Challenges in Scaling Up VL in Resource Limited Settings

Collins Otieno Odhiambo

KEMRI/CDC
Outline

- **Background**
  - VL role in HIV treatment monitoring
  - VL testing situation in Kenya
  - Barriers to VL scale-up

- **Strategies for scale-up**
  - DBS
    - Kenya experience
    - Meta analysis
    - DBS challenges
  - POC
    - Kenya evaluation

- **Considerations and conclusions**
New WHO Recommendations: VL

- Viral load (VL) recommended as the preferred approach to monitor treatment success and diagnose ARV treatment failure in adults and children (Strong recommendation, low quality of evidence)

- Viral load should be monitored at 6 months, 12 months, then 12 monthly

- Treatment failure is defined by persistently detectable VL above 1,000 copies/ml

- Where viral load monitoring is unavailable, the use of clinical and CD4 monitoring is recommended.
Viral Load Capacity in Kenya

- Rapid ART scale-up since 2004
  - ~ 800,000 patients on ART in Kenya
- Clinical/Immunologic monitoring were mostly used
- Viral Load testing was based on priority (targeted)
- VL testing made available to confirm treatment failure prior to ARV switch
  - Currently moving from targeted VL to routine VL testing
Barriers to VL Scale-up in Resource Limited Settings
Barriers of VL Scale-up

- High Costs of Equipments & Reagents
- Technical complexities of current platforms
- Limited Quality Assurance systems
- Lack of clear guidelines on VL requests leading to unnecessary or late testing
- Unreliable supply chain for kits/consumables
- Turn around time of results
- Infrastructural challenges
- Transport and cold chain logistics
Strategies for scale-up

- Dried Blood Spots
Why DBS?

- Facilitates sample collection from decentralized settings thereby increasing VL access
  - Stability of RNA in plasma is dependent on freezing after separation, but stable in DBS at ambient temperatures (Munoz et al. 2005, Reigadas et al. 2009)

- Simpler and cheaper collection
  - Minimum expertise required
  - Relatively low amount of blood is required

- Does not require cold chain & is non-hazardous thereby simplifying shipment to centralized facilities

- Can easily ride on the existing EID infrastructure
Meta-analysis Methodology

- Extensive literature review for all studies comparing DBS to plasma for viral load testing using several search engines and terms
- 38 published/unpublished studies identified met inclusion criteria; primary data included from 27 studies
- Resulted in >6,500 paired data points for the primary viral load technologies currently available
- Used a bivariate random effects model to determine bias, accuracy, precision and misclassification to account for between-study variation

Vojnov et al, 2014
## Meta-analysis Results

<table>
<thead>
<tr>
<th>Assay assessed</th>
<th>Sensitivity (mean %)</th>
<th>Specificity (mean %)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Abbott Molecular: Abbott RealTime HIV-1 (manual, m24sp and m2000sp) assays with m2000rt platform</strong></td>
<td>95.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>91.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1529</td>
</tr>
<tr>
<td><strong>Biocentric: Generic HIV Charge Virale</strong></td>
<td>94.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>531</td>
</tr>
<tr>
<td><strong>bioMérieux: NucliSENS EasyQ&lt;sup&gt;®&lt;/sup&gt; HIV-1 v2.0</strong></td>
<td>84.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>94.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1062</td>
</tr>
<tr>
<td><strong>Roche Molecular Systems: COBAS&lt;sup&gt;®&lt;/sup&gt; AmpliPrep/COBAS&lt;sup&gt;®&lt;/sup&gt; TaqMan&lt;sup&gt;®&lt;/sup&gt; HIV-1 Test, version 2.0 [free virus elution protocol]</strong></td>
<td>81.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>96.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>229</td>
</tr>
<tr>
<td><strong>HIV-1 RNA 1.0 Assay (kPCR)</strong></td>
<td>90.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>87.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>144</td>
</tr>
</tbody>
</table>

WHO TWG 2014
### Sensitivity, specificity, positive predictive value, and negative predictive value (95% Confidence Intervals) of DBS compared with paired plasma specimen viral load, patient support centers, Nyanza, Kenya

