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Comparison between Sysmex CyFlow Counter and BD FACSCanto II for counting CD4$^+$ Cells in Indonesia

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ABSTRACT

CD4$^+$ count is essential in evaluating the immunological status of HIV+ patients and the need for prophylaxis therapy against opportunistic infections. CyFlow Counter is a novel Sysmex instrument to count CD4$^+$ cells and reports the results in absolute and percentage values (aCD4$^+$, %CD4$^+$). However, it has not been evaluated in Indonesia. This study aimed to compare the Sysmex CyFlow Counter with BD FACSCanto II. Samples were collected from leftover EDTA blood samples of patients with CD4$^+$ count tested in Dharmais Cancer Hospital. The aCD4$^+$ and %CD4$^+$ from CyFlow Counter were compared against FACSCanto II using correlation, Bland-Altman, and mean difference test. Sensitivity, specificity, and misclassification rates were also analyzed with aCD4$^+$ count threshold of 200 cells/µL. A total of 70 EDTA blood samples from Dharmais Cancer Hospital were analyzed with BD FACSCanto II and Sysmex CyFlow Counter, with 20 subjects having CD4$^+$ count of 150-299 cells/µL, 28 having 300-449 cells/µL, and 22 having 450-550 cells/µL. CyFlow Counter had a good correlation with FACSCanto II in aCD4$^+$ and %CD4$^+$ (r = 0.892 [p=0.000], r=0.955 [p=0.000], respectively). There was no significant mean difference between CyFlow Counter and FACSCanto II (p=0.097 for aCD4$^+$ and p=0.611 for %CD4$^+$). Bland-Altman test results showed a high agreement (94.29%) with a mean difference of -32.29 cells/µL for aCD4$^+$ and a high agreement (98.57%) with a mean difference of -0.76% for %CD4$^+$. Sensitivity, specificity, and total misclassification rates were 83.33%, 100.00%, and 3.33%, respectively. Sysmex CyFlow Counter CD4$^+$ count results were comparable to FACSCanto II.

Keywords: HIV, CD4$^+$ count, absolute, percentage, CyFlow Counter, FACSCanto II

INTRODUCTION

CD4$^+$ cell count is an important test for measuring a patient's immune and clinical status and risk of opportunistic infections and guiding diagnostic decision-making, especially for patients with advanced HIV disease.[1] A study in Indonesia in 2016 showed an inverse correlation between CD4$^+$ cell count and neopterin, a marker of immune activation.[2]

Sysmex CyFlow Counter is a semi-automatic instrument to identify absolute and percentage CD4$^+$ count (aCD4$^+$, %CD4$^+$). CyFlow Counter uses a green laser with three optical parameters: SSC and two fluorescence channels. It also uses an accurate volumetric absolute counting system.

Sysmex CyFlow Counter has been evaluated by the World Health Organization (WHO) in Belgium, resulting in good precision and correlation against BD FACSCanto II.[3] However, it has yet to be evaluated in Indonesia. The objective of this study was to compare the Sysmex CyFlow Counter with BD FACSCanto II. The result of this study can be used to help decide its use in Indonesia.

METHODS

The study design was cross-sectional. The study population consisted of patients in Dharmais Cancer Hospital, Indonesia, who came to the laboratory to have their CD4$^+$ count tested with BD FACSCanto II in April 2021. Inclusion criteria were CD4$^+$ count of 150-299 cells/µL, 300-449 cells/µL, and 450-550 cells/µL with at least 20 subjects for each group. Leftover EDTA blood samples were collected and analyzed using Sysmex CyFlow Counter. Samples with insufficient volume were excluded from the study.

Within-run and between-days precision test was carried out using Streck Control Material Low and for aCD4$^+$ and %CD4$^+$. Ten consecutive runs were performed in one day for the within-run precision test and one each day for ten consecutive days for the between-days precision test.

Absolute and percentage CD4$^+$ count were measured for each subject. Results from Sysmex CyFlow Counter were compared with BD FACSCanto II using correlation and the Bland-Altman test in accordance with a study in 2017 about statistical

method comparison instruments for choosing a new CD4 technology (Table 1).[4] The Sensitivity, specificity, and misclassification rates were also analyzed for aCD4$^+$ following the study. The CD4$^+$ count threshold used in this study was 200 cells/µL, consistent with the WHO 2017 guideline definition of advanced HIV disease.[5] The threshold values of 350 and 500 cells/µL used in the study in 2017 were not applied in this study because the initiation of antiretroviral therapy does not consider CD4$^+$ count anymore. The mean difference was determined based on several CD4$^+$ count methodologies in comparison studies.[6,7]

Table 1. Analytic methods

Method	Principle
Correlation	Relationship
Bland-Altman	Agreement
Sensitivity, specificity	Accuracy
Misclassification rate	Accuracy
Mean difference	Difference

This study has been declared ethically feasible by the Research Ethics Committee of Dharmais Hospital, Jakarta, with the number 023/KEPK/III/2021.

