HIV immunological and virological monitoring tools

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5th INTEREST workshop
Dar es Salam
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Monitoring HIV disease progression and treatment

When to start ART?

When to switch ART?

CD4 count

VL
Adherence
Toxicity
HIV-DR

Presented at the 5th INTEREST workshop – 10 – 13 May 2010, Dar-es-Salaam, Tanzania
Standards of care for HIV monitoring

**In rich settings**

- CD4 count
- HIV VL

- CD4 by flow cytometry
- PCR-based VL

are the state-of-the-art monitoring tools.

**In poor settings, WHO recommends**

- CD4 count desirable
- VL optional
Expensive instruments

Well-trained operators

Expensive reagents

Established laboratory infrastructure

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Centralized lab

District or regional lab

Health Center (no lab)

Lack of resources
Further challenges to monitor HIV in resource-poor settings

Harsh environmental conditions

HIV variability

Low reliability

Adapt technology

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Developments in CD4 counting technology

A reduce the price and complexity of laboratory-based assays on bench-top instruments

Novel gating strategies
• Panleucogating
• CD4 primary gating

Simplified flow cytometry instruments
• 2 to 1 platform
• Internal QC
• CD4-dedicated

B Manual, microscopy-based magnetic techniques

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## Simplified bench-top flow cytometers

<table>
<thead>
<tr>
<th></th>
<th>Param</th>
<th>technology</th>
<th>Price test ($)</th>
<th>through put</th>
<th>Reag. storage</th>
<th>certification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Facscount</strong></td>
<td>CD4 Abs %</td>
<td>Flow cytometry</td>
<td>25</td>
<td>&lt;50 samples per day</td>
<td>2-8</td>
<td>FDA</td>
</tr>
<tr>
<td><strong>Guava EasyCD4 System</strong></td>
<td>CD4 Abs</td>
<td>Microcapillar flow cytometry</td>
<td>2.3</td>
<td>High</td>
<td>2-8</td>
<td>FDA approval for version EZCD4</td>
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<tr>
<td><strong>Partec CyFlow</strong></td>
<td>CD4 Abs</td>
<td>Volumetric flow cytometry</td>
<td>1.75</td>
<td>High</td>
<td>2-8</td>
<td>CE marked</td>
</tr>
</tbody>
</table>
Remaining limitations of simplified flow cytometers.

- Instrument operation is lab-based
  - skilled and motivated staff
  - Cold chain
  - Power supply

- Patient needs to come back
  - No task shifting

- Do not readily provide CD4 % (children!)

- Not always cheap enough

- Not robust enough (dust, heat, humidity)

- Not portable enough
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Facscalibur = 100kg
Facscount = 25.9kg
Guava easy CD4 = 17.8kg
Cyflow counter = 11.5kg
Manual magnetic CD4 counting techniques

- Dynal/ Coulter
- Simple instrumentation
- Well correlated with Flow cytometry

**BUT** labour intensive
- no CD4%
- Intensive training

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Centralized lab

State-of-the-art or simplified FC

District or regional lab

simplified FC or Manual techniques

Health center (no lab)

?
Need to develop CD4 Point of care (POC) instruments for rural settings

- Simpler/faster manipulation
- Hand-held or instrument-free
- No waste
- Independent of power supply
- No cold chain required
- No phlebotomy
- CD4 abs and %
- Cheap (1-2$)

