Transfer of diagnostic and monitoring technologies into resource poor setting

Workshop sponsored by the Forum for Collaborative HIV Research
April 22, 2002
Washington DC

Summary of Findings for CD4 assays
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Professor of Immunology/Microbiology
Rush Medical Center
Chicago, IL
This part of the workshop focused on current status of two representative technologies for measuring CD4 as a model for validating these types of assays for use in resource poor settings.
Workshop Strategy

• Focus on two models for technologies with data available, using non flow cytometric methods

• Other groups are focusing on modified flow cytometric approaches
  – See Skillsbuilding Workshop SB 68 Thursday 14:30
Coulter® Manual CD4 Count Kit

- Anti CD14 Antibody blocking bead
- Anti CD4 Antibody detection bead
- CD4+ T cells
- Absolute CD4 count

Statistics:
Manual CD4 vs Flow
Slope: 0.92
Intercept: +0.05
Cor. Coef.: 0.93

Hemacytometer Count
- Depth: 0.1mm
- Distance: 3mm
### Summary of Cyto-Sphere Studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Population</th>
<th>Correlation to Flow Cytometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Landay US/ Uganda 1993</td>
<td>376 HIV+</td>
<td>( r = 0.912 )</td>
</tr>
<tr>
<td>Carella US 1995</td>
<td>117 HIV+</td>
<td>( r = 0.93 )</td>
</tr>
<tr>
<td>Gernow Denmark/Ivory Coast 1995</td>
<td>44 HIV+ Denmark, 79 HIV+ Ivory Coast</td>
<td>( r = 0.93 ), ( r = 0.74 )</td>
</tr>
<tr>
<td>Johnson US 1995 (Immuno VCS)</td>
<td>46 HIV+</td>
<td>( r = 0.90 )</td>
</tr>
<tr>
<td>Zwerner US 1997</td>
<td>140 HIV+</td>
<td>( r = 0.98 )</td>
</tr>
<tr>
<td>Didier Paris 2001</td>
<td>55 HIV+</td>
<td>( r = 0.453 )</td>
</tr>
</tbody>
</table>
### Predictive value of Cyto-Sphere Assay Compared with Flow Cytometry

<table>
<thead>
<tr>
<th></th>
<th>Cyto-Sphere Assay</th>
<th>Flow Cytometry CD4+ T cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤200</td>
<td>&gt; 200</td>
</tr>
<tr>
<td>≤200</td>
<td>70</td>
<td>13</td>
</tr>
<tr>
<td>&gt;200</td>
<td>6</td>
<td>293</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>76</td>
<td>306</td>
</tr>
</tbody>
</table>

- 96% predictive value CD4 count > 200 x 10^6/l.
- 92% predictive value CD4 count ≤200 x 10^6/l.
250 µl whole blood
Monocyte depletion
for 10 minutes

Isolation of T4
and T8 lymphocytes
for 10 minutes

T4

T8

Wash

Cell lysis

Cell counting
Light microscopy
## Summary of Dynabead Studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Population</th>
<th>Correlation to Flow Cytometry</th>
</tr>
</thead>
</table>
| Lyamuya Dar es Salaam 1996 | 91 HIV- 98 HIV+ | $r = 0.827$ HIV-  
$r = 0.974$ all CD4 HIV+  
$r = 0.898$ 0-199 CD4 (n = 38)  
$r = 0.809$ 200-499 CD4 (n = 55)  
$r = 0.925$ >500 CD4 (n = 25) |
| Didier Paris 2001    | 46 HIV+          | $r = 0.913$                                  |
| Diagbouga West Africa 2002 | 301 HIV+   | $r = 0.890$                                  |
Correlation with Flow Cytometry: 96%
Reproducibility

Dynabeads, T4 - T8 Quant. (cells/μl)

Sample no. (three independent tests per sample)
RESULTS (correlation coefficients, $p < 10^{-4}$ for all $r$)

Run 1

Run 2

Run 3

Run 4

Run 5

TruCount® Flow cytometry
Crowe et al 2002

$R = 0.96$

$n = 54$

$R = 0.69$

$N = 27$
Moving Cyto-Sphere and Dynabead Testing into the Field

- Flow cytometry should be the reference method
- The tests should be validated in the context of an antiretroviral trial
- In that context, the assays should ideally provide real-time results so that treatment decisions can be implemented without having to wait for the infected study participant to return
- Quality assurance and quality control must be addressed in validation studies
- Adequate training must precede the trials
What are the Critical Factors Influencing the Ability to Deploy CD4 Testing?

