleman et al., 2023; Lippi et al., 2011). Improvements in automation, staffing, and specimen transport systems could reduce turnaround times and safeguard accuracy (Giavarina & Lippi, 2017). This is particularly relevant in low- and middle-income countries, including Indonesia, where logistical barriers and workforce limitations frequently prolong turnaround times. Establishing strict SOPs that limit hemoglobin testing to within two hours of blood collection is therefore critical, in line with ongoing Ministry of Health initiatives to improve laboratory service quality and reduce preanalytical errors nationwide (Kemenkes RI, 2024). This highlights the need for national laboratory guidelines to define acceptable preanalytical timeframes for hematological testing explicitly.

The main strength of this study lies in its controlled experimental design, which directly addresses the short-term stability of hemoglobin, a rarely investigated parameter. However, several limitations should be noted. The study was conducted in a single center with a modest sample size and included only healthy volunteers, which may limit generalizability to oncology patients. In addition, only room temperature conditions were assessed, and Hb was the sole hematological parameter examined.

Future research should expand to more diverse populations, including oncology patients, and examine different storage conditions and additional hematological indices. It would also be valuable to investigate how such preanalytical variability translates into clinical decision-making, particularly in transfusion practice.

These results underscore the need for timely laboratory practices to preserve the reliability of hemoglobin measurement. They also provide an evidence base that supports improvements in laboratory quality assurance and cancer patient management, forming the foundation for the concluding recommendations of this study.

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CONCLUSION(S)

This study shows that hemoglobin (Hb) concentrations in EDTA-anticoagulated blood samples decrease significantly with short-term preanalytical delays of 2–6 hours. Even modest delays can introduce clinically relevant errors, particularly in oncology, where transfusion thresholds are defined within a narrow Hb range.

The findings highlight the urgency of minimizing turnaround time to ensure reliable Hb results for cancer patient management. The novelty of this study lies in demonstrating the impact of short-term delays, an often-overlooked aspect of preanalytical quality assurance. Although limited by its single-center design, small sample size, and focus on healthy volunteers, the evidence strongly supports the recommendation that Hb measurement should ideally be performed within two hours of blood collection. Future studies should include oncology populations and evaluate additional storage conditions to strengthen laboratory practice guidelines.

Conflict of Interest

The author(s) declare that they have no conflict of interest.

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