Lab-free
## POC CD4 counting devices

<table>
<thead>
<tr>
<th>Device</th>
<th>Detection</th>
<th>Price test</th>
<th>Throughput</th>
<th>Reag. storage</th>
<th>Validation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PointCARE Now</td>
<td>CD4/ WB/ LY Abs and %</td>
<td>10$</td>
<td>High?</td>
<td>No cold chain</td>
<td>FDA</td>
</tr>
<tr>
<td>PIMA</td>
<td>CD4 Abs %</td>
<td>6$</td>
<td>High?</td>
<td>No cold chain</td>
<td>Comparable to BD facscalibur</td>
</tr>
<tr>
<td>LabNow</td>
<td>CD4 Abs</td>
<td>?</td>
<td>High?</td>
<td>No cold chain</td>
<td>Comparable to BD facscalibur</td>
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<tr>
<td>Daktari</td>
<td>CD4 Abs</td>
<td>?</td>
<td>High?</td>
<td>No cold chain</td>
<td>Performance evaluation ongoing</td>
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<tr>
<td>Zyomix</td>
<td>CD4 Abs</td>
<td>2$</td>
<td>High?</td>
<td>No cold chain</td>
<td>Field studies not available</td>
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</table>
The development of simplified HIV viral load technology has not advanced much.
<table>
<thead>
<tr>
<th>tests</th>
<th>Target</th>
<th>Price test ($)</th>
<th>Linear range</th>
<th>Hr/ test</th>
<th>Subtype/group</th>
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</thead>
<tbody>
<tr>
<td><strong>Roche</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cobas Taqman (vers. 1 &amp; 2)</td>
<td>LTR, gag</td>
<td>30-100</td>
<td>100-3x10^6</td>
<td>6</td>
<td>Group M A-H</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100-3x10^6</td>
<td></td>
<td>Group O (vers.2)</td>
</tr>
<tr>
<td><strong>BioMérieux</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>NucliSENS EasyQ HIV-1 (vers. 1.1)</td>
<td>gag</td>
<td>40-60</td>
<td>100-3x10^6</td>
<td>8</td>
<td>Group M A-D</td>
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<tr>
<td><strong>Siemens</strong></td>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td>VERSANT HIV RNA (vers. 1.0; kPCR)</td>
<td>pol</td>
<td>30-75</td>
<td>31-11x10^6</td>
<td>22</td>
<td>Group M A-G CRF-AE Group O</td>
</tr>
<tr>
<td><strong>Abbot</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RealTime HIV-1 Pol Integ</td>
<td>Pol Integ</td>
<td>20-40</td>
<td>40-10x10^6</td>
<td>6</td>
<td>All</td>
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<tr>
<td><strong>Biocentric</strong></td>
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<td></td>
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<td></td>
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<tr>
<td>Generic HIV VL</td>
<td>LTR</td>
<td>10-20</td>
<td>300-10x10^6</td>
<td>4</td>
<td>All</td>
</tr>
</tbody>
</table>
Centralized lab

District or regional lab

Health center (no lab)

Commercial PCR-based VL assays
Alternative HIV viral load: surrogate markers

Activated CD8

CD38 on CD8: immune activation
- Marker of disease progression.
- Possibility to combine with CD4 count.
- All HIV clades.

P24 protein
Ultrasensitive p24 assay
- All HIV clades
- ELISA format
- 5-19$

Reverse transcriptase activity
Exavir
- All HIV clades
- ELISA format
- 14-23$

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But challenges remain

**Ultrasensitive p24 assay**
- Unclear clinical significance of non virion-associated p24
- Trained staff
- Instrumentation

**Exavir**
- Time-consuming (48-72 hours)
- Sensitivity affected by RT mutations

**CD38 on CD8 LY**
- Lack of specificity (background inflammation)
- Complex standardization from lab to lab

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HIV viral load Point of care: we are not there yet

- Miniaturized PCR chips (HIV RNA)
- Miniaturized immunoassay (p24)
- Portable HIV RNA amplification
- Others...

Are still in their early phase of development.
What are the remaining gaps?

Centralized lab
- Commercial or in-house PCR VL
- (simplified) Flow cytometers

District or regional lab
- Simplified Flow cytometers/manual techniques or POC
- VL on closed PCR platform or POC

Health center (no lab)
- POC

What are the remaining gaps?
Beyond the need for further technical development...

What more can we do to help these devices hold their promises once they are available on the field?