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# Comparative Evaluation between the New BD FACS Count System and Standard BD FACS Count System by Enumeration of Absolute TCD4 Lymphocytes in Adults: Preliminary Results

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## Abstract

**Background:** The purpose of this study was to evaluate the performance of the new BD FACS Count System compared to the standard BD FACS Count system for count of lymphocytes TCD4.

**Methods:** It was a comparative study conducted in Centre MURAZ research institute. The New BD FACS Count System dedicated to enumerate absolute and percentage of TCD4+ was compared to the standard BD FACS Count System dedicated to enumerate only absolute number of TCD4+, TCD8+, TCD3+ and the CD4/CD8 ratio. Results were analyzed by Meth Val software.

**Results:** The New BD FACS Count System compared favorably with the BD FACS Count System for absolute TCD4+, resulting in an overall correlation coefficient of 0.99 for the patients evaluated.

**Conclusion:** The New BD FACS Count System is simple to perform as the old system and was an excellent alternative method to manage adults HIV in resource limited settings.

**Keywords:** BD FACS count system; TCD4+ absolute; HIV; Adults; Resource limited settings; Burkina Faso

## Introduction

TCD4+ cells are the target cells for human immunodeficiency virus (HIV). Patients TCD4+ levels is the most important parameter for assessing HIV progression, help to determine risk for opportunistic infections, evaluate if the patient should be placed on antiretroviral therapy (ART) and indicate also if the therapy provided is efficacy [1]. WHO/UNAIDS recommended since 2010 the use of ART treatment cut-off of less than 350 TCD4+/µl for adults and adolescents then since 2013, the limit of TCD4+ to treat has been update at ≤ 500/µl [2,3]. However, US Centers for Disease Control and Prevention (CDC) has established a treatment cut-off TCD4+ percentage of <25% for infants under 11 months of age, <20% for children up to 3 years of age, and <15% for children between 3 and 5 years of age [4]. Conventional flow cytometry is the most accepted gold standard to enumerate absolute and percentage of TCD4+ for adults and infants HIV infection management. But, they are very expensive and complex for resource limited settings. The standard BD FACSCount™ System was developed as an alternative method and dedicated for absolute counting of TCD4+, TCD8+, TCD3+, CD4/CD8 ratio and without simultaneous percentage of lymphocyte. The new BD FACSCount™ system is dedicated to provide simultaneously absolute and percentage results of TCD4+ for adults and infants HIV management. The main study conducted by Pattanapanyasat et al. with the new BD FACS Count was observed good performance with this device in comparison with the gold standard flow cytometry [5].

Before using the new BD FACS Count System for routine TCD4+ management in Burkina Faso, preliminary study was conducted at Centre MURAZ research institute with a few samples of specimen. The purpose of the study was to evaluate the performance of the new BD FACS Count System in comparison with the standard BD FACS Count system for absolute TCD4+ counting as a tool for adults TCD4+ management.

## Materials and Methods

### Design

A small comparative study was conducted in 2010 at Centre MURAZ to compare the standard and the new software of BD FACS Count System for their capacity to deliver the same results of absolute TCD4+ using adult's blood. It was a study to perform an in house comparative evaluation prior to switching the new reagents and the new software of BD FACS Count System before using for routine TCD4+ counting.

Adult's HIV-1, negative and unknown serology participants were included in the study to carry out for absolute TCD4+ and TCD4+ percentage by both two systems of BD FACSCount.

### Subjects

K3EDTA venous whole blood sample were collected from 3 HIV-1 seropositive, 6 HIV seronegative and 1 unknown HIV statuses, and then processed for lymphocytes enumeration within 6 hours. Participant's age was between 22 and 40 years.

