Viral Load Testing in Africa: 23 years later?
Lessons learnt, Future Challenges and Opportunities

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University of the Witwatersrand &
Head of National Priority Programs NHLS

Acknowledgements: Sergio Carmona, Lesley Scott, Gayle Sherman, Leigh Berrie
Massive Clinical Changes in Global ARV Treatment Programs

- **Treatment Simplification**
  - Fixed dose combination (single pill) (e.g. TDF/FTC/EFV)
  - Massive Price Reductions in ARVs
- **Trend towards initiation at higher CD4s** and **greater value placed on use of viral load** (23 years after first description)
- Life-Long treatment for pregnant women?
- Treatment in all children <5 years
- Treatment as Prevention? Discordant couples, PEP and PREP
  - HPTN 052
  - ACTG 5202
  - PREP
- **Integrated response**
  - Move towards Consolidated Guidelines: WHO
  - HIV and co-morbidities: TB and Hepatitis B
- De-centralization of care and Task shifting
Clinical uses for viral load assays

• Early identification of treatment failure:
  • When to switch? Threshold? Frequency of testing?
  (CD4 poor predictor of virological failure)
• Targeted adherence in first-line therapy
  • Questions remain whether 3 months or 6 months is most useful?
• Early infant diagnosis
• Sentinel surveillance
• Use prevents complex resistance profiles developing
• **Program management**: community viral load or % virological failure delineating treatment outcomes or sites at high risk for HIV drug resistance and intervention

CLEARER EVIDENCE BASED GUIDELINES NEEDED AS PART OF INTERNATIONAL AND NATIONAL ART TREATMENT GUIDELINES
Low level of HIVDR in a clinical trial setting (CIPRA) with frequent VL monitoring: n=812

- Frequent monitoring: 3 monthly
- Virological failure 83/812 patients (10.2%)
- 61/83 failing patients developed resistance (73.5%)
- Average time on ART 24 months
<table>
<thead>
<tr>
<th>Site</th>
<th>Malawi (Hosseinipour et al., 2009