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Research paper

Performance evaluation of the MBio Diagnostics point-of-care CD4 counter

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ABSTRACT

The measurement of the absolute CD4 T-cell count is critical in the initial evaluation and staging of HIV-infected persons, yet access to this technology remains limited in many low resource settings where disease burden is highest. Here we evaluate the performance of a prototype point-of-care device (POC) to quantify CD4 T cells from MBio Diagnostics, Inc. Whole blood samples, both venous and capillary (finger stick), were collected from known HIV-infected participants at the University of California, San Diego Antiviral Research Center, and tested using the MBio system and conventional flow cytometry. A total of 94 venipuncture and 52 capillary samples were processed and statistical analyses included comparison to flow cytometry results. For the venipuncture samples, Bland–Altman analysis resulted in a mean bias of −10 cells/μL (−23 to +3 cells/μL, 95% CI), and limits of agreement (LOA) of −132 and +112 cells/μL. For the capillary samples, Bland–Altman resulted in a mean bias of −4 cells/μL (−31 to +23 cells/μL, 95% CL), and LOA of −195 and +186 cells/μL. For the San Diego study cohort, the prototype MBio system showed negligible quantitative bias relative to flow cytometry. Higher variability was observed in the capillary samples relative to venipuncture, but system precision for both capillary and venipuncture samples was good. There was also close agreement between results from the same participant when tested with two different systems, different operators and different locations. This preliminary evaluation suggests that the MBio CD4 device holds promise as a POC system for quantitation of CD4 T cells in limited-resource settings.

1. Introduction

Destruction of CD4 helper T cells is the hallmark of HIV infection. Thus, the CD4 T cell count is an important measurement used for disease staging, management of prophylaxis for opportunistic infections, and together with HIV viral load testing, determining need for and monitoring of antiretroviral therapy. Flow cytometry provides accurate measurements of CD4 T cells and is the current standard-of-care in most settings. While there are examples of implementation of effective flow cytometry solutions at the national level(Glencross et al., 2008a, 2008b), access to timely, cost-effective CD4 counts is still limited in many high disease burden, low resource settings.(Peter et al., 2008; Taiwo and Murphy, 2008; WHO, 2012).

Despite significant advances in HIV care, adequate laboratory infrastructure for HIV diagnosis and monitoring remains a major global health challenge, particularly in resource-limited areas(Vitoria et al., 2009; WHO, 2011). With each step in the HIV testing and treatment process, loss to follow up rates increase. Pre-treatment loss to follow up rates can exceed 50%

Abbreviations: POC, Point-of-care; LOA, Limits of agreement; AVRC, Antiviral Research Center; UCSD, University of California San Diego; %SIM, Percentage similarity; CV, Coefficient of variation; ART, Antiretroviral therapy; WHO, World Health Organization.

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in some areas and are a major challenge in HIV care (Djomand et al., 2003; Amuron et al., 2009; Micek et al., 1999; Losina et al., 2010; Rosen and Fox, 2011). Recent studies show that access to point-of-care (POC) CD4 T cell counts improve patient retention and initiation of antiretroviral therapy in health clinics in resource-limited settings (Jani et al., 2011a). The increasing prevalence of HIV infection worldwide, along with changing criteria for treatment will further increase the demand for more available, reliable, and cost-effective methods for T-cell enumeration. Point-of-care T cell quantitation is an important step in the decentralization and integration of HIV treatment, and thus is a major priority in the next phase of HIV care (WHO, 2011). While advances have been made in POC diagnostics, many still have disadvantages that potentially limit their usefulness. These limitations include cost, requirements for technical expertise, quality assurance, and throughput, and vary between different technologies(Zachariah et al., 2011; Boyle et al., 2012).

