

# CD45 assisted PanLeucogating for Accurate, Affordable Dual Platform CD4+ T cell Enumeration

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**Background:** American and European guidelines for dual-platform (DP) flow cytometry recommend absolute CD4 T cell counts to be calculated from two parameters: the absolute lymphocyte counts obtained on a haematology analyzer and the percentages of CD4+ cells among lymphocytes (CD4%/lympho) obtained by flow cytometry. Nevertheless, the identification of lymphocytes is error-prone: a poor match between these common denominators in the two systems is the main source of inaccuracy. In contrast, total leucocyte counts (white cell counts; WCC) and CD4% among the autogated CD45+ leucocytes (CD4%/leuco) can be determined with greater accuracy.

**Methods:** We have introduced 'PanLeucogating', i.e. using total leucocytes as the common denominator for improving the precision of DP absolute CD4 counting. Correlations and Bland-Altman tests were used for statistical analysis.

**Results:** First, 22 stabilized whole blood samples were provided by U.K. NEQAS and the higher accuracy and precision of CD4 counts were documented using PanLeucogating when compared with Lymphogating. Next, 183 fresh and 112 (TransFix™) fixed whole blood samples were used

to compare DP methods and single platform (SP) methodology, including both volumetric and bead-based techniques. Particularly high correlation and comparable precision of absolute CD4 counts were observed between the SP volumetric method and DP PanLeucogating ( $R^2 = 0.990$ ; bias  $6 \pm SD 17\%$ ). The SP volumetric method showed lower levels of agreement with the DP Lymphogating ( $R^2 = 0.978$ ; bias  $14 \pm SD 37\%$ ) and with the SP Bead-based method ( $R^2 = 0.960$ ; bias  $4 \pm SD 31\%$ ).

**Conclusions:** These observations, taken together, clearly show that DP leucocyte counts (WCC) should replace lymphocyte counts as the "common denominator" although CD4%/lympho values can, as an extra step, be also readily provided if requested. When coupled with Quality Control for WCC on haematology analyzers, the DP method with CD45 PanLeucogating represents a robust CD4 T cell assay that is as accurate as the SP volumetric technique. This DP method uses only two, CD45 and CD4, antibody reagents and can be run on any pair of haematological analyzer plus flow cytometer.

With millions of people HIV infected, the AIDS epidemic has placed a crushing burden on the limited health care budgets in developing countries like South Africa. The only flicker of hope is through clinical trials, the introduction of economies to therapy, and the promotion of vaccination. All of these aims require affordable CD4 counting as part of a service infrastructure. Laboratory protocols for HIV/AIDS disease monitoring, however, follow US and UK based guide

lines (1-9), and these protocols provide for precise CD4 counting (17) but are expensive and inappropriate for the Third World (10,11). Alternative technologies tested to replace expensive flow cytometry have, however, not been widely implemented due to their complexity and sparse quality control (12-16). Consequently, to make flow cytometry simpler and more affordable is the optimal solution (10,11,17).

During routine CD4 enumeration two different concepts are applied. The first utilizes a dual platform (DP) comprising two instruments viz. a haematology analyzer for absolute lymphocyte counting (ALC) and a flow cytometer for defining the percentage of CD4 cells in a matching lymphoid population, referred to as the 'common

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