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Review

Evaluation of a flow cytometry method for CD4 T cell enumeration based on volumetric primary CD4 gating using thermoresistant reagents

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ABSTRACT

Laboratory follow-up of HIV patients in resource-limited settings requires appropriate instruments for CD4 T cell enumeration. In this study, we evaluated the application of a simplified, mobile and robust flow cytometry system, the Apogee Auto 40 analyzer (Auto40) using thermoresistant reagents, for CD4 T cell enumeration. We measured the absolute CD4 counts in fresh whole blood samples from 170 Senegalese subjects, including 129 HIV-positive (HIV+) patients and 41 HIV-negative (HIV−) controls. Based on volumetric primary CD4 gating, cells were stained with commercially available reagents (Easy MoAb CD4;Bio-D, Valenzano, Italy) and analyzed on the Auto40. The results were compared with those from the FACSCount system (Becton Dickinson, San Jose, USA). Repeatability analysis was performed on duplicate testing of 49 samples on both FACSCount and Auto40. The intra-run precision was measured by 10 replicates using 3 clinical blood samples with low, intermediate and high CD4 concentrations.

The results from the two instruments were in good agreement. The percent similarity between the results of both instruments was 99% ± relative standard deviation of 12.7%. The concordance correlation coefficient was 0.99. The absolute bias and limits of agreement (LOA) between the two instruments, calculated by Bland–Altman analysis, were clinically acceptable (bias: +4 cells/μl; LOA: −111 to +120 cells/μl). The clinical agreement between the two instruments at a cutoff of 200 CD4 cells/μl was 94%. The repeatability of measurements on the Auto40 was also similar to that observed with FACSCount system (bias +0.1 cells/μl, coefficient of variation 2.5% *vs* bias −1.1 cells/μl, coefficient of variation 2.9% respectively). In conclusion, our results indicate that the Auto 40 system, using thermoresistant reagents, is suitable for CD4 T cell enumeration and will be a helpful tool to improve HIV laboratory monitoring in resource-limited settings.

Abbreviations: ART, antiretroviral therapy;CHUN Fann, Fann National University Teaching Hospital;IHS, Institute of Social Hygiene;DGDC, Belgian Directorate General for Development Cooperation.

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Contents

1. Introduction

Antiretroviral therapy (ART) in HIV patients is a complex process requiring close surveillance by health care providers and careful adherence to the therapeutic regimen (World Health Organization (WHO, 2009)). Although the availability of antiretroviral drugs has widely improved, the correct identification of patients requiring treatment and subsequent monitoring of treatment efficacy depend on access to laboratory facilities. Despite the significant progress over the last 10 years, the absence of adequate laboratory facilities in resource-limited countries remains a major challenge. To successfully implement the ART program in these settings, HIV follow-up requires precise but simplified, low-cost and, more importantly, robust assays for assessing the virologic and immunological responses to treatment (Mandy et al., 2008).

Flow cytometry-based counting of lymphocyte subpopulations using fluorescent-labeled monoclonal antibodies is the most widely used tool, and is generally accepted as the method of choice for this type of analysis (Brando et al., 2000). However, flow cytometry instruments are often oversized and too expensive to perform simple CD4 measurements. Moreover, well-trained technicians are needed to operate these instruments, which are very sensitive to humidity and dust and require regular maintenance. In addition, the corresponding reagents are often sensitive to environmental temperature variations during shipment and storage. Thus, the requirement of a cold chain during transport and storage complicates the logistic organization and increases the running costs (Mandy et al., 2008).

A number of alternative CD4 counting systems have been introduced in countries with limited resources (Dieye et al., 2005; Pattanapanyasat et al., 2008). However, there is still a continuous need to evaluate the accuracy, precision and validity of novel low-cost CD4+ T cell counting technologies.

The Auto 40 system (Apogee Flow Systems, Hemel Hempstead, UK) is an instrument for CD4 cell enumeration. It is a mobile flow cytometer that uses the Easy MoAb CD4 thermoresistant reagents (Bio-D, Valenzano, Italy). The efficacy of this monoclonal antibody has been previously evaluated in a multicenter study, conducted in five different European laboratories (Barbesti et al., 2005). This system works according to a no-lyse no-wash procedure. The analysis of the results is performed in accordance with the method of primary CD4 gating (Janossy et al., 2000).

The Apogee A40 analyzer was originally developed for military applications. Recently, it has been adapted for CD4 counting to the Auto40 system. It consists of a blue diode laser with a side scatter and a fluorescent detector. It can optionally be equipped with up to three fluorescent detectors, together with a sheath fluid recycling cassette to enhance its radius of independence. The device automatically computes both relative and absolute CD4 cell counts, based on volumetric measurements, using a syringe moved by a step-by-step motor.

In the present study, the Auto40 system was compared with a well-established, commercially available dedicated CD4 instrument, the FACSCount (Becton Dickinson, San Jose, CA), in a reference lab in Dakar, Senegal.

2. Materials and methods

2.1. Blood samples

In total, 170 consecutive whole blood samples were collected from patients presenting for follow-up at the Fann University Teaching Hospital (CHUN Fann) or Institute of Social Hygiene (IHS). Among these patients, 129 were HIV-positive (119 HIV-1, 8 HIV-2 and 2 HIV-1/2), and 41 were HIV-negative. Negative subjects were recruited as volunteers in the hospital. The HIV status was validated by two rapid HIV-1/2 diagnostic tests (Determine®, Abbott Laboratories, Illinois, USA and ImmunoComb® II, HIV-1&2 Bispot, Orgenics, Yavne, Israel). This study has been approved by the Senegalese ethical committee and all patients signed an informed consent.

2.2. CD4 T cell numeration

Whole blood samples were analyzed in the Immunology Unit (Laboratory of Bacteriology and Virology) at Le Dantec University Hospital, Dakar, Senegal, within 3 h after venipuncture.

The reference procedure for absolute CD4 counting used in this study was the FACSCount system (Becton Dickinson, San Jose, USA) and its dedicated commercial reagents for absolute CD4 counting. Briefly, 50 µl of K_3EDTA anti-coagulated whole blood was stained with fluorescent anti-CD3 and anti-CD4 antibodies. FACSCount Test tubes contained fluorescent microbeads with a known concentration to calculate the