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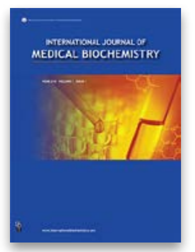

Letter to the Editor

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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Dear Editor,

We eagerly and thoroughly enjoyed reading your journal's first edition of the new year. We were particularly interested in the flow cytometry-related paper and the letter to the editor in this issue [1, 2]. We would like to take this opportunity to express our appreciation to the participating researchers and technical collaborators.

Various theoretical and practical approaches are involved in the interpretation of flow cytometry analyses. The analysis is subject to interpretation because it might differ based on the scientific discipline and methodology used, even though the basic ideas are always the same.

In keeping with the aforementioned concepts, we would like to provide some remarks and contributions to illustrate our methodology through the insightful article of the International Journal of Medical Biochemistry titled "Impact of preanalytical storage on the accuracy of CD3, CD4, and CD8 testing results using the BD FACSLyric™ Clinical Flow Cytometry System."

We shall try to draw conclusions through some remarks because the article did not go into detail about the testing procedures. For example, how was the absolute count of T lymphocyte subsets determined using the gating strategy? First, we assume that the flow was monitored (SS-Time), then the doublets were removed (FS Peak- FS Int), followed by the use of a viability dye (7-AAD) to identify live cells, and finally, we think that the CD45 pan-leukocyte marker (CD45 against SSC) was used for gating.

We were unable to draw firm conclusions regarding the fluorescent signal strength of the cells and the tube design, which may differ depending on the laboratory, because we were unable to observe the tube design of the lab (CD45-FITC/CD4-PE/CD3-PerCp, etc.). Thus, we can add that APC is more stable among the fluorescent dyes, whereas FITC is more light-sensitive. Consequently, it should either be evaluated right away after application or left in the dark until examination is complete. Although the platform utilized to calculate absolute cell counts is not explicitly mentioned in the article, it appears that the researchers employed the single-platform approach with beads (fluorescent microspheres) to accomplish so. A precise and exact WBC count is crucial when employing the dual-platform approach to provide absolute counts. WBC count × lymphocyte × antibody positivity is the formula for absolute count/µL. The gating technique is crucial in this situation. If there are issues with the WBC count, the dual-platform method is not recommended [3].

Giving the blood-to-bead ratio (1:1, vol/vol, etc.) could have given more information about the researchers' analysis approach if their approach had been single-platform. However, if the article's approach was dual-platform, the Australasian Cytometry Society recommends that the T-cell absolute count be determined using lymphocytes for samples older than 48 hours and WBC counts for samples younger than 48 hours [4]. The "Lymphosum" method is advised to increase accuracy in dual-platform analysis of absolute T-cell counts. The formula T cells+B cells+NK cells=100%±5 is used to confirm this [5].

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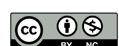

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