PanLeucogating
Akin Abayomi

Cost effective CD4 counting at a central laboratory.

The Barbados experience.

CCAS Surinam 2008
Appreciation

Frank Mandy
George Janossy
Phil McCoy
John Codrington
Clive Landis
CCAS executive and faculty
Fellow colleagues
Sponsors and Vendors
People of Surinam for hosting us
“How can you heal me if you don’t like me”  George Barron

It is recorded in history that Jesus had compassion on the multitude and many were healed.

We need a global community of health care providers who are infused with compassion.
Caribbean Water fall calculation

Prevalence of 3% in the Caribbean popu of ~40 Million

230,000 found with HIV??

50,000 need ARVs
How to identify the 230,000

- Need to engage mass VCT programs
- VCT won't be attractive without capacity to:
  - Stage patients @ presentation
  - Monitor with CD4
  - Offer ARVs and other OIP
  - Post ARV Monitoring (CD4/VL/CD38)

In other words comprehensive care
HIV Infection is characterized by a steady decline in the number of CD4 cells.
Protocols: Predicate vs Sustainable

• Two tubes, 4 colours each
  – Several levels of quality
  – High purity and high recovery
  – Expensive
  – Current guidelines

• One tube, two or even one colour
  – Cheap
  – Questionable accuracy
Annual cost be to Caricom to measure three CD4’s per year [Predicate method]

- 230,000 @ 25USD = $17 million
Granulocytes

Basophils

Eosinophils

Neutrophils

Monocytes

Lymphocytes
Lymphocyte Subsets

Total Lymphocytes

T Lymphocytes (CD3+CD4+)

T Lymphocytes

B Lymphocytes

NK Cells

NK Cells

CD56+CD16+CD3-

CD3+CD8+

T Cytotoxic

B Lymphocytes

CD19+
What is flow cytometry?
Some basic definitions

- Gating
- Homogenous vs Heterogenous
- Dual vs single platform (beads or Volumetric)
- Purity and recovery
- Primary gating
FLUORESCENCE vs SIDE SCATTER

T-CELLS

B Cells

Monocytes

T-Cells
The recovery of the gate is the percentage of cells of interest in the sample that are within the gate. Cells may fall outside the gate.
The purity of the gate is the percentage of cells within the gate that are the cells of interest. There may be contaminants.
Lymphocyte Subsets

Total Lymphocytes

- T Lymphocytes (CD3+CD4+)
- B Lymphocytes (CD19+ CD3-)
- NK Cells (CD56+CD16+CD3-)

T Lymphocytes
- CD3+CD8+ T Cytotoxic

B Lymphocytes
- CD19+ CD3-
WHAT ENVIRONMENT ARE YOU WORKING IN?

WHAT IS APPROPRIATE TECHNOLOGY FOR YOUR LOCATION?
WHO suggest use TLC

• Use of Total Lymphocyte Count derived from a haematology cell counter.

• If TLC is < 1,200 then suggests CD4 is also in the region of 200 or below.

• We looked at that also
Results

Figure 1:
Scatter of Absolute CD4 count and lymphocyte count

Cd4am=M
Cd4af=F
Results

The regression equation is:

\[ \hat{y} = 132 + 231(\text{lym1}) + 419(\text{lym2}) - 86(\text{sex}) \]

Where

\( \hat{y} \) is derived mean of predicted CD4

lym1 = 1, for lymphocyte count 1000-2000, 0 otherwise

lym2 = 1 for lymphocyte count >2000; 0 otherwise

sex = 0 : female, 1 : male
Machine C

Lymphosum

TLC

$r^2 = 0.8705$
Machine B

\[ r^2 = 0.02477 \]

Lymphosum

TLC

Lymphosum vs. TLC data for Machine B with a correlation coefficient of 0.02477.
Findings!

• Initial findings suggest that absolute CD4 counts can be predicted by lymphocyte counts provided!!!!!

• These findings support the algorithm provided by the WHO

• Which suggest that a TLC of 1200 /µL may be suggestive of a CD4 of 200 Cells /µL
HIV CAUSES 2 TYPES OF AIDS

THE ONE WE ARE MORE FAMILIAR WITH IS THE “ADULT ACQUIRED IMMUNE DEFICIENCY SYNDROME”
HIV HAS ACCELERATED THE CAPACITY FOR:

- **ADVANCEMENT IN**
- **INFRASTRUCTURE**
- **DIAGNOSTICS AND**
- **SPECIALIST SERVICES**
A central referral health care facility

• Should be able to provide flow for
  – More than just CD4 counting
    • Leukaemia lymphoma
    • Fine needle aspiration
    • Infectious disease
    • Immunological assays
    • Research
    • Forensics
So at QEH and in Barbados we opted for an open 4 colour system with a long term vision for sustainability and service to the medical community at large.
Principle of Cell Counting by Flow Cytometry
Rolls Royce CD4
“The Principle”

• High Purity

• High Recovery
The lymphocyte recovery of the gate is the percentage of lymphocytes in the sample that are within the gate.