<table>
<thead>
<tr>
<th>CAP/CTM</th>
<th>Plasma viral load</th>
<th>Detectable</th>
<th>Undetectable</th>
<th>Total</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DBS viral load</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Detectable</td>
<td>150</td>
<td>48</td>
<td>198</td>
<td>100.0 (97.6 - 100.0)</td>
<td>4.0 (0.5 - 13.7)</td>
<td>75.8 (69.2 - 81.6)</td>
<td>100.0 (15.8 - 100.0)</td>
</tr>
<tr>
<td></td>
<td>Undetectable</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>150</td>
<td>50</td>
<td>200</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abbott m2000</td>
<td>DBS viral load</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Detectable</td>
<td>137</td>
<td>7</td>
<td>137</td>
<td>93.9 (88.8 - 97.2)</td>
<td>88.0 (82.2 - 92.4)</td>
<td>100.0 (97.4 - 100.0)</td>
<td>85.3 (73.8 - 93.0)</td>
</tr>
<tr>
<td></td>
<td>Undetectable</td>
<td>11</td>
<td>52</td>
<td>63</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>148</td>
<td>52</td>
<td>200</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Comparisons between CAP/CTM DBS and Abbott DBS tests at different clinical cut-offs using CAP/CTM plasma as the gold standard

<table>
<thead>
<tr>
<th>Cut-point (Viral cps/ml)</th>
<th>Test</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Correctly classified (%)</th>
<th>LR+</th>
<th>LR-</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥1000</td>
<td>CAP/CTM</td>
<td>100</td>
<td>3.9</td>
<td>75.0</td>
<td>1.04</td>
<td>0.0</td>
</tr>
<tr>
<td>≥1000</td>
<td>Abbott</td>
<td>97.3</td>
<td>90.4</td>
<td>95.5</td>
<td>10.1</td>
<td>0.03</td>
</tr>
<tr>
<td>≥2000</td>
<td>CAP/CTM</td>
<td>100</td>
<td>17.3</td>
<td>78.5</td>
<td>1.20</td>
<td>0.00</td>
</tr>
<tr>
<td>≥2000</td>
<td>Abbott</td>
<td>96.6</td>
<td>90.4</td>
<td>95.0</td>
<td>10.1</td>
<td>0.03</td>
</tr>
<tr>
<td>≥3000</td>
<td>CAP/CTM</td>
<td>98.0</td>
<td>36.5</td>
<td>82.0</td>
<td>1.54</td>
<td>0.06</td>
</tr>
<tr>
<td>≥3000</td>
<td>Abbott</td>
<td>95.3</td>
<td>94.2</td>
<td>95.0</td>
<td>16.5</td>
<td>0.05</td>
</tr>
<tr>
<td>≥4000</td>
<td>CAP/CTM</td>
<td>97.3</td>
<td>54.0</td>
<td>86.0</td>
<td>2.11</td>
<td>0.05</td>
</tr>
<tr>
<td>≥4000</td>
<td>Abbott</td>
<td>94.6</td>
<td>98.1</td>
<td>95.5</td>
<td>49.2</td>
<td>0.06</td>
</tr>
<tr>
<td>≥5000</td>
<td>CAP/CTM</td>
<td>96.0</td>
<td>82.7</td>
<td>92.5</td>
<td>5.54</td>
<td>0.05</td>
</tr>
<tr>
<td>≥5000</td>
<td>Abbott</td>
<td>93.2</td>
<td>98.1</td>
<td>94.5</td>
<td>48.5</td>
<td>0.07</td>
</tr>
</tbody>
</table>
DBS use under field conditions

- Assess VL performance of DBS prepared in clinical settings using three simplified spotting modalities

- Assess diagnostic accuracy of detecting virologic failure (VF) defined as plasma VL $\geq 1000$ copies/ml compared to plasma VL

Schmitz et al, 2014
Methods

Venipuncture (Venous) → Finger Stick (Capillary) → Directly → Microcapillary
### Results

Table 2. Sensitivity, Specificity, Kappa agreement, and Misclassification by DBS type and threshold compared to plasma on Abbott m2000 platform among adults and children on ART