- **Cost of the technologies**
  - instrument/machinery
  - reagents

- **Laboratory infrastructure**
  - human resources e.g. scarcity of technicians, level of training
  - material resources e.g. electricity, refrigeration
Model for CD4 Testing in Resource-Poor Settings

• Reference center >>>
  • how many?

• Provincial or district level>>>

• Primary care or rural setting>>>

• flow cytometry
  • high cost
  • resource-intensive
  • high precision

• dynabeads/cytospheres
  • lower cost
  • less resource intensive
  • more labor time
  • lower precision

• Ship or fix samples
  • least resource intensive
  • low tech
Future Directions

- Development of plan to evaluate low cost CD4 technologies in clinical trials in resource poor setting
- Develop strategy for implementing technologies in reference, district, and primary care settings
- Engage organizations such as WHO, CDC, and NIH in implementing studies and developing training programs
Comparison of CD4 counts using Dynal assay when samples are tested 6, 24 and 72 hours after collection compared with flow cytometry within 6 hours

Crowe et al 2002
QC sample testing in Melbourne and Bali, Indonesia

Crowe et al 2002
Coulter® Manual CD4 Count Kit

- Anti CD14 Antibody blocking bead
- Anti CD4 Antibody detection bead

CD4+ T cells

monocytes

Hemacytometer Count

Absolute CD4 count
Assays that can classify HIV+ subjects into ranges such as $>$ or $<$ 200 cells/µl would represent a great advantage in countries with no CD4 technology.
Successful implementation of a low cost method for enumerating CD4+ T lymphocyte in limited-resource settings

Serge Diagbouga, Corine Chazallon, Michel D. Kazatchkine, Philippe Van De perre, André Inwoley, Souleymane M’Boup, Mireille prince David, ténin Aoua Thiéro, Robert Soudré, Jean-Pierre Aboulker, Laurence Weiss.
In HIV-infected patients, CD4 cell count:

• is the best predictor of the risk of Opportunistic Infections and clinical progression,

• has to be regularly monitored to timely initiate Anti Retroviral treatment and assess its efficacy on immune reconstitution,

• has to be performed on fresh whole blood, e.g. ideally in field laboratories
DYNABEADS® TECHNIQUE: Features

• Does not require sophisticated technology,

• Equipment and reagents are less expensive than those of Flow Cytometry (FC),

• Is easy to use in the field,

• Yielded encouraging results in preliminary comparisons studies with the reference FC technique.
**DYNABEADS<sup>®</sup> TECHNIQUE**

**Principle**

A 3 step-technique

- **Step 1: Depletion in monocytes**: Dynabeads method uses magnetic beads coated with anti-CD14 mAb to capture and isolate CD14+ monocytes from whole blood.

- **Step 2: CD4+ T lymphocyte isolation**: The CD4+ T lymphocyte are selected using magnetic beads coated with anti-CD4 mAb.

- **Step 3: CD4+ T lymphocyte enumeration**: The isolated CD4+ T lymphocyte are lysed, stained with acridine orange, the nuclei are then counted directly using fluorescent microscope; results are expressed as numbers of CD4+ T lymphocyte/µl of whole blood.
METHODOLOGY
Evaluation of comparison

• Multi-site Study
  - coordinated by one laboratory (Bobo-Dioulasso) using FC as reference technique,
  - including 6 sites in 5 countries Bobo-Dioulasso and Ouagadougou (Burkina Faso), Abidjan (Côte d’Ivoire), Bamako (Mali), Lomé (Togo), Dakar (Sénégal)

• Training: one single 3-days local training for all involved technicians including:
  - Dynabeads® Technique
  - Methodology of whole blood staining for analysing CD4+ T lymphocyte using FC
• 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
METHODOLOGY

Additional experiments

• Evaluation of the reproducibility of both techniques:
  - Dynabeads® in 130 duplicate samples
  - FC in 65 duplicate samples (20 from the reference site)

• Evaluation of the impact of the delay in sample handling
  Dynabeads® technique performed in 28 samples immediately following taking blood and 4, 8, 12 and 24 hours later
Set-up of the two Techniques

**Dynabeads™ Technique:**
- Fresh whole blood sample
- Processed in the field independently and in blinded manner to FC
- Ambient temperature
- 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
STATISTICAL METHODS

Evaluation of:

- **Systematic median difference** between the Dynabeads® and the FC techniques

- **Amplitude of the within-sample difference** between the results of the two techniques

- **Agreement** between the two techniques in classifying patients according to clinically relevant thresholds of CD4 counts (e.g. 200 CD4/µl)