### Procedures

**BD FACS Count System (V1.4, Becton Dickinson, San Jose, CA): standard/reference:** Standard BD FACS Count System with

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software V1.4 was used as reference method for the determination of absolute TCD4+cell counts according to the manufacturer's operating procedure. Its reagent kit is provided in a two-tube format containing the antibodies tube of CD4/CD3 reagents with reference beads and tube of CD8/CD3 reagents with reference beads. In standard FACSCount method, 50µl of EDTA uncoagulated whole blood was added to the two-tubes (CD4/CD3 reagent tube, CD8/CD3 reagent tube) using a pipette. They were vortexed for 5 seconds and incubated in the dark at room temperature for 60 minutes. Then, 50 µl of a fixative solution was added to the tubes. The tubes were vortexed, and the non lysed stained sample was analyzed in FACSCount using the standard software.

**BD FACS count system (V1.3, Becton Dickinson, San Jose, CA): new/field:** The new version of BD FACS Count is the same device than the standard one but rune with new software and new reagents for simultaneous counting of absolute and percentage of TCD4+. The reagent kit is provided in a one-tube format containing a mixture of three monoclonal antibodies (CD4/CD14/CD15 conjugated respectively with PE/PE-Cy5/PECy5), a nuclear DNA fluorescent dye, and a known number of fluorescent micro beads. Staining with the new FACSCount, CD4 reagent was performed by adding 50 µl of EDTA-uncoagulated whole blood to the CD4 reagent tube using a pipette. The mixture was vortexed for 5 seconds and incubated in the dark for 30 minutes at room temperature. Then 50 µl of the fixative solution was added to the tube. The tube was vortexed, and the non lysed stained sample was analyzed in FACSCount using the new software [5].

## Statistical Analysis

Meth Val software (Method Validator Software 1.1.9.0, Philippe Marquis, Metz, France) was used to analyze data. Absolute TCD4+ counts obtained by the new FACSCount system were compared to the standard one by linear regression analysis and coefficient of variation. Bland–Altman statistical bias method was used to determine the level of agreement between the results obtained by the new and the standard systems. For the precision, 5 replicates of whole blood from an individual were pipette and analyzed by the new system. Coefficient of variation (CV%) was calculated for absolute TCD4+.

## Results

As it is shown in table1 and figure1, a high and significant overall correlation (r=0, 99, slope of the best linear fit=1,107) was observed between absolute TCD4+ count obtained with the standard software and the new one using the same device of BD FACS Count System. A negative intercept (Y-intercept= -36) was detected. Bland–Altman plots of the comparison between the new and the standard software gave a bias of 39 TCD4+/µl (LOA from -21, 7 TCD4+/µl to 99, 7 TCD4+/µl). For the intra-lab reproducibility of the new system, coefficient of variation obtained was 1, 24% for absolute TCD4+ using the new software.

## Discussion

The new software BD FACS Count Systems is saved time, easy to use and have the same performance as the standard software for absolute TCD4 enumeration. The original software includes CD4 and CD8 T-cell absolute value with the automated reports. The new software eliminates CD8 T-cell counts but includes CD4 T-cell percentage in addition to absolute count in the automated reports. To accommodate for the percentage values of CD4 T-cells, the reagent configuration is different and more expensive with the second option.

With the new software, the detectable range for absolute TCD4+ is 50 to 5000 /µl and the percentage of TCD4+ range is 5% to 65%. The results of the absolute TCD4+ compared favorably with standard FACSCount with an overall correlation coefficient of 0.99. Correlation was done with nine results because one result of TCD4+ obtained by the standard method was superior by 2000TCD4+/µl. The CV was under 2% for TCD4+ obtained with the new system. But, unfortunately the limit of this study was the small number of participants (10) and we think that, the fact that CD4 T-cell absolute values were similar is obvious when using the same instrument with two reagents and the two softwares. For adults HIV infected individuals, percentage of CD4 T-cells is not a requirement. So, it's necessary to perform the new system with children participants under five years for TCD4+ percentage value in resource limited settings.

## Conclusion

The new BD FACS Count system is reliable for adults HIV management in resource limited settings. But, to be performed at best because the evaluation is incomplete, the reliability and the reproducibility of the New BD FACS Count system require further evaluation in larger longitudinal studies in resource limited context based on specimens from children under the age of 5 years.

### Conflicts of Interest

The authors declare no conflict of interest.

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