The Comparison of Hemoglobin and Leukocytes Examination Between Whole Blood and Pre-Diluted Modes on Hematology Analyzer

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Abstract

Hematological testing plays a vital role in assessing the condition of blood and its components, such as hemoglobin and leukocytes. One of the commonly used methods is the automatic hematology analyzer, which operates with two measurement modes: whole blood for standard sample volumes (±1 mL) and pre-diluted for non-standard volumes (>20 µL to <1 mL), typically due to pre-analytical factors. This study aims to compare hemoglobin levels and leukocyte counts between the whole blood and pre-diluted modes. The study applied an observational analytic study with a cross-sectional design involving 30 randomly blood samples. Each sample was analyzed using both modes, and the results were statistically evaluated using a paired t-test. The findings revealed a significant difference in hemoglobin levels between the whole blood and pre-diluted modes ($p = 0.002$), while no significant difference was observed in leukocyte counts ($p = 0.40$). Although the difference in hemoglobin levels was statistically significant, it was not clinically relevant, and both modes obtained results that were acceptable for clinical interpretation.

Keywords: Hemoglobin, hematology analyzer, leukocytes, pre-diluted, whole blood

1. Introduction

Clinical laboratory testing encompasses several areas, including clinical chemistry, clinical microbiology, clinical parasitology, clinical immunology, and hematology. Among these various tests, hematology is the most frequently performed. This test aims to assess the condition of blood and its components, including hemoglobin and leukocytes (Mansyur & Arief, 2015). Although hemoglobin and leukocytes are distinct blood cell components, they are related. Hemoglobin plays a role in the exchange of oxygen and carbon dioxide in body tissues (Hariani Nurjanah et al., 2023), while leukocytes function as the body's defense against foreign substances that can cause infection or disease. Hemoglobin and leukocyte levels can be measured using various methods, both manual and automated (Bain, 2014).

Automated methods can use a hematology analyzer, the gold standard for hematology diagnosis. This test can be performed in two modes: whole blood when the sample volume meets the standard (1 ml) and pre-diluted when the volume does not meet the standard (Hidayat, 2020). In sampling cases, sometimes there can be situations where blood samples do not always meet the sample volume standard. This is particularly true for overweight individuals who struggle to detect veins, infants under five and three, or those who are less cooperative when drawing blood through a small vein, which can potentially result in inadequate blood volume.

When the blood sample volume is insufficient, whole blood sampling is performed. There is a risk of errors because the sample cannot be drawn optimally by the device. Therefore, pre-diluted sampling is an alternative analysis method that can be used to maintain accurate results, even with samples that do not meet the volume standard (Gina, 2020).

Research conducted by Shintia (2018) showed differences in hemoglobin levels, leukocyte counts, and platelet counts between whole blood and pre-diluted blood samples using a Sysmex XP 100 type 3 Hematology Analyzer. The leukocyte count was higher in pre-diluted sampling compared to whole blood sampling. Meanwhile, research by Gina Kamilia et al. (2020) revealed that hemoglobin levels, leukocyte counts, and platelet counts in both modes showed significant differences, although the values were still within the normal range. This study was conducted to compare hemoglobin levels and leukocyte counts between whole blood and pre-diluted modes on a Hematology Analyzer.

2. Research Method

This study employed a quantitative method with an observational analytical design and a cross-sectional approach. The subjects were respondents undergoing routine blood tests in the laboratory. A sample size of 30 was obtained through simple random sampling. The examination was conducted using two modes on the Sysmex XP 100 hematology analyzer: whole blood and pre-diluted, to compare hemoglobin levels and leukocyte counts. The collected data were then analyzed using a paired t-test.

3. Results and Discussion

3.1. Results

Based on the data in Table 1, the descriptive analysis of hemoglobin and leukocyte examinations using whole blood and pre-diluted modes, the average hemoglobin levels were 11.823 g/dl for whole blood and 11.483 g/dl for pre-diluted modes, respectively. Meanwhile, the average leukocyte levels were recorded at 7357/µl in whole blood and 7187/µl in pre-diluted modes.

Table 1. Descriptive analysis of hemoglobin and leukocyte test results using whole blood and prediluted modes

Variable	Min	Max	Mean	SD
Hemoglobin WB	7.4	15.0	11.823	.9465
Hemoglobin PD	7.1	15.1	11.483	.8769
Leukocytes WB	1.9	15.0	7.357	.0682
Leukocytes PD	2.1	14.8	7.187	.9898

Based on Table 2, the significance values for the Shapiro-Wilk normality test for whole blood hemoglobin ($p = 0.095$) and pre-diluted ($p = 0.379$), and whole blood leukocytes ($p = 0.478$) and pre-diluted ($p = 0.347$), were all >0.05. This indicates that the data distribution in all four groups meets the assumption of normality.

