

## Affordable CD4<sup>+</sup> T cell Counts by Flow Cytometry II. The Use of Fixed Whole Blood in Resource-poor Settings

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### Abstract

We tested the feasibility and precision of affordable CD4<sup>+</sup> T-cell counting in resource-poor settings using a recently standardised fixative, TransFix™ in whole blood (WB) by flow cytometry (FCM). The precision of the assays was established under optimal conditions for single-platform FCM such as the volumetric CytoronAbsolute and the bead-based FACSCan. Fresh WB samples from HIV-seropositive and seronegative patients were tested in Tanzania and South Africa, fixed and sent to the U.K. for re-analysis 7 days later. Correlation, bias and limits of agreements were analysed by linear regression and the Bland-Altman test. Absolute CD4<sup>+</sup> T-cell counts remained stable for at least 10 days when TransFix was added to WB in 1/10 dilution at 20-25°C, and for 7 days when added in 1/10 or 1/5 dilution to samples stored to mimic 'tropical' conditions at 37°C. Higher temperatures such as 42°C were tolerated for only short periods since the recovery had decreased to 63% by day 3. The reproducibility of lymphocyte subset analysis remained unchanged by TransFix with Coefficient of Variations <6% for all T cell subsets. Absolute CD4<sup>+</sup> T-cell counts and CD4<sup>+</sup> T cell% values on fixed samples in the U.K. showed a high correlation with the results using fresh samples in Tanzania ( $r=0.993$  and  $0.969$ , respectively) and with the samples handled in Johannesburg ( $r=0.991$  and  $0.981$ ) with minimal bias. Primary CD4 gating using only a single CD4 antibody also remained accurate in TransFixed samples ( $r=0.999$ ). Thus TransFix permits optimal fixation and transport of WB samples in the developing world for FCM to local regional laboratories and for quality assurance in international centres. When used together with inexpensive primary CD4 gating, TransFix will allow reliable and affordable CD4<sup>+</sup> T-cell counting by FCM in resource-poor setting

### 1. Introduction

The absolute numbers of CD4<sup>+</sup> lymphocytes and their percentage values within the total lymphocyte populations (CD4<sup>+</sup> T%/lymphocytes) are the two most frequently requested flow cytometric assays which retain clinical significance in HIV infections in six clinical/diagnostic settings: (i) to assess the degree of immune deterioration and speed of progression towards AIDS (Taylor et al., 1989; Phillips et al., 1991); (ii) to improve AIDS surveillance through CD4<sup>+</sup> T-cell count reporting (Taylor et al., 1989; McAnulty et al., 1997); (iii) to group HIV seropositive naïve patients into cohorts according to their baseline CD4<sup>+</sup> T-cell counts prior to initiating therapy (Miller et al., 1999); (iv) to properly choose the timing for prophylaxis of opportunistic infections in AIDS (Taylor et al., 1989; Phillips et al., 1991), (v) to monitor, in terms of CD4<sup>+</sup> T cell recovery, the efficacy of antiretroviral (Autran et al., 1997) and/or cytokine IL-2 therapy (Youle et al., 2000), and (vi) to check CD4<sup>+</sup> T-cell counts during trials of protective and therapeutic vaccines.

CD4<sup>+</sup> T-cell counts and viral load assays are complementary tests during the clinical management of HIV disease and opportunistic infections, particularly when the appropriate timing of treatment is important (Miller et al., 1999). Nevertheless, CD4<sup>+</sup> T-cell counts are not available in developing countries, where the catastro-

phic impact of HIV infection and AIDS is felt, for three main reasons. Firstly, current assays to count CD4<sup>+</sup> T cells by FCM are expensive and require high technology that is unaffordable. Secondly, HIV-positive WB samples need to travel far to reach expert laboratories, and the quality of tests cannot be guaranteed by the time these samples reach their destination. As European guidelines (Barnett et al., 1997) state that CD4<sup>+</sup> T-cell counts should be performed within 18-24 h, these tests remain impractical in most areas of the world, except in developed countries with special fast delivery services. Thirdly, until recently insufficient resources have been made available to cover the costs of anti-retroviral therapy (ART) and/or vaccination (Yamey, 2000); thus in the absence of therapy requests for repeated CD4<sup>+</sup> T-cell counts in HIV-seropositive patients remained clinically unjustified.

There have been efforts to address this situation. Occasionally, short-term grants have been provided for purchasing expensive Western technology in attempts to ameliorate HIV related long term problems – only to observe beneficial effects evaporating as soon as the funds stopped. Fourteen years after setting up our collaboration in Tanzania, the CD4<sup>+</sup> T-cell counting costs are still covered by grants from the developed world. Efforts have also been made to count CD4<sup>+</sup> T cells with non-FCM methods but this has remained largely impractical with only sporadic quality control.