

POSTER PRESENTED AT THE AIDS MEETING IN BARCELONA ON 9TH JULY 2002
Less expensive CD4+ T cell monitoring using Panleucogating

Wilja Mandy¹, George Janossy², Deborah Glencross³, David Barnett⁴, Jonathan Mermin¹, Robert Downing¹
¹CDC-Uganda, Uganda Virus Research Institute, PO Box 49, Entebbe, **Uganda**
²(HIV) Immunology & Mol. Pathol., Royal Free & Univ. College Medical School, London, **UK**
³Department of Molecular Medicine & Haematology, University of Witwatersrand, Jo'burg, **South Africa**
⁴UK NEQAS for Leucocyte Immunophenotyping, Royal Hallamshire Hospital, Sheffield, **UK**

Background

With the sharp drop in the cost of ARV drug therapy the number of patients accessing ARVs has increased dramatically. In Uganda, patients pay for their own health care and monitoring. A number of labs provide CD4+ T cell monitoring, mostly in Kampala. With increasing access to ARVs, the demand for patient monitoring at regional and district levels is likely to increase. Both the high cost of laboratory testing and the shortage of competent labs are major constraints to widening access to ARV therapy. No cheap, simple tests for CD4+ T cell enumeration have been approved for use in Uganda. As a result, immune monitoring services have been provided centrally by a few, well-developed labs using calibration bead-based flow cytometry. Costs are high at between \$20-30 per sample. We investigated the use of cost-sparing dual-platform [DP] methods for immune monitoring which can reduce the cost to under \$5 per sample. DP methods use data from both a haematology analyzer [CBC] and a flow cytometer to determine absolute count CD4+ T cell count. Calibration beads are not required which in itself reduces costs by ~50% and allows reagent-sparing protocols to be used.

Flow cytometry and panleucogating

FACSCount, a dedicated CD4+/CD8+ T cell counting system, designed for resource-poor settings, has been the mainstay of immune monitoring in many resource-poor settings. The instrument itself costs around \$30,000 and the overall reagent cost per sample is around \$15.0; this compares to an initial investment of around \$100,000 for a flow cytometer [FACScan, Becton Dickinson] - reagent costs using beads [TruCOUNT] are about the same as for FACSCount. The FACScan does however have a major advantage over FACSCount - whereas reagent costs for FACSCount cannot be reduced, alternative protocols are available for FACScan which bring reagent costs down to ~\$2 per sample. These DP protocols require a white cell count [WCC] and/or %lymphocyte [differential count] which adds ~\$0.8 to the cost.

In the standard **DP-lymphocyte gating** protocol [DP-LyG], the absolute CD4+ count is calculated from the relative number of CD4+ T lymphocytes [CD3+] and the total lymphocyte count [%lymphocytes x WCC]. In the **pan-leucogating** [PLG] protocol, the reference population is the total leucocyte population [CD45+] which is the same as the WCC; the latter is a better controlled parameter on a haematology analyzer than %lymphocytes making PLG more reliable than DP-LyG for CD4+ cell counting.

Methods

We compared absolute CD4+ cell count by PLG and by DP-LyG against FACSCount, our standard method, on 337 specimens from HIV-infected and uninfected persons [Fig 1]. TriTEST reagents were used with MultiSET software for DP-LyG and AffordCD4 reagents for PLG. Mabs were titrated to determine the optimal dilution for use.

Results

Results for PLG were less scattered than for DP-LyG and over the whole CD4+ range, correlation with FACSCount results was slightly better for PLG [R²=0.96] than for DP-LyG [R²=0.91]. Moreover, there were fewer outliers [paired % difference >50% from mean] with PLG [4%] than with DP-LyG [13%]. At CD4+ counts <200 cells/ul, the mean paired % difference [MPD] in CD4+ cell counts [excluding outliers] was 7.7% between FACSCount and DP-LyG [Fig 2, Panel A] and 0.9% between FACSCount and PLG [Fig 2, Panel B]; at CD4+ counts >200 cells/ul, the respective values were 0.7% [Fig 3, Panel A] and 2.8% [Fig 3, Panel B].

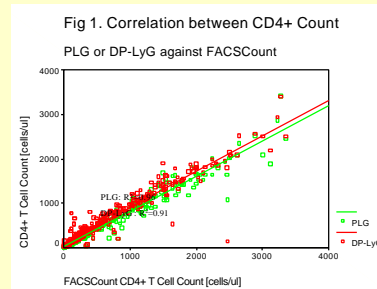


Fig 2.

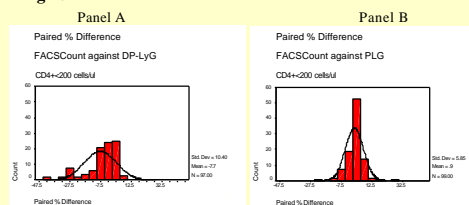
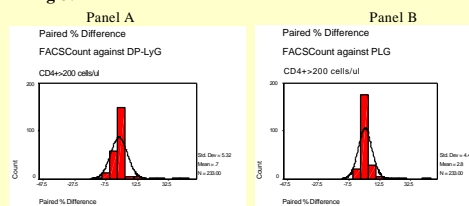


Fig 3.



Reagent costs for CD4+ T cell counting

FACSCount	US\$ 15.0
TriTEST with TruCOUNT	US\$ 16.0
TriTEST without TruCOUNT	US\$ 8.0*
TriTEST without TruCOUNT [titrate Mabs]	US\$ 2.0*
Panleucogating	US\$ <2.0*
* Requires WCC	US\$ 0.8

Conclusions

In Uganda, immune monitoring services are currently provided using flow cytometry [FACSCount, TriTEST with TruCOUNT beads or equivalent] at a few well-developed laboratories

Instrument and maintenance costs are high but there is no alternative until cheaper, low-tech methods become available

Costs to the patient for CD4+ cell counting are prohibitive at \$15 - \$30 but can be reduced to under \$5 using DP methods and reagent-sparing protocols

Panleucogating, performs as well as the DP-lymphocyte referencing method and at a fraction of the cost of FACSCount or TriTEST with TruCOUNT beads. In addition, due to CD45 gating inherent in the Panleucogating protocol, this assay is particularly robust. DP methods for CD4+ T cell enumeration can be implemented in resource-poor countries to give good quality services and substantial savings for patients

Further reading: the issue 2: (15th April, 2002) of Clin. Cytometry 50: (2002)



Further information: cdc-uganda@cdc.gov