Analytically, a normal distribution can be recognized by its symmetrical data pattern, where the mean, median, and mode are nearly identical. Approximately ±68% of the data falls within one standard deviation of the mean, ±95% falls within two standard deviations, and ±99.7% falls within three standard deviations (Notoatmodjo, 2018). These characteristics provide a strong basis for applying the paired t-test as an appropriate comparative analysis method.

Table 2. Hemoglobin Normality Test

	Kolmogorov-Smirnov			Shapiro Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
HB WB	0.136	30	0.163	0.941	30	0.095
HB PD	0.158	30	0.200	0.963	30	0.379

In both data sets, the normal distribution pattern was followed, with the following characteristics: Most hemoglobin values are concentrated around the mean, and the number of average data is relatively the same. The results of the mean, median, and mode values of whole blood hemoglobin and pre-diluted hemoglobin data tend to be close to or the same, so that the distribution curve is symmetrical (Darmayani et al., 2016). Approximately 68% of hemoglobin values were within one standard deviation of the mean, 95% were within two standard deviations, and almost all (99.7%) were within three standard deviations of the mean (Notoatmodjo, 2018).

Based on the results of the paired t-test on hemoglobin levels using the whole blood and pre-diluted modes in Table 3, a p-value of 0.002 ($p < 0.05$) was obtained. This result indicates a significant difference between the two examination modes.

Table 3. Hemoglobin comparison test

		SD	STD.Error Mean	Sig(-tailed)
Pair	HB WB - HB PD	0.5612	0.04188	0.002

Based on the results of a paired t-test on the difference in hemoglobin levels between whole blood and pre-diluted hemoglobin, the standard deviation (SD) was 0.5612 g/dL. This value indicates that the variation in the difference between paired data is relatively low, indicating consistency in the results of the differences between samples. There were no extreme fluctuations that could indicate the presence of significant outliers (Andriyani et al., 2019; Amelia et al., 2023).

The Standard Error of the Mean (SEM) of 0.04188 g/dL indicates high precision in estimating the average difference obtained. With a small SEM, it can be concluded that this average difference is a stable and reliable estimate of the difference between the two measurement methods (Darmayani et al., 2016).

A significance value (p-value) of 0.002 (<0.05) confirms that the detected difference is statistically significant. In practice, this means there is a consistent systematic difference between hemoglobin measurements in the whole blood mode and in the pre-diluted mode. The negative direction of the difference (HB PD < HB WB) indicates that measurements in the pre-diluted mode tend to be lower than in the whole blood mode. This pattern is consistent with the assumption of a hemodilution effect due to the addition of a diluent solution in the pre-diluted mode, which lowers the measured hemoglobin concentration. Thus, these results overall demonstrate a consistent and measurable method difference (Gina et al., 2020).

Table 4. Leukocyte Normality Test

	Kolmogorov-Smirnov			Shapiro Wilk		
	Statistic	**df**	**Sig.**	**Statistic**	**df**	**Sig.**
Leukocyte WB	0.122	30	0,200	0.968	30	0.478
Leukocyte PD	0.112	30	0,200	0.962	30	0.347

Based on Table 4, the results of the normality test for leukocyte counts using the Shapiro-Wilk method showed a significance value of 0.478 for the whole blood method and 0.347 for the pre-diluted method. Because both significance values are greater than 0.05, it can be concluded that the data are normally distributed.

The leukocyte counts in both datasets follow a normal distribution pattern, characterized by the following: Most leukocyte counts are concentrated around the mean, and the number of medians is relatively equal. The mean, median, and mode of the whole blood and pre-diluted leukocyte counts tend to be close to or equal, resulting in a symmetrical distribution curve. Approximately 68% of leukocyte counts fall within one standard deviation of the mean, 95% within two standard deviations, and almost all (99.7%) fall within three standard deviations of the mean. A paired t-test was then performed (Notoatmodjo, 2018).

