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Technical Report

Impact of preanalytical storage on the accuracy of CD3, CD4, CD8 testing results using the BD FACSLyric™ Clinical Flow Cytometry System

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Abstract

Objectives: The development of flow cytometry has facilitated the phenotypic characterization of different populations of lymphocytes. The number of technical platforms performing this analysis is limited and requires transportation of samples from the field site to the laboratory. The aim of our study was to evaluate the agreement of absolute CD3+, CD4+ and CD8+ T cells count measurements from whole blood specimens stored at room temperature (15–25°C) tested 24 h and 72 h post-collection.

Methods: Forty-one EDTA-anticoagulated blood samples stored at room temperature (15–25°C) after sampling were assayed after 24 h and 72 h, respectively. The BD FACSLyric™ system was used to identify and enumerate cell subsets.

Results: After 72 h, CD3+, CD4+, and CD8+ T lymphocytes, data showed non-significant differences with p-values of 0.766, 0.855, and 0.754, respectively. The Boxplot showed substantial convergence between the two measurement periods.

Conclusion: Our data indicated that whole blood can be stored for up to 72 h at room temperature before analysis without affecting the result.

Keywords: Flow cytometry, lymphocyte subset counts, preanalytical storage

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The analysis of peripheral blood for lymphocyte subsets is explored in various diseases such as autoimmune diseases, viral infections, leukemia, renal transplant, and acquired immune deficiency syndrome [1, 2]. The CD4 antigen on T cells is a target of HIV infection and the decrease in the number of CD4+ T cells is closely related to disease progression. The CD8 antigen is expressed on the suppressor/cytotoxic T cell population. Increased activation and proliferation of CD8+ T cells correlate with acute stages of HIV infection [3]. The development of flow cytometry has facilitated the phenotypic characterization of different types of lymphocytes by laboratories. In France, a limited number of laboratories perform this analysis, blood collection is most often done in a peripheral laboratory located at a different address from the technical platform performing the analysis, and therefore, samples must be transported. The accuracy of CD3+, CD4+, and CD8+ T lymphocytes testing results depends on the quality of the blood sample provided to the laboratory. According to the technical data sheet (BD Multitest™ CD3/CD8/CD45/CD4, BD Biosciences®, San Jose, USA), anticoagulated blood stored at room temperature (20–25°C) must be stained within 48 h of draw.

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The aim of our study was to evaluate the agreement of absolute counts of CD3+, CD4+, and CD8+ T lymphocytes from whole blood specimens stored at room temperature (15–25°C) tested 24 h and 72 h post-collection.

Materials and Methods

Ethical approval

The laboratory investigations were carried out in accordance with the General Data Protection Regulation (EU Regulation 2016/679 and Directive 95/46/EC) and the French data protection law (Law 78–17 of 6 January 1978 and Decree 2019–536 of 29 May 2019), which does not require a review by an ethics committee for the secondary use of samples collected for health-care purposes. In such case, the use of elements and products of the human body for a medical or scientific purpose other than that for which they were removed or collected is possible (article L.1211-2 of the French Public Health Code). The "Labosud Database" is registered at the French National Commission on Informatics and Liberty, CNIL, under record No. 2073511v0.

Inclusion of participants

From February 2022 to April 2022, we studied the accuracy of CD3+, CD4+, and CD8+ T lymphocytes results of forty-one EDTA-anticoagulated blood samples stored at room temperature (15–25°C) after sampling and assayed after 24 h and 72 h, respectively. Thirty-four subjects were treated for acquired immune deficiency syndrome, five were monitored for leukemia and two patients with pulmonary viral infections. The samples were analyzed in the different runs using the same instrument which had been verified according to the accreditation criteria of ISO 15189 [4].