MBio Diagnostics, Inc. is developing a simple and cost-effective CD4 T-cell counting system that would allow for decentralization of testing and treatment in resource-limited settings. The system is designed to be compatible with batch operation, such that a single operator using a single system could process 60 to 80 samples in a day (~10/h). There are two overall purposes for the current study. First, by placing the prototype system in a clinical setting, development engineers obtained operational feedback from users in an intended use setting. This pre-marketing evaluation provided input for design improvements. Second, the study provided a system performance assessment relative to flow cytometry using fresh whole blood samples from a cohort of HIV-infected participants. The assay protocol used in this preliminary study is a laboratory protocol requiring a skilled operator. MBio Diagnostics anticipates simplification of that assay protocol as the product moves toward final format.

2. Methods

2.1. System description

The MBio Diagnostics CD4 quantification system, referred to here as the MBio SnapCount™ System, combines single-use, disposable cartridges with a simple reader instrument. Based on the principle of static imaging cytometry with fluorescent immunostaining, the system utilizes a novel, laser-based illumination approach combined with MBio's proprietary planar waveguide technology. A detailed description of the optical system is beyond the scope of this manuscript. A related system based on the MBio planar waveguide technology has been previously described for multiplexed immunoassay applications (Lochhead et al., 2011).

The disposable cartridges for the CD4 quantification system are simple, single channel devices with passive fluidics (no pumps or valves). All flow in the device is by capillary action. The sample preparation protocol (described in detail below) includes addition of whole blood to a proprietary reagent prior to transfer to the assay cartridge. Cartridges are processed on the bench top, independent of the reader instrument. As a result, multiple samples can be processed in parallel; a single operator can process approximately 15 cartridges per hour with the system described here.

2.2. Study participants

HIV-infected male and female individuals were recruited for this study from the Antiviral Research Center (AVRC) and the Owen Clinic at the University of California, San Diego (UCSD) Medical Center between May 2011 and October 2011. All participants provided written informed consent. Inclusion criteria included documented HIV infection. Exclusion criteria included anemia or other contraindication to venipuncture. No participants were excluded on the basis of gender, race or ethnicity, or socioeconomic or treatment status. The Institutional Review Board at UCSD approved this study.

2.3. Sample collection

After enrollment, participants provided 3 separate 3 mL whole blood specimens, collected via venipuncture into evacuated K_2EDTA BD Vacutainer® tubes. One tube was used to process the specimen on the MBio SnapCount™ device at the AVRC. A second tube was sent to the reference flow cytometry laboratory. A third tube was sent to MBio Diagnostics in Boulder, Colorado via next day shipping to be tested on a second SnapCount™ device, for device and operator comparisons. Participants also provided a finger stick (capillary) sample when an operator was available for immediate MBio system testing. Capillary samples were collected using CAPIJECT® safety lancets (1.5 mm width blade, 2.0 mm depth; Terumo Medical Corporation, New Jersey, USA), and 10 μL MicroSafe capillary tubes (SafeTec, Pennsylvania, USA).

2.4. Flow cytometry

Reference flow cytometry CD4 counts for each sample were generated at the Immunogenetics Laboratory at the Veteran's Health Administration of San Diego using one of the three Vacutainer® tubes. The Immunogenetics Lab uses a dual platform approach with a Becton Dickinson FACSCalibur flow cytometer. Flow cytometric results were reported after completion of the SnapCount™ analysis.

2.5. SnapCount CD4 T cell enumeration

The laboratory assay protocol used in this study was as follows. All steps were performed at ambient temperature, which for this study was between 20 °C and 25 °C. For venous samples, 10 μL of whole blood was transferred from the Vacutainer® tube to a microtube containing pre-measured proprietary liquid stain reagent using an adjustable pipetter. For the capillary blood collection, a disposable plastic capillary tube (MicroSafe) was used to transfer 10 μL of whole blood from the study participant's finger to a microtube containing the pre-measured proprietary liquid stain reagent.

Once venous or capillary samples were added to the microtubes for staining, they were briefly mixed by either aspirate-dispense with a pipetter or by vortexing. Immediately after mixing, 35 μL of the diluted blood sample was transferred to the inlet port of an MBio cartridge using an adjustable pipetter. Each cartridge was incubated on the bench top for 20 min, a fixative was added, and then the cartridges were inserted into the SnapCount™ Reader for analysis within 1 h.