- **Correlation coefficient** between the two techniques

- **Coefficient of variation** for each technique
RESULTS

Description of the population

- 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.
• The overall median systematic difference between Dynabeads\textsuperscript{R} and FC was $-16$ cells/\mu l (CI 95: -22, -8)

• By CD4 strata, the median systematic differences between the two techniques were:
  - +7.5 cells for CD4 cell counts < 200
  - -23 cells for 200 < CD4 cell counts < 349
  - -43.5 cells for 350 < CD4 cell counts < 499
  - -96.5 cells for 500 < CD4 cell counts < 999
  - -269 cells for CD4 cell counts > 1000
• The overall median amplitude of the within-sample difference between the two techniques was 56 cells/µl (interquartile 22-137)

• By CD4 strata, the median amplitudes of the difference between the two techniques were:
  25 (11-51) for CD4 cell counts < 200
  54 (24-95) for 200 < CD4 cell counts < 349
  90 (37-165) for 350 < CD4 cell counts < 500
RESULTS (continued)
Agreement between the two techniques in classifying patients at the threshold of 200 CD4 cells/µl

<table>
<thead>
<tr>
<th>Flow Cytometry</th>
<th>&lt; 200</th>
<th>≥ 200</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 200</td>
<td>242</td>
<td>50</td>
</tr>
<tr>
<td>≥ 200</td>
<td>24</td>
<td>341</td>
</tr>
</tbody>
</table>

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

74  Discrepants pairs of results

<table>
<thead>
<tr>
<th>&lt; 50</th>
<th>50 - 100</th>
<th>≥ 100</th>
</tr>
</thead>
<tbody>
<tr>
<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

### DYNABEADS<sup>R</sup> Technique

<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>

### 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 FC (●) is presently available (07/2002)

- Mali
- Niger
- Senegal
- Nigeria
- Côte d'Ivoire
- Burkina Faso
- Ghana
- Togo
- Cameroon
- Guinea
- Mauritania
- Sierra Leone
- Liberia
- Togo
West African Sites where Dynabeads® technique (*) was implemented
CONCLUSION

In the context of the increasing availability of anti Retroviral Drugs in Africa, and of the growing need for the countries to have access to CD4+ T lymphocyte enumeration, the results of this study support the implementation of the Dynabeads® technique in the laboratories which are not equipped with flow cytometer.

A continuous monitoring of the quality of CD4 cell enumeration is required.
CONCLUSION

Further steps of the work

✓ Implementation of a network of African laboratories having a quality insurance program for the CD4+ T lymphocyte enumeration

• Automating Dynabeads<sup>R</sup> CD4 enumeration in labs equipped with hematology Analyzers (n and %)

• Optical versus m fluorescence studies:
  - comparison of data
  - Cost/effectiveness evaluation

• Evaluation of Dynabeads<sup>R</sup> in ARV Clinical trials
LABORATORIES INVOLVED:

- Bacteriology – Virology Laboratory, Le Dantec Hospital, Dakar, Senegal, Pr S. MBoup
- National HIV/STD Center, CHU-Tokoin, Lomé, Togo, Pr M. Prince David, Dr Y.A. Segbena
- Ambulatory Treatment Center (CTA) / OPALS, Ouagadougou, Burkina Faso, Pr R. Soudré, Dr P.T. Sanou
- National Research Institute of Public Health (INRSP,) Bamako, Mali, Dr A.T. Tioro, Pr F. Bougoudogo
- CeDRes, Abidjan, Côte d’Ivoire, Dr A. Inwoley, Dr F. Rouet
- Department of Immunology, Hôpital Européen Georges pompidou, Paris, Pr L. Weiss, Pr M. Kazatchkine
- Department of Immunology, Centre Muraz, Bobo-Dioulasso, Burkina Faso, Dr S. Diagbouga, Pr Philippe van De Perre

- Statistical analyses by INSERM SC 10, Dr J.P. Aboulker, Dr C. Chazallon
### RESULTS (continued)

**Influence of concentration of anti-CD4 mAb on Dynbeads® results obtained from 15 samples**

<table>
<thead>
<tr>
<th>Amount of Anti-CD4 mAb</th>
<th>Median of CD4 COUNTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 µl</td>
<td>798</td>
</tr>
<tr>
<td>30 µl</td>
<td>810</td>
</tr>
<tr>
<td>35 µl</td>
<td>874</td>
</tr>
<tr>
<td>40 µl</td>
<td>836</td>
</tr>
</tbody>
</table>
Dynabeads<sup>R</sup> material
Trained technicians in Centre Muraz, Bobo-Dioulasso