Table 1. Statistical description of CD3+, CD4+ and CD8+ T cells results after 24 and 72-h sampling and stored at room temperature (15–25°C)

	CD3+ T (cells/µL)				CD4+ T (cells/µL)				CD8+T (cells/µL)			
	Storage 24 h at 15–25°C	Storage 72 h at 15–25°C	Mean bias %	t-test 24 h versus 72 h	Storage 24 h at 15–25°C	Storage 72 h at 15–25°C	Mean bias %	t-test 24 h versus 72 h	Storage 24 h at 15–25°C	Storage 72 h at 15–25°C	Mean bias %	t-test 24 h versus 72 h
Number of samples	41	41	−2.2%	p=0.766	41	41	−2.2%	p=0.855	41	41	−3.0%	p=0.754
Minimum value	515	524			231	222			281	302		
Maximum value	3022	3001			1557	1542			1760	2047		
Mean	1772	1809			744	757			1000	1028		
SD	559	567			318	320			406	418		

SD: Standard deviation

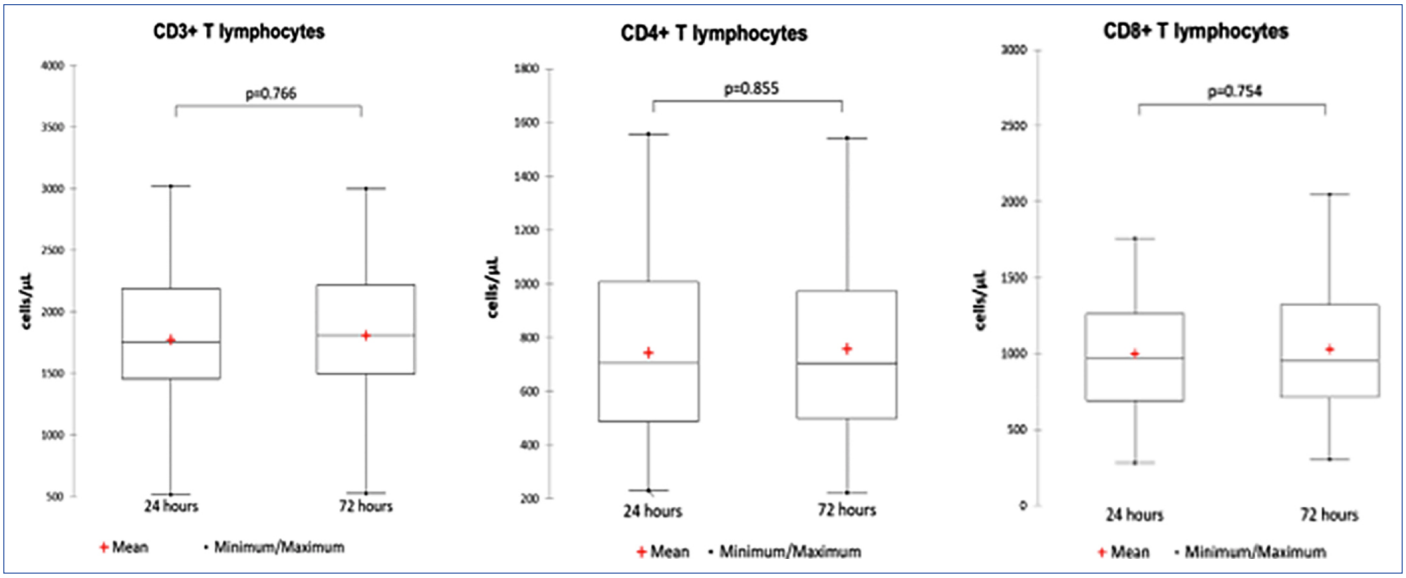

Figure 1. Boxplots of CD3+, CD4+, CD8+ T lymphocytes results during storage at 24 and 72 hours.

Analysis

The BD FACSLyric™ system, using flow cytometry, identifies and enumerates cell subsets. Antibodies directed against to specific cell proteins are labeled with a fluorescent dye and incubated with the cell suspension. The suspension flows through the cytometer and is measured by a laser which excites the fluorescent antibodies. The fluorescence is captured, and the resulting data are analyzed to reveal information about the cells. Multiple antibodies, each labeled with a different fluorochrome, can be used in a single tube to simultaneously identify different cell populations. The imprecision of the method for counting CD3+, CD4+ and CD8+ T lymphocytes showed CVs of 4.7%, 5.8%, and 5.4%, respectively.