<table>
<thead>
<tr>
<th>Threshold (≥ copies/ml) Plasma:DBS</th>
<th>Sample type (n)</th>
<th>Sensitivity (%) (95% CI)</th>
<th>Specificity (%) (95% CI)</th>
<th>Kappa (95% CI)</th>
<th>False positives (%)</th>
<th>False negatives (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000:1000</td>
<td>V-DBS (733)</td>
<td>88.8 (84.2-92.4)</td>
<td>92.6 (89.9-94.7)</td>
<td>0.81 (0.76 - 0.85)</td>
<td>7.4</td>
<td>11.2</td>
</tr>
<tr>
<td></td>
<td>M-DBS (724)</td>
<td>86.9 (82.0-90.9)</td>
<td>94.2 (91.7-96.1)</td>
<td>0.81 (0.77 - 0.86)</td>
<td>5.8</td>
<td>13.1</td>
</tr>
<tr>
<td></td>
<td>D-DBS (732)</td>
<td>85.8 (80.8-89.9)</td>
<td>93.6 (91.1-95.6)</td>
<td>0.80 (0.75 - 0.84)</td>
<td>6.3</td>
<td>14.2</td>
</tr>
<tr>
<td>1000:3000</td>
<td>V-DBS (733)</td>
<td>84.7 (79.7-89.0)</td>
<td>97.7 (96.0-98.9)</td>
<td>0.85 (0.81 - 0.89)</td>
<td>2.2</td>
<td>15.2</td>
</tr>
<tr>
<td></td>
<td>M-DBS (724)</td>
<td>84.8 (79.7-89.1)</td>
<td>97.1 (95.2-98.4)</td>
<td>0.84 (0.80 - 0.88)</td>
<td>2.9</td>
<td>15.1</td>
</tr>
<tr>
<td></td>
<td>D-DBS (732)</td>
<td>83.3 (78.1-87.8)</td>
<td>97.3 (95.5-98.6)</td>
<td>0.84 (0.79 - 0.87)</td>
<td>2.7</td>
<td>16.7</td>
</tr>
<tr>
<td>1000:5000</td>
<td>V-DBS (733)</td>
<td>81.5 (76.1-86.1)</td>
<td>98.1 (96.5-99.1)</td>
<td>0.83 (0.78 - 0.87)</td>
<td>1.8</td>
<td>18.4</td>
</tr>
<tr>
<td></td>
<td>M-DBS (724)</td>
<td>81.6 (76.1-86.2)</td>
<td>98.1 (96.5-99.1)</td>
<td>0.83 (0.78 - 0.87)</td>
<td>1.9</td>
<td>18.4</td>
</tr>
<tr>
<td></td>
<td>D-DBS (732)</td>
<td>79.3 (73.7-84.2)</td>
<td>98.6 (97.1-99.4)</td>
<td>0.81 (0.77 - 0.86)</td>
<td>1.4</td>
<td>21.0</td>
</tr>
</tbody>
</table>
Challenges using DBS

- Among treated patients contribution of cell-associated & pro-viral DNA leading to low specificity which may lead unnecessary treatment switch

- Variation of results in different assay
  - Lower limit of detection
  - Extraction and amplification technologies
  - Target region for amplification

- Turn around time
Strategies for scale-up

- Point of care devices
Benefits of viral load POC devices

- Portability: Increasing accessibility to rural areas
- Low cost increasing affordability
- Simplicity of use enhancing task shifting from highly skilled laboratory technicians
- Limited infrastructure needs e.g. electricity
- Fast turn-around time with immediate results
  - Leads to reduction in loss to follow-up
  - Reduction in patient time and costs-return visits of the results
  - Improves care due to fast clinician decision making
POC Technology Pipeline - Viral Load

- Liat™ Analyser
- IQuum
- Alere Q
- Alere
- EOSCAPE HIV™ Rapid RNA Assay System
- Wave 80 Biosciences
- Gene Xpert
- RT CPA HIV-1 Micronics
- Ustar
- Gene-RADAR
- Cavidi AMP
- Nanobiosy
- ALL
- BioHelix
- Truelab PCR
- Molbio/bigTe
- LYNX Viral Load Platform
- NWGHF
- Viral Load Assay with BART
- Lumora

