

# Affordable CD4<sup>+</sup> T cell counts by flow cytometry

## III. CD45 gating for optimal volumetric analysis

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**Summary.:** Flow cytometers currently supported by industry are capable of providing precise diagnostic service for CD4 T cell enumeration in order to monitor HIV infection but are not optimal and too expensive to run under resource-poor conditions where such a service is most needed. We have therefore combined the concepts of volumetric flow cytometry (i.e. measuring cell numbers in unit volumes of blood), primary CD45 and CD4 gating (i.e. automated gating for directly counting leucocytes and CD4<sup>+</sup> T cells amongst them) and the use of generic monoclonal antibodies (MAbs, such as CD45, CD4 and CD8) in order to introduce a more efficient and economical flow cytometric arrangement.

Absolute counts and percentage values (among leucocytes and lymphocytes) were generated using CD45/CD4 and CD45/CD8 combinations. We have then tested, in comparison with industry standards, how precise and how efficient the newly developed analytical settings were. Bland-Altman analyses were performed.

CD4 counts, absolute and percentage values, showed  $R^2 > 0.96$  correlations and no bias. CD8 counts also correlated well and showed 4.6% bias in favour of the CD3<sup>+</sup>,CD8<sup>+</sup> test but no significant alterations in the CD4/CD8 ratios. One single assistant could conveniently run 400 samples per day, a performance that extrapolates to run the huge output of 100,000 CD4 tests per year.

On the basis of precision, high efficacy and low costs, volumetric flow cytometers equipped with auto-biosamplers and operating with generic MAbs in double-colour immuno-fluorescence (IF) using Windows-based autogating and communication software have been found to be optimal for resource-poor settings. These instruments operate without the additional hidden costs of using microbeads or without requiring 'double platforms' with two tandem instruments.

**Keywords:** volumetric flow cytometry, leucocyte differentials, panleucogating, CD45, absolute CD4 counts, primary CD4 gating, CD4 lymphocytes, CD8 lymphocytes, human immunodeficiency virus (HIV), resource-poor settings

Dedicated flow cytometers are designed to enumerate lymphocyte subpopulations, including CD4<sup>+</sup> T cells, and their proportions among lymphocytes. In clinical service these instruments deliver absolute CD4<sup>+</sup> T cell counts with high level of accuracy (25,27,30,31). Nevertheless, the various cytometric systems differ in their complexity and costs (reviewed in ref.9), and it is now documented that routine CD4 T

cell enumeration can be simplified without compromising quality (14,18,33). This process of optimisation (Table I) is in line with recent aspirations to develop cost-effective services for patients who receive anti-retroviral therapy (ART) in resource-poor settings (14,17,19,23).

In clinical flow cytometry dedicated instruments using 'single-platforms' (SP) are preferred due to their convenience and the proven precision of the protocols used (25,27,30,31). 'Single platforms' can operate on a volumetric principle by counting CD4<sup>+</sup> T cells in a unit volume of blood (25,27), e.g. in microchambers (27) or by employing precision sy-

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