Statistical analysis

A Shapiro–Wilk test demonstrated that the distribution of results was parametric and the comparison between populations was evaluated using a paired Sample t test (differences between periods were considered significant at $p<0.05$). To illustrate the impact of the results according to the methods used, boxplots have been used.

Analytical agreements between 24 h and 72 h after sampling were analyzed using a scatter plot with a Passing Bablok regression analysis. Correlation between methods was assessed using the Pearson correlation test ($p<0.05$ was considered statistically significant). In the regression analysis, limits of agreement were plotted according to the minimum total error formula developed by Frazer [5] using the biological variation of peripheral blood T-lymphocytes defined by Falay et al. [6] The acceptable accuracy counting CD3+, CD4+, and CD8+ T lymphocytes should be within the total allowable error range of ±24.4%, ±20.4%, and 25.4%, respectively. Analytical agreement results between 24 h and 72 h after sampling were analyzed using Bland-Altman Plots [7] expressed as percentages of the values on the axis ([time 24 h-time 72h/Average %]).

Results

For CD3+ T lymphocytes, the results at 24 h varied between 515 and 3022 cells/µL with a mean of 1772±559, and at 72 h, they ranged from 524 to 3001 with a mean of 1809±567. Regarding CD4+ T cells, the first measurements were between 231 and 1557 cells/µL with a mean of 744±318, and the second ranged from 222 to 1542 with a mean of 757±320. The results for CD8+ T lymphocytes at 24 h varied between 281 and 1760 cells/µL with a mean of 1000 ± 406, and at 72 h they ranged from 302 to 2047 with a mean of 1028 ± 418 (Table 1). After 72 h, CD3+, CD4+, and CD8+ T lymphocytes, data showed non-significant differences with p-values of 0.766, 0.855, and 0.754, respectively. The Boxplot showed substantial convergence between the two measurement periods (Fig. 1).