Table 5. Leukocyte Comparison Test

		SD	**STD.Error Mean**	**Sig(-tailed)**
Pair	Leukocyte WB - Leukocyte PD	0.4324	0.790	0.040

Based on Table 5, the results of the paired t-test on leukocyte parameters show a standard deviation (SD) value of 0.4324 × 10^3/µL, which indicates that the variation in differences between samples is relatively small and consistent across all data. The standard error of the mean (SEM) value of 0.790 × 10^3/µL illustrates the level of error in estimating the average difference that is still within the acceptable precision limits for the study sample size (n = 30). A significance value of p = 0.040 (<0.05) indicates a statistically significant difference between leukocytes in whole blood and pre-diluted modes. A negative difference indicates that the average in pre-diluted mode is lower than in whole blood mode (Jurastiwi, 2017).

3.2. Discussion

The significant difference in hemoglobin levels between the two examination modes can be explained by the hemodilution mechanism. The pre-diluted mode uses a smaller blood sample volume than whole blood and requires the addition of a diluent solution (cell pack) containing phosphate-buffered saline (PBS). This dilution process, while intended to maintain cell stability, indirectly reduces the measured hemoglobin concentration due to a decrease in the proportion of erythrocytes to the total fluid volume (Andriyani et al., 2019).

This finding aligns with research by Amelia et al. (2023), showing that dilution can decrease hematological parameters, particularly the erythrocyte count, which is the basis for calculating hemoglobin levels. Furthermore, hemoglobin stability is also significantly influenced by pre-analytical factors, such as the waiting time before examination and sample storage temperature (Bain, 2014). In the pre-diluted mode, an excessive anticoagulant ratio can alter the integrity of the erythrocyte membrane, increasing the risk of hemolysis, and ultimately affecting the measurement results (Widianto, 2019).

This difference in hemoglobin values is similar to the findings in leukocyte parameters, although quantitatively the difference is relatively smaller. Paired t-test results indicate a statistically significant difference between leukocyte counts in the whole blood and pre-diluted modes. In the pre-diluted mode, the addition of a diluent solution can separate and disperse leukocytes inhomogeneously, resulting in some cells not being detected by the flow cytometry sensor on the hematology analyzer (Siska, 2020).

Jurastiwi (2017) adds that each manual or automatic mixing step has the potential to cause some cell loss due to adhesion to the tube wall or damage to the cell membrane. However, clinically, the leukocyte values produced by both modes remain within the normal reference range (4,650–10,300/µL) as established by the Clinical and Laboratory Standards Institute (CLSI, 2018), so the difference does not have significant implications for diagnostic interpretation.

The similarity in the patterns of differences in hemoglobin and leukocytes suggests that the effect of the pre-diluted method is systematic, although the magnitude of the differences differs. In hemoglobin, this difference has potential clinical implications, especially in respondents with values approaching the lower limit of normal, whereas in leukocytes, although statistically significant, the mean values of both modes remain within the normal reference range (CLSI, 2018), so the clinical implications are relatively minimal.

The findings of this study corroborate a previous study (Shintia, 2018; Gina et al., 2020), which states that the pre-diluted method can be a valid alternative when the whole blood method is not feasible in cases of limited sample volume, such as in neonates, pediatrics, or when veins are difficult to access. Although there was a statistically significant difference, the resulting values were still clinically acceptable, considering the context of the problem.

From a laboratory management perspective, the agreement of the results for both parameters with the reference value limits strengthens the argument that the pre-diluted method can be an effective solution to reduce sample rejection rates due to insufficient sample volume, without compromising the reliability of result interpretation. However, implementation of this method requires strict standard operating procedures (SOPs), including minimum blood volume limits, diluent composition, and maximum examination time since sample collection, to minimize bias in the results (Notoatmodjo, 2018).

Numerical data analysis also supports this conclusion. For hemoglobin parameters, the average values for the whole blood and pre-diluted modes were 11.828 and 11.483 g/dL, respectively, while the leukocyte parameters, the average values were 7.357 and 7.187 × 10^3/µL. When compared with the reference values for hemoglobin for men (12.5–16.7 g/dL) and women (12.0–15.6 g/dL), and leukocytes (4,650–10,300/µL) (Gina et al., 2020), the results for both modes were close to or within the normal range. These findings indicate that although the identified differences were statistically significant, they did not significantly impact clinical interpretation. Therefore, the pre-diluted mode can be used as an alternative examination option when the whole blood mode is not possible (Shintia, 2018; Gina et al., 2020; CLSI, 2018).

4. Conclusion

There are significant differences between the whole blood and prediluted modes for hemoglobin and leukocyte testing. However, the prediluted mode can still be used as an alternative test because it does not affect the interpretation of the results.

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