The lymphocyte purity of the gate is the percentage of cells within the gate that are lymphocytes. The remainder may be monocytes, granulocytes, red cells, platelets, and debris.
Percentage lymphocyte recovery cannot be determined without using a panel of monoclonal antibodies that identify T-, B-, and NK-cells.
Addition of CD45 to a single tube containing CD3, CD4, and CD8 allows the identification of lymphocytes based on CD45 and side scatter and the enumeration of CD4+ and CD8+ T-lymphocytes.

CD19+ B-cell values may be essential for assessing the immune status of pediatric patients.

Use of a second tube containing a natural killer (NK) cell marker together with CD3 and CD19 can help to assess the recovery and purity of the lymphocytes within the CD45/side-scatter gate.
TETRA PANEL CD45/CD4/CD8/CD3

First Tube

CD45 Gating

Monos exclusion

Refine with CD3-CD4+
CD3+ CD8+

CD3
TETRA PANEL CD45/CD56/CD19/CD3

Second tube

CD3-CD56+
CD3-CD19+

CD3+

CD3+CD56+CD19=TLC
General consensus is that:

Results obtained from Rolls Royce protocols are not more accurate than a simple Primary CD4 gating even in ageing samples

Janossy, G., Jani, I., Göhde, W., BJH, 2000, Vol. 111

Bergeron M., Mandy F. Cytometry, 2002, 50

Denny T. Cytometry part B, 2008, 74B
Performance of the Panleucogating Protocol for CD4+ T Cell Enumeration in an HIV Dedicated Laboratory Facility in Barbados.
PLG?

• Originally designed for dual platform
• And capacity to analyse old samples
• Converted to Single platform
• Made Pan Leucogate redundant
• Essentially now prim CD4 gating protocol
• Can do % for paediatrics if required
A synopsis

• 150 blood samples analyzed

• Good correlation between two protocols in the clinically relevant range

• Bland Altman showed good agreement

• Cost analysis has actually showed a saving of USD 17.86 per test for reagents
Figure 1: (A) A panleucogate is drawn using CD45 and side scatter fluorescence to identify all white cells. (B) Events in the panleucogate staining CD4 positive show a clear separation between CD4 positive and negative cells. (C) Percentage of CD4 positive cells in the lymphocyte gate. This sample has a CD4 count of 154 cells/μL and CD4 % of 10.4.
CD4 count = 608 cells/µL; CD4 % = 45.6
CD4 count = 620 cells/µL; CD4 % = 20.3
CD4 count = 431 cells/µL; CD4 % = 17.2
CD4 count = 475 cells/µL; CD4 % = 27.2
Comparison of the Panleucoh gating technique with four-colour heterogenous gating for enumeration of CD4+ T cell counts by flowcytometry

- Excellent correlation observed between absolute counts obtained using PLG & TetraONE system.

![Graph showing absolute counts obtained with Panleucoh gating in single platform & dual platform settings](image)

Absolute counts obtained with Panleucoh gating in Single platform & Dual platform settings

$R^2 = 0.97$

$R^2 = 0.96$
Comparison

Bland-Altman analysis comparing TetraOne vs. PLG single and dual platforms

Single

Absolute CD4 counts (cells/µl)

Difference (CD4 cells/µl TetraOne vs. PLG)

Bias: +4.60 cells/µl

+ 2 S.D.

- 2 S.D.

250 500 750 1000

-100

-75

-50

-25

0

25

50

75

100

250 500 750 1000

-100

-75

-50

-25

0

25

50

75

100

Bland-Altman analysis comparing TetraOne vs. PLG single and dual platforms
Acceptable CD4?
Co morbidity, TB, Hepatitis, HTLV, Malaria, Gastritis, lymphomas

Error in CD4 purity = Error in CD4 recovery
# Cost Implication for CD4 analysis

<table>
<thead>
<tr>
<th>TEST</th>
<th>COST</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLG + Ancillary Quality Control reagents</td>
<td>US$ 8.00</td>
</tr>
<tr>
<td>Annual budget after 2006</td>
<td>US$ 52,000</td>
</tr>
<tr>
<td>Four colour monoclonal antibody + Ancillary Quality Control reagents</td>
<td>US$ 25.86</td>
</tr>
<tr>
<td>Annual budget prior to 2006</td>
<td>US$ 119,000</td>
</tr>
</tbody>
</table>
Annual cost be to Caricom to measure three CD4’s per year. Predicate vs Alternative

• 230,000 @ 25USD = $17 million

• 230,000 @ 8USD = $ 5.5 million

• 230,000 @ 2USD = $1.4 million *
THE NEXT BOLT?