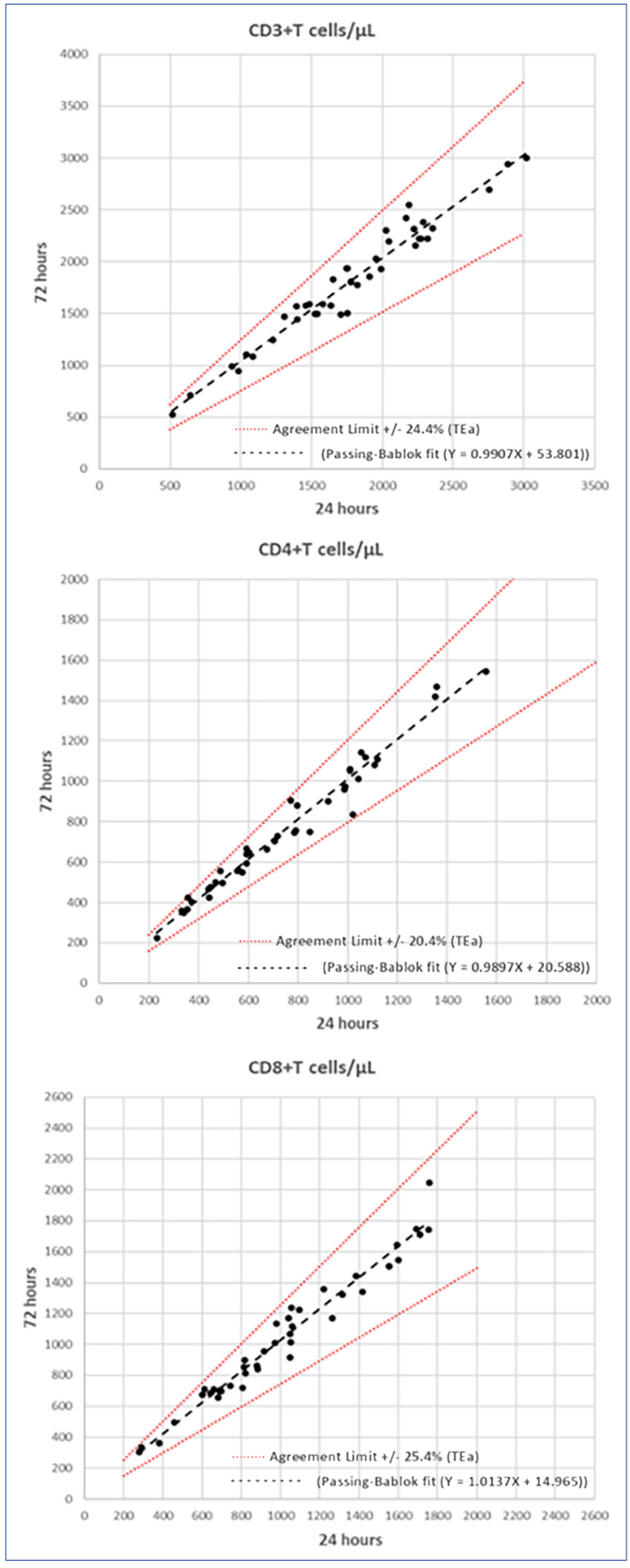

Figure 2. Comparison of results obtained after 24 hours and 72 hours of sampling for CD3+, CD4+ and CD8+ T lymphocytes.

For CD3+ T cells, the Pearson correlation coefficient (r) showed a significant and strong (r=0.98 [p<0.001; 95% confidence interval [CI]: 0.91–1.05]). The regression slope was 0.991 (p<0.001; 95% CI: 0.920–1.062) and the y intercept was 53.8 (p<0.001; 95% CI: −78.2–185.8). For CD4+ T populations, the Pearson correlation coefficient (r) indicated a significant and strong correlation (r=0.99 [p<0.001; 95% CI: 0.93–1.04]). The regression slope was 0.990 (p<0.001; 95% CI: 0.933–1.046) and the intercept was 20.6 (p<0.001; 95% CI: −24.9–66.0). The correlation coefficient of Pearson (r) for CD8+ T lymphocytes showed a significant and strong correlation (r = 0.98 [p<0.001; 95% CI: 0.92–1.04]). The regression slope was 1.014 (p<0.001; 95% CI: 0.950–1.077) and the intercept was 14.9 (p<0.001; 95% CI: −53.4–83.3). As regards to the three parameters studied, the analytical agreements showed that all results were within the total allowable margin of error (Fig. 2). A Bland-Altman plot showing the agreement results between the different analysis times is shown in Figure 3. For CD3+ T cells, the mean percentage difference between 24 and 72 h was −2.2 % (95% CI −15.3% to +10.9%). Regarding CD4+ T cells, the mean percentage difference was −2.2% (95%CI −16.1% to +11.7%). Finally, for CD8+ T lymphocytes, the mean percentage difference was −3.0% (95%CI −17.5% to +11.5%). The Bland-Altman plot method only defines the intervals of agreements, it does not say whether those limits are acceptable or not. Acceptable limits (red lines) based on biological considerations showed that the bias % of each test were included in the minimum total allowable error range (Fig. 3).