RESULTS AND DISCUSSIONS

A total of 70 samples were collected, with 20 subjects having CD4$^+$ count of 150-299 cells/µL, 28 having 300-449 cells/µL, and 22 having 450-550 cells/µL. Within-run precision test using Streck Control Material Low yielded a CV of 7.06% for aCD4$^+$ and 4.87% for %CD4$^+$, while those using Streck Control Material Plus resulted in a CV of 5.80% for aCD4$^+$ and 1.05% for %CD4$^+$. Between-day precision test using Streck Control Material Low yielded a CV of 8.53% for aCD4$^+$ and 7.31% for %CD4$^+$, while the ones using Streck Control Material Plus resulted in a CV of 8.85% for aCD4$^+$ and 2.00% for %Cd4$^+$.

Saphiro-Wilk normality test on the aCD4$^+$ using Sysmex CyFlow Counter and BD FACSCanto II showed abnormal distribution in both variables (p=0.030 and 0.003, respectively). Because of the abnormal distribution, the correlation between both measurements was analyzed using the Spearman correlation test, which later revealed a correlation coefficient of 0.892 (p=0.000). In addition, because of the abnormal distribution, the mean difference between both measurements was analyzed using the Mann-Whitney U test, which showed no significant difference (p=0.097).

Bland-Altman test revealed a mean bias of -32.29 cells/µL (95% limits of agreement were 69.10 and -133.68, Figure 1). Four subjects were outside the limit of agreement, with aCD4$^+$ of 396 and 270, 329 and 496, 356 and 493, 370 and 542 cells/µL using Sysmex CyFlow Counter and BD FACSCanto II, respectively (Table 2).

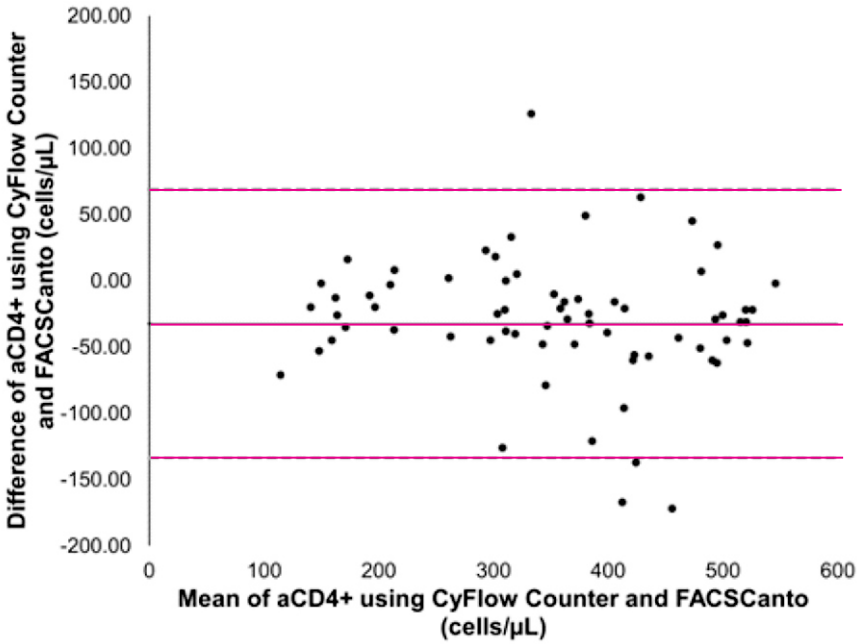

Figure 1. Bland-Altman plot of aCD4$^+$ between CyFlow Counter and BD FACSCanto II

Saphiro-Wilk normality test on the %CD4$^+$ using Sysmex CyFlow Counter and BD FACSCanto II showed normal distribution in both variables (p=0.929 and 0.565, respectively). Because of the normal distribution, the correlation between both measurements was analyzed using the Pearson correlation test, which revealed a correlation coefficient of 0.955 (p=0.000). In addition, because of the normal distribution, the mean difference between both measurements was analyzed using an independent T-test, which showed no significant difference (p=0.611).

Bland-Altman test revealed a mean bias of -0.76% (95% limits of agreement were 4.44 and -5.95, Figure 2). One subject was outside the limits of agreement, with %CD4$^+$ of 26% using Sysmex CyFlow Counter and 41.5% using BD FACSCanto II (Table 2).

Sensitivity and specificity were calculated for aCD4$^+$ using a threshold of 200 cells/µL. An aCD4$^+$ value less than the threshold was considered positive. The calculation revealed a sensitivity of 83.33% and a specificity of 100.00% (Table 3). The misclassification rate was also determined and yielded a false positive rate of 0.00%, a false negative rate of 3.33%, and a total misclassification rate of 3.33%.

