

Affordable CD4⁺ T cell counts on 'single-platform' flow cytometers I. Primary CD4 gating

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Summary. Here we demonstrate the flow cytometric concept of 'primary CD4 gating' utilizing three different CD4 MAbs conjugated with five different fluorochromes. CD4⁺ lymphocytes were defined by an autogate in a single histogram of CD4 FI ('y' axis) versus side light scatter ('x' axis). A wide range of absolute counts for >600 individuals, including HIV+ patients, were compared with those obtained by 'state-of-the-art' single-platform flow cytometers such as the volumetric Ortho CyturonAbsolute™ and the Becton-Dickinson FACSCalibur™ using Tru-Count® beads. The correlation between CD4 counts obtained with primary CD4 gating and the full test panel on the Ortho Cyturon was excellent ($R^2=0.999$). Bland-Altman statistics showed a mean difference of -2 cells/mm³ (CI95% = -3

to -1 ; limits of agreement -27 to $+23$). In addition, a second tube stained with a CD8 MAb to count CD8⁺ lymphocytes can be incorporated. We conclude that primary CD4 gating on single-platform volumetric flow cytometers is one of the most economical and flexible technologies for routine cost-conscious service work, particularly during the follow-up of patients undergoing anti-HIV therapy and/or vaccination in the developing world.

Keywords: CD4 T lymphocyte, Absolute CD4 Counts, Primary CD4 gating, Flow Cytometry (FCM), Human immunodeficiency Virus-1 (HIV), Resource-poor Settings

Clinical flow cytometry (FCM) is a precise technology in which morphological (scatter)- and immunofluorescence (IF) properties of stained leucocyte (WBC) populations as well as their absolute counts are measured in the whole blood (WB) using 'lyse-no-wash' procedures (Brando *et al*, 2000; Mercolino *et al*, 1995; Nicholson *et al*, 1997). Standard protocols have been introduced, and precision has been increased by reverse pipetting and by setting up parallel samples to identify occasional pipetting errors (reviewed by Brando *et al*, 2000). The quality of routine clinical assays can now be maintained at such a high level that biological factors may influence lymphocyte counts more severely than residual technical variations. Such biological factors include diurnal There are significant differences between the methods used to determine absolute cell numbers during FCM. 'Dual-platforms' rely on flow cytometric ('FACScan') percentages and haematological WBC data whereas There are significant differences between the methods used to determine absolute cell numbers during FCM. 'Dual-platforms' rely on flow cytometric ('FACScan') percentages and haematological WBC data whereas rest and exercise (Campbell *et al*, 1997; Fei *et al*, 1993), stress and hormonal effects (Dhabhar *et al*, 1995; Infante *et al*, 1996) as well as IL-2 therapy (Kaplan *et al*, 1991).

There are significant differences between the methods used to determine absolute cell numbers during FCM. 'Dual-platforms' rely on flow cytometric ('FACScan') percentages and haematological WBC data whereas 'single-platforms' are dedicated flow cytometers with direct absolute counting

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facility. Recently, 280 laboratories collaborated to compare 'dual-' and 'single-platform' systems. Interlaboratory variations of absolute counts on 'single-platform' were low: around 13.7 % Coefficient of Variation (%CV). Among these the volumetric CyturonAbsolute™ showed the lowest mean %CV (7.1%). 'Dual-platform' values varied widely (23.4 %CV; range 14.5-43.4%) (Barnett *et al*, 1999). O'Gorman and Nicholson (2000) have also observed that interlaboratory variations were primarily derived from the use of different haematological analysers to obtain lymphocyte count. Thus currently in the clinical service 'single-platform' FCM can be regarded as the state-of-the-art (or 'predicate') technology (Brando *et al*, 2000)

'Single-platform' methods are accurate but still complex. Non-volumetric cytometers such as FACSCalibur™ or Coulter XL™ use two tubes with eight monoclonal antibodies (MAbs) plus known numbers of beads added for providing absolute counts (Nicholson *et al*, 1997). Volumetric cytometers such as the Cyturon use three tubes with nine antibodies to give absolute values for CD4+ and CD8+ T cells as well as CD3⁺, CD16⁺, CD19⁺ values for a T+B+NK lymphocyte count ('ImmunoSum'; Connelly *et al*, 1995).

Main leucocyte populations such as lymphocytes, monocytes and PMN cells can be distinguished by their morphology reflected by their forward- and side light scatter features. Nevertheless, MAbs can identify these cells even more precisely. First Loken *et al*. (1990) have defined the correlation between CD45/CD14 binding intensity and light scatter parameters of WBC types using back-gating. 'Primary' immunological gating can also be performed with lineage specific MAbs exploiting the superb specificity of these reagents. Mandy *et al*. (1992) established primary CD3 gating for T lympho-