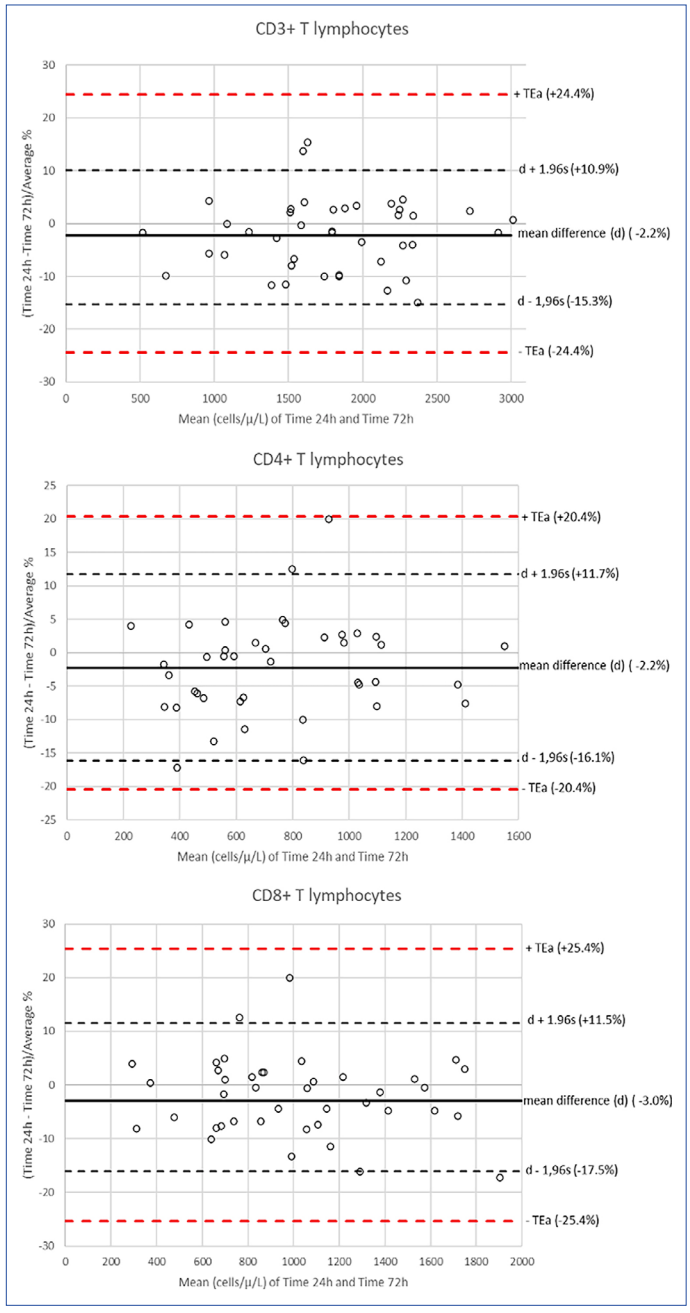

Figure 3. Bland-Altman plot of the difference between Time 24h and Time 72h, expressed as percentages of the values on the axis [(time 24h – time 72h)/Average %)], vs. the mean (cells/µL) of the two measurements.

Discussion

In 1993, Paxton and Bendele [8] showed that increases of temperature resulted in greater variation and that 4°C could be an acceptable storage parameter for whole blood where extreme ambient temperatures may be a problem. In 2004, Jalla et al. [9] described non-significant alteration in T-cell subsets after storage at elevated room temperature (38–45°C) for 24 h. Other data indicated that, when tests cannot be done immediately, samples for T cell measurements could be conveniently held at a temperature of around 17°C for up to 48 h with minimum changes in the level of expression of each subset [10, 11].

Obtaining high quality flow cytometry data from peripheral blood samples and the need to transport the samples present additional challenges. It requires careful harmonization of logistical procedures between laboratories, including temperature control in extreme climatic environments. Processing of diagnostic samples can be delayed by transportation as well as by personnel and equipment availability. This can have several consequences for the outcome of flow cytometric analysis. In this study, no significant change in the accuracy of CD3+, CD4+ and CD8+ T lymphocytes was observed when whole blood was stored at room temperature for 72 h before analysis. These results could facilitate the phenotypic characterization of different T lymphocytes populations by laboratories, especially for very remote populations. These results relate exclusively to the method used and cannot necessarily be extended to other technologies.

Conclusion

When blood samples cannot be analyzed immediately, as it is often the case for CD3+, CD4+, and CD8+ T lymphocytes subsets, our data indicated that whole blood can be stored for up to 72 h at room temperature (15–25°C) before analysis without affecting the results.

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