Quality Assurance / Quality Control of CD4 and viral load assays in the ressources-limited settings

What is available / needed in Burkina Faso?
FCHIVR Meeting, Warsaw, October 2003

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Diagbouga et al., AIDS 2003;17:2201-2208
ANRS 1226 Study

- Comparison of the Dynabeads\textsuperscript{R} and the FC reference techniques using 657 pairs of CD4 counts obtained independently and in a blinded manner on 2 aliquots of the same blood sample from a total of 301 patients

- Five one-day runs organized every three months from December 2000, to December 2001

- FC External Quality Control was organised (QASI Program, Frank Mandy & John Fahey)
Quality Control used in ANRS 1226 Study

- **Dynabeads® Technique:**
  - Fresh whole blood sample
  - Processed in the field independently and in blinded manner to FC
  - Ambient temperature (20-25°C)
  - Reagents stored at +4°C
  - Lecture in full Malassez cell

- **Flow Cytometry Technique:**
  - Whole blood sample stained and fixed in the field
  - Shipping at ambient temperature to the coordinator center (delay 12 to 24h), except for Dakar
  - All samples analysed on tubes TruCount using FACSCan in the coordinator center
  - FC values used as reference in all analyses
301 HIV-infected patients seen between December 2000 and December 2001 in one (n = 112), two (n = 61), three (n = 75), four (n = 40) or five (n = 13) occasions in 12 outpatients clinics.
RESULTS (continued)
Agreement between the two techniques in classifying patients at the threshold of 200 CD4 cells/µl

<table>
<thead>
<tr>
<th>Dynabeads&lt;sup&gt;R&lt;/sup&gt;</th>
<th>Flow Cytometry</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 200</td>
</tr>
<tr>
<td>&lt; 200</td>
<td>242</td>
</tr>
<tr>
<td>≥ 200</td>
<td>24</td>
</tr>
</tbody>
</table>

Proportion of discrepant results using strict definition: 74/657 = 11.3 (8.9, 13.7)

74 Discrepant pairs of results

<table>
<thead>
<tr>
<th></th>
<th>&lt; 50</th>
<th>50 - 100</th>
<th>≥ 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 50</td>
<td>22</td>
<td>21</td>
<td>31</td>
</tr>
</tbody>
</table>

Discrepant with a difference ≥ 100: 31/657 = 4.7 (3.1 - 6.3)
### RESULTS (continued)

**Coefficient of variation**

<table>
<thead>
<tr>
<th>Sites</th>
<th>Site 1</th>
<th>Site 2</th>
<th>Site 3 Ref site</th>
<th>Site 4</th>
<th>Site 5</th>
<th>Site 6</th>
<th>All Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>CV</td>
<td>14.6</td>
<td>7.0</td>
<td>9.7</td>
<td>6.0</td>
<td>5.3</td>
<td>9.8</td>
<td>8.4</td>
</tr>
<tr>
<td>n</td>
<td>16</td>
<td>7</td>
<td>49</td>
<td>24</td>
<td>20</td>
<td>14</td>
<td>130</td>
</tr>
</tbody>
</table>

**DYNABEADS\textsuperscript{R} Technique**

**Flow Cytometry Technique**

<table>
<thead>
<tr>
<th></th>
<th>Reference site</th>
<th>Sites Distant from Reference site</th>
</tr>
</thead>
<tbody>
<tr>
<td>CV</td>
<td>8.3</td>
<td>19.7</td>
</tr>
<tr>
<td>n</td>
<td>20</td>
<td>45</td>
</tr>
</tbody>
</table>
RESULTS:
Impact of the delay in sample handling on Dynabeads® Technique

Numbers and Proportions of samples exhibiting a decrease in CD4 cell counts ≥ 20%

<table>
<thead>
<tr>
<th>Time</th>
<th>n</th>
<th>% (95 CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hour 4</td>
<td>0 / 28</td>
<td>0 (0, 12.3)</td>
</tr>
<tr>
<td>Hour 8</td>
<td>3 / 28</td>
<td>10.7 (2.3, 28.2)</td>
</tr>
<tr>
<td>Hour 12</td>
<td>5 / 28</td>
<td>17.9 (6.1, 36.9)</td>
</tr>
<tr>
<td>Hour 24</td>
<td>14 / 28</td>
<td>50.0 (30.7, 69.4)</td>
</tr>
</tbody>
</table>
West African Sites where Dynabeads® technique (*) was implemented
Launching in September

