Laboratory monitoring of ART in developing countries

Praphan Phanuphak, M.D., Ph.D.

Faculty of Medicine, Chulalongkorn University
The Thai Red Cross AIDS Research Centre
HIV-NAT, Bangkok, Thailand
Aims of laboratory monitoring

• To follow and predict disease progression
• To judge when to start therapy
• To see how good is the treatment
• To judge when to change and what to change to

Nevertheless, the best is clinical monitoring. Thus, lack of ideal laboratory monitoring cannot be used as reason for not to start ART
What the clinicians want to know?

- What is the most essential monitoring test?
- What is the alternative next to the ideal?
- How to make it cheap and accessible?
- How to use the less essential tests and how to make them cheap and accessible?
CD4

- The single most important monitoring test
- Each newly infected individual should have a baseline CD4
- Absolute lymphocyte count is far from perfect
- It can be cheap
Absolute Lymphocyte Count vs CD4 Count

n = 487
r = 0.6
How to make CD4 cheap?

- Proportionate reduction of staining monoclonals and blood volume
- CD4 only staining
- Non-flow technologies including IF microscopy
- Bulk purchase of equipment and reagents
- Home-made monoclonals including free offer
- Shared utilization of existing facilities
Sample and reagent volume in staining performance testing

<table>
<thead>
<tr>
<th>Sample and Reagent Volume</th>
<th>Experimental setting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard</td>
</tr>
<tr>
<td>Whole blood (µl)</td>
<td>100</td>
</tr>
<tr>
<td>Monoclonal Ab (µl)</td>
<td>20</td>
</tr>
<tr>
<td>Working FAC lysing (ml)</td>
<td>2</td>
</tr>
</tbody>
</table>
## Summary of statistics for specimen analyzed

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>All Subjects Median absolute count (median %)</th>
<th>N</th>
<th>Normal Subjects Median absolute count (median %)</th>
<th>N</th>
<th>Patient subjects Median absolute count (median %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CD4 (median)</td>
<td>CD8 (median)</td>
<td>CD4 (median)</td>
<td>CD8 (median)</td>
<td>CD4 (median)</td>
</tr>
<tr>
<td>Standard reagent staining</td>
<td>111</td>
<td>643 (32)</td>
<td>748 (35)</td>
<td>922 (39)</td>
<td>736 (29)</td>
<td>268 (15)</td>
</tr>
<tr>
<td>Half reagent Staining</td>
<td>111</td>
<td>647 (31)</td>
<td>736 (35)</td>
<td>918 (40)</td>
<td>717 (29)</td>
<td>269 (16.5)</td>
</tr>
<tr>
<td>One-fourth reagent staining</td>
<td>111</td>
<td>655 (30)</td>
<td>734 (36)</td>
<td>926 (40)</td>
<td>712 (28)</td>
<td>222* (13)</td>
</tr>
</tbody>
</table>

* p value > 0.05
Correlation of % CD4 between standard and half staining

$\rho = 0.988$

$r^2 = 0.975$
Correlation of %CD4 between standard and one-fourth staining

- Correlation coefficient (r) = 0.972
- Coefficient of determination (r²) = 0.945
Investigational Simplified CD4 Counts
Non-FACS Assay

EDTA blood 1:4 dilution
+ magnetic beads CD4

Placing the test tube in the MPC

Pipette the supernatant

Count CBC
Compare CD4 between Flow cytometry and Magnetic Beads

CD4 by flowcytometry (cells/cu.mm)

CD4 by magnetic beads (cells/cu.mm)

Abs. CD4 by Flow

Linear (Abs. CD4 by Flow)

$r = 0.81$
### Bead CD4 at 1:4 dilution

<table>
<thead>
<tr>
<th>FACS CD4 cut-off</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>n1/n2*</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>86</td>
<td>82.3</td>
<td>81/79</td>
</tr>
<tr>
<td>250</td>
<td>79.7</td>
<td>83.5</td>
<td>70/90</td>
</tr>
<tr>
<td>200</td>
<td>68.3</td>
<td>86</td>
<td>60/100</td>
</tr>
<tr>
<td>150</td>
<td>71</td>
<td>92.2</td>
<td>45/115</td>
</tr>
<tr>
<td>100</td>
<td>75</td>
<td>94</td>
<td>32/128</td>
</tr>
</tbody>
</table>

*(n1 = CD4 < cut-off, n2 = CD4 > cut-off)*

**Interpretation:**
At the cut-off of CD4 < 200, the sensitivity became less than 80%.