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Zingue D^{1*}, Hien H^{1,2}, Drabo A¹, Nouctara M¹, Kabore A¹, Ouedraogo O¹ and Meda N^{1,3}

¹Centre Muraz, Ministry of Health/Burkina Faso, West Africa

²Institut de Recherche en Sciences de la Santé (IRSS/DRO)/Burkina Faso, West Africa

³UFR Sciences de la Santé, Université de Ouagadougou/Burkina Faso, West Africa

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

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; Materials and Methods
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+/l for adults and adolescents then since 2013, the limit of TCD4+ to treat has been update at 500/l [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].

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

3 EDTA 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

*Corresponding author: Zingue D, Centre Muraz, Ministry of Health/Burkina Faso, PO Box 390, Burkina Faso, West Africa, Tel: 226 20 97 01 02; Fax: 226 20 97 04 57; E-mail: zinguedezemon@yahoo.fr

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so ware 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 FACSCCount 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 lytic solution was added to the tubes. The tubes were vortexed, and the non lysed stained sample was analyzed in FACSCCount using the standard software.

BD FACS count system (V1.3, Becton Dickinson, San Jose, CA):