- ESTHER (French Foreing Office)
- MAP-II & TAP (World Bank)
- GFATM (UN)
- Several NGO (i.e. MSF)
- Private initiatives
### CD4 Assays available in Burkina Faso (October 2003)

<table>
<thead>
<tr>
<th>CD4 Assays</th>
<th>Sites of implementation</th>
</tr>
</thead>
<tbody>
<tr>
<td>FACS Can (BD)</td>
<td>3</td>
</tr>
<tr>
<td>FACS Count (BD)</td>
<td>3 + 2 CHU</td>
</tr>
<tr>
<td>Cy-Flow (Partec)</td>
<td>1</td>
</tr>
<tr>
<td>CD4 Manual Count (Coulter)</td>
<td>1</td>
</tr>
<tr>
<td>Dynal T4 Quant (Dynal)</td>
<td>8 + 40 districts, CHR</td>
</tr>
</tbody>
</table>
## Viral load Assays available in Burkina Faso (October 2003)

<table>
<thead>
<tr>
<th>VL Assays</th>
<th>Sites of implementation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplicor (Roche)</td>
<td>1</td>
</tr>
<tr>
<td>b DNA (Chiron)</td>
<td>1</td>
</tr>
<tr>
<td>PCR Real Time</td>
<td>1 + 2 CHU</td>
</tr>
<tr>
<td>P 24 Ag assay</td>
<td>1?</td>
</tr>
</tbody>
</table>
What is needed in Burkina Faso?

- Implementation of a network of laboratories having a quality assurance / quality control program for the CD4+ T lymphocyte enumeration and the viral load measurements to guarantee the national initiative for access to ARV
OBJECTIVES OF PILOTE STUDY

- To identify, evaluate, validate and compare, available tools for quality assurance / quality control of CD4 measurements using Dynal T4 Count and CD4 Manual Count
- To validate in the field the usefulness of the better tool in a network of national laboratories
- To build a network of national laboratories (Web site, Internet) using routinely these techniques
Quality Assurance procedures

- Handbooks of laboratory safety
- Consensus conferences and international guidelines
  - UK NEQAS, NIH, IQA, QASI, NCCLS, CQAP, CAP
- Checking quality of blood specimens
- Avoid microbial contamination of reactive
- Use appropriate anticoagulant for blood collection
- Homogenase reagents in solution before using
- Data storage
- Processing of blood specimens
Quality Control Procedures

- Internal quality control
- External quality control
- Inter-laboratory variability
- Inter-technician variability
- Intra-laboratory variability
- Delay in sample handling
- Reproducibility
Quality Control procedures

- **Direct supervision (TB/WHO/UICTMR)**
  - Sites Survey each six months
  - Comparison of data obtained in situ by supervisors and sites

- **Optimalising Flow Cytometry technique used**
  - Using precise pipettes
  - Using the same reactives and buffers from the reference site at each run of quality control
Quality control tools

- Evaluation of CD4 assays using human stabilised cells
  - Ortho Absolute Control (Ortho Diagnostics Systems, Raritan, NJ)
  - StatusFlow mid and low (R & D Systems, Minneapolis, MN)
  - FluoroTrol-CD4 tri level low, mid, and normal (BioErgonomics, MN)
  - CD-Chex Plus Low and normal CD4 (Strek Laboratories, Omaha, NE)
  - Immuno-Trol Cells (Beckman Coulter, Miami, FL)
  - Coulter Cyto-Trol – Control Cells kit

- Evaluation of CD4 assays using fresh blood additioned with fixatives
  - Cyto-check
  - Transfix (David Barnett & Viv Granger)

11/4/03
Quality control Tools

- Transfix used in several dilutions (1/5, 1/10)
- Transfix used in several temperatures (4°C, 25°C, 37°C, 45°C)
- Transfix used in situ in the lab and after transportation of blood specimens