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New Concepts in Affordable CD4+ T Cell Enumeration for Resource-poor Settings (AffordCD4 – 2 Years Experience)

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Aims

The 'AffordCD4' project* is an international collaborative effort led by academics, with the aim of :

- introducing affordable CD4 tests in resource-poor settings
- developing simple sustainable technology in the laboratories that operate in poor countries

The achievement of the above goal will be followed by:

- the implementation of other HIV and infectious diseases assays (e.g. flow cytometry based multiplexed assays, viral load surrogate markers) using a common technological platform

* (<http://www.AffordCD4.com>)

Current achievements

- Introduction of **generic reagents** (CD4, CD8 and CD45) [Ref. 1-3]
- Introduction of simplified, accurate and robust counting assays such as **PanLeucogating** using CD4, CD8 and CD45 antibodies (see Fig.1)
- Promotion of international **quality assurance** to evaluate new assays. By this means it has been shown that CD45 assisted PanLeucogating is more accurate, robust and cost-effective than previously recommended double-platform protocols [Ref. 4]

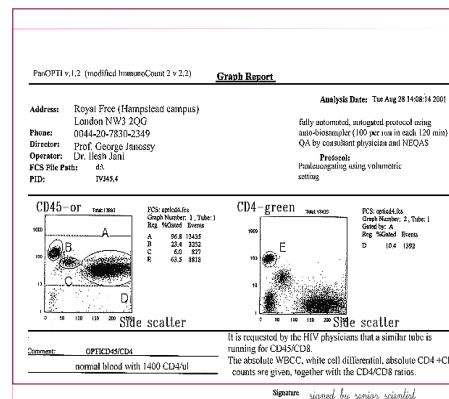


Figure 1

1. www.AffordCD4.com
2. Janossy G, Glencross DK, Jani IV, et al. AffordCD4: How to decrease the prices of counting CD4 T cells by flow cytometry while increasing accuracy, efficiency and quality – lecture at the workshop : *Monitoring and Diagnostic Tools for the Management of Antiretroviral Therapy in Resource-Poor Settings*, Bethesda, Washington, U.S.A. on 11-13th November 2001. www.projint.org/presentations/bethesda.html
3. Janossy G, Jani IV, Bradley NJ, Bikoue A, Pitfield T, Glencross DK. Affordable CD4+ T cell counts by flow cytometry III. CD45 gating for volumetric analysis. *Clin Diag Lab Immunol* 2002 (In press).
4. Glencross DK, Scott LE, Jani IV, Barnett D, Janossy G. CD45-assisted PanLeucogating for accurate, cost-effective dual-platform CD4+ T-cell enumeration. *Cytometry (Clin. Cytometry)* 2002; **50**: 69-77.

Current achievements (II)

- Introduction of CD45 assisted PanLeucogating in South Africa (see Fig.2 for accolade)
- Introduction of CD45 assisted PanLeucogating in other African settings:
 - Mandy W et al. at Entebbe, Uganda [see Ref. 5]
 - Kampala, Uganda (Fig.3)
- Introduction of the short-term stabiliser *TransFix* for sample transportation (up to 10 days) and quality assurance [Ref. 6]



Figure 2

5. Mandy W, Janossy G, Glencross DK, Barnett D, Mermin J, Downing R. Less expensive CD4 T cell monitoring using panleucogating. Poster at the XIV AIDS Conference, 9th July, 2002. MoPeB3105.
6. Jani IV, Janossy G, Iqbal A, et al. Affordable CD4+ T cell counts by flow cytometry. II. The use of fixed whole blood in resource-poor settings. J Immunol Methods 2001; 257:145-54.

CD45 based protocols in double- and single-platform assays

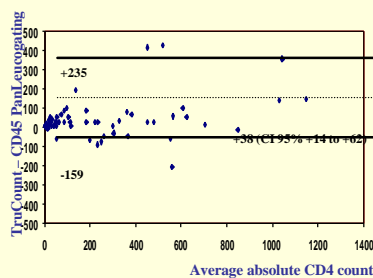


Figure 3a Bland-Altman plot comparing absolute CD4 counts yielded by the double-platform CD45 assisted PanLeucogating with those obtained by a bead-based single-platform assay in **Kampala, Uganda (by S.B.T & P.M)**. The same outliers were seen for absolute CD8 counts. So the irregularities can be attributed to bead-related abnormalities or to errors in haematological WBC counting. **The use of PanLeucogating in Kampala has resulted in ~75% assay price reduction and a huge increase in the laboratory service output.**