*Estimated as of March 2013 - timeline and sequence may change. Dotted line indicates that no market launch date has been set by the company.*
SAMBA background

- **Simple AMplification Based Assay (SAMBA) nucleic acid-based point of care (POC) platform**
  - Qualitative EID test (Positive/Negative)
  - Semi-quantitative viral load monitoring test (>1000 copies)
    - Plasma
    - Leuco-depleted whole blood, without venous puncture and centrifugation
Primary Objectives

- **Phase 1:** Validate in-laboratory performance of the POC SAMBA for country product approval

- **Phase 2:** Feasibility of using POC SAMBA system among clinical site staff at selected health facilities

- **Phase 3:**
  - Impact at 6 weeks of life on time to ART initiation
  - Impact on patients retention in care and treatment
  - Cost-effectiveness
SAMBA VL Whole Blood/Plasma Evaluation

Whole blood collected from participants on HAART

Samples shipped to KEMRI/CDC facility

Leuco-depletion Whole blood

SAMBA

Plasma separation

Roche CAP/CTM

Discordant Samples

Abbott M2000
VL Results
## Plasma VL SAMBA vs. Roche + Abbott (Combined Gold Standard)

<table>
<thead>
<tr>
<th>Copies/ml</th>
<th>Combined Gold std</th>
<th>Combined Gold std</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 1,000</td>
<td>91</td>
<td>2</td>
<td>93</td>
</tr>
<tr>
<td>&lt;1,000</td>
<td>6</td>
<td>98</td>
<td>104</td>
</tr>
<tr>
<td>Total</td>
<td>97</td>
<td>100</td>
<td>197</td>
</tr>
</tbody>
</table>

Sensitivity at Clinical cutoff of 1000 copies: 93.8% (CI; 87.0-97.7%)

Specificity at Clinical cutoff of 1000 copies: 98.0% (CI; 93.0-99.8%)

Concordance: SAMBA vs Roche + Abbott = 95.9% (189/197)
Leuco-depleted Whole blood VL SAMBA vs. Roche + Abbott (Combined Gold STD)

<table>
<thead>
<tr>
<th>Viral Load (cp/ml)</th>
<th>Roche &gt; 1,000</th>
<th>Roche &lt; 1,000</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAMBA &gt; 1,000</td>
<td>35</td>
<td>8</td>
<td>43</td>
</tr>
<tr>
<td>SAMBA &lt; 1,000</td>
<td>0</td>
<td>162</td>
<td>162</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>170</td>
<td>205</td>
</tr>
</tbody>
</table>

Sensitivity: 100.0% CI (90.0%, 100.0%)

Specificity: 95.3% CI (90.9%, 97.9%)

Overall concordance: SAMBA vs Roche + Abbott = 96.1% (197/205)
Findings

- High sensitivity and specificity obtained with SAMBA VL assays
- Comparable results obtained from different countries
- SAMBA device is much easier to handle and simpler sample processing
- SAMBA reagents do not require cold chain transport or cold storage
Integrated approach (Centralized vs POC)

- Potential limitation for POC’s and centralized systems calls for an integrated approach that ensures a greater impact, quality and effective use of both systems.

- Laboratory systems are most preferred in areas with high test needs due to higher throughput as compared to POCs.

- POC’s however are likely to leverage turn around time and increase patient retention to care and can be most suitable in outreach clinics.
Conclusion

- Need for comprehensive integrated approach on VL testing in RSL
- Plasma use, preferred on sites near centralized systems, while DBS and POC’s can be used in remote and far areas

- Need for establishment of QA guidelines on DBS and POC VL testing
- Need for MOH driven in-country VL testing
Acknowledgements

Clinton Health Access Initiative

Lara Vojnov
Trevor Peter
Marta Prescott
Jessica Joseph
Charles Kasipo

Rosanna Peeling
Maurine Murtagh
Ben Cheng
DBS VL Consortium

CDC
Chunfu Yang
Clement Zeh

National Health Laboratory Services – South Africa
Wendy Stevens
Sergio Carmona
Thank you