Table 3. Sensitivity and specificity

		BD FACSCanto II	
		Pos	Neg
Sysmex CyFlow Counter	Pos	10	0
	Neg	2	58

According to the WHO protocol for laboratory evaluation of lymphocyte subset enumeration technologies, aCD4$^+$ is required to have a CV of less than 15% for aCD4$^+$ ≤ 200 cells/µL and less than 10% for aCD4$^+$ > 200 cells/µL.[8] The within-run and between-days precision tests in this study using Streck Control Material Low (target value 171 cells/µL) yielded a CV of 7.06% and 8.53%, respectively, which were below the limit of 15%. Within-run and between-days precision tests using Streck Control Material Plus (target value 1196 cells/µL) resulted in a CV of 5.80% and 8.85%, respectively, which were also below the limit of 10%.

The correlation test of aCD4$^+$ showed a robust correlation (correlation coefficient 0.892, p=0.000),

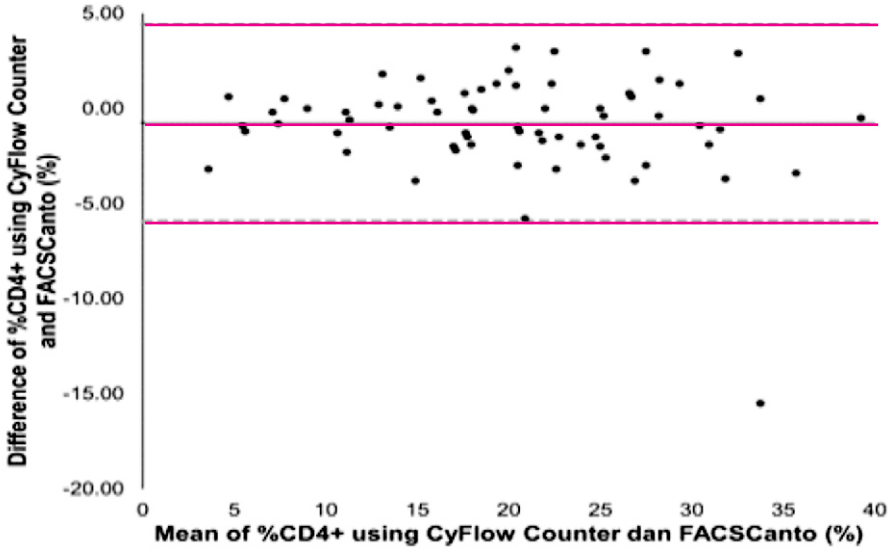

Figure 2. Bland-Altman plot of %CD4$^+$ between CyFlow Counter and BD FACSCanto II

Table 2. Difference between subjects with aCD4$^+$ and/or %CD4$^+$ outside of limits of agreement

Subject	aCD4$^+$ (cells/µL)		%CD4$^+$ (%)	
	CyFlow Counter	BD FACSCanto II	CyFlow Counter	BD FACSCanto II
1.	**396**	**270**	16	14.4
2.	**329**	**496**	18	23.8
3.	**356**	**493**	22	22
4.	**370**	**542**	**26**	**41.5**

and the mean difference test showed no significant difference (p=0.097). In addition, the correlation test of %CD4$^+$ also delivered a robust correlation (correlation coefficient 0.955, p=0.000), and the mean difference test showed no significant difference (p=0.611).

The Bland-Altman test for %CD4$^+$ showed that 1 of 70 subjects had a difference outside the limit of agreement (Figure 2, Table 2), still resulting in a high agreement of 98.57%. A lower %CD4$^+$ in the subject compared to the reference method might be caused by a smaller gating when the sample was tested with CyFlow Counter.

The Bland-Altman test for aCD4$^+$ showed that 4 of 70 subjects had a difference outside the limit of agreement (Figure 1, Table 2), still resulting in a high agreement of 94.29%. Lower aCD4$^+$ found in 3 subjects but higher aCD4$^+$ found in 1 subject might be caused by the difference in absolute lymphocytes measured by CyFlow Counter and the reference method. CyFlow Counter uses a single platform method, while the reference method uses a double platform one. This is in line with the WHO prequalification study for CyFlow Counter, which showed a tendency towards a negative bias compared to the dual platform method.[9] However, the differences in the four subjects did not cause any difference in determining the need for prophylaxis treatment for cryptococcal disease and special counseling.[5]

The sensitivity and specificity of Sysmex CyFlow Counter to determine advanced HIV disease were 83.33% and 100.00%, respectively, with a false positive rate of 0.00%, a false negative rate of 3.33%, and a total misclassification rate of 3.33%. These results were better than those of a study in 2017, although the thresholds used were different.[4] In the previous study, sensitivity for <350 and <500 cells/µL were 84.90% and 96.00%, respectively; while specificity for <350 and <500 cells/µL were 93.20% and 95.80%, respectively. In addition, total misclassification rates for <350 and <500 cells/µL were 12.60% and 3.93%, respectively.

CONCLUSIONS AND SUGGESTIONS

Sysmex CyFlow Counter CD4$^+$ count results were comparable to FACSCanto II. Further studies with extreme samples (aCD4$^+$ <150 cells/µL or > 550 cells/µL, older than 24 hours) were suggested to evaluate its limitation.

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