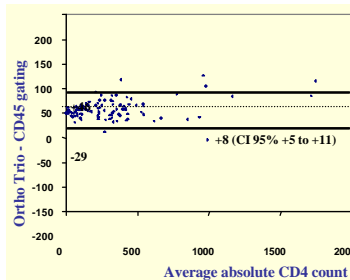
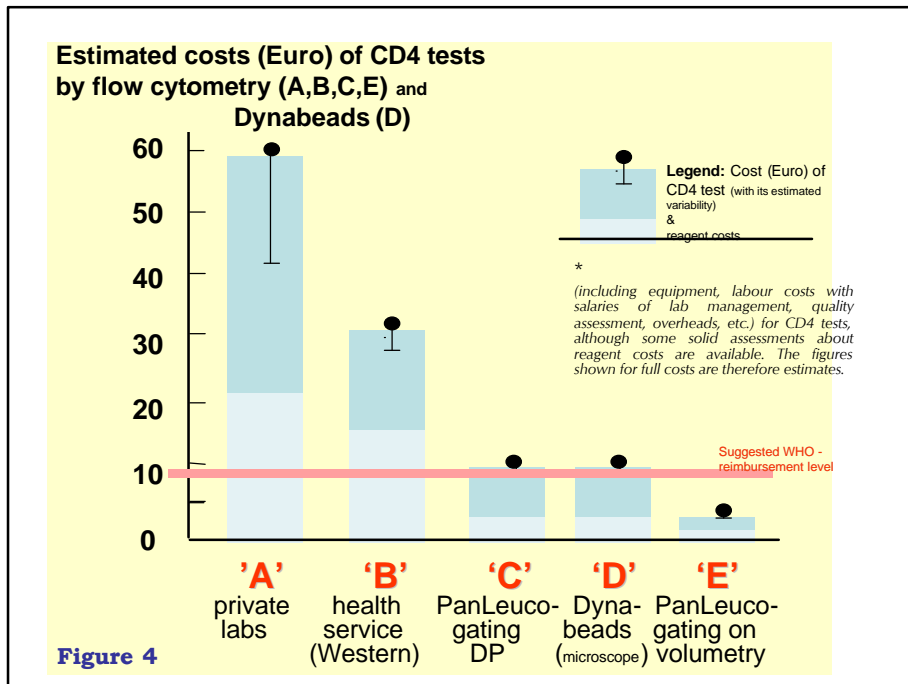


Figure 3b Bland-Altman plot comparing absolute CD4 counts yielded by a single tube, two-colour CD45/CD4 protocol with those generated by a state-of-the-art three tubes, three-colour assay. **Both assays were performed on a volumetric single-platform cytometer.** On this equipment, a CD45/CD4 plus CD45/CD8 combination allowed the accurate reporting of 16 parameters (including total WBC and differential) with a throughput of at least 200 samples/day.



Legend to Fig.4:

- In private labs the prices vary, reaching 'rip-off' levels in some ('A').*
- The estimates in Western countries using e.g. TruCount bead technology on single platform ('B') are clearly unaffordable in resource-poor settings, and the cost of FACSCoat tests are also high (due to expensive reagents).
- 'A' and 'B' need to be replaced by PanLeucogating ('C') on double platforms (DP; see Ref. 4). This protocol may utilise generic monoclonal antibodies or commercial antibodies following careful titration.
- The economical arrangements ('C') have already been introduced in a number of Regional Centres in African countries. The flow cytometric technology can operate on drastically reduced costs. These low costs are comparable to those achieved by the non-flow methods ('D' [Ref. 9,10])
- The choice between 'C' (PanLeucogating) and 'D' (Dynabead method - the most promising microscopic non-flow technology advocated for resource-poor setting on the basis of affordability) will not be decided on the prices of reagents alone but also on other factors.
- The optimal arrangement can be achieved by single platform volumetric instruments ('E'). We currently run cost-effective gating technology on second-hand volumetric flow cytometers [Ref.3] to show the feasibility of economical, large scale regional CD4 service.

9. Didier, J.M. et al. Comparative assessment of five alternative methods for CD4+ T- lymphocyte enumeration for implementation in developing countries. *J Acquir Immune Defic Syndr* 2001; 26:193-5.

10. Lyamuya, E.F. et al. Evaluation of the FACSCoat, TRAx CD4 and Dynabeads methods for CD4 tests. *J. Immunol Methods* 1996; 195:103-12.

Discussion

- The price-structure of CD4 enumeration has dramatically changed in the last two years (Fig.4) as illustrated by the examples in Johannesburg, Entebbe and Kampala.
- The next important step is to concentrate on the development of simple and affordable equipment:
 - These should be small, volumetric cytometers operating with inexpensive light sources [Ref. 7]
 - The equipment should be able to perform both cellular tests and multiplexed immunoassays [Ref. 8]
 - An interim solution can be provided by the use of available, second-hand volumetric cytometers [Ref.3]

7. Janosy G, Jani IV, Kahan M, Barnett D, Mandy F, Shapiro H. Precise CD4 T cell counting using red diode laser excitation: for richer, for poorer. *Cytometry (Clin. Cytometry)* 2002; **50**: 78-85.
8. Jani IV, Janosy G, Brown DW, Mandy F. Multiplexed immunoassays by flow cytometry for diagnosis and surveillance of infectious diseases in resource-poor settings. *Lancet Infect Dis* 2002; **2**: 243-503.

Conclusions: AffordCD4

- Inexpensive methods are available for CD4 T cell enumeration, both for using flow cytometers and other approaches
- Therefore the choice of the appropriate technology for CD4+ T cell enumeration will not be decided on the prices of tests alone
 - but also on other features of the assays such as
 - **availability of appropriately priced simple flow cytometer**
 - **availability of quality assurance**
 - **efficiency, accuracy and reproducibility**
 - **robustness to perform in aged samples**
- Further improvements in the technology of flow cytometry, using simple volumetric flow cytometers, are imminent but require help from industry
- In our view, industry should be more helpful in designing these simple modern cytometers, to improve technology globally. Countries with little money should not be punished by being forced to use second-rate solutions, expensive machines, dear reagents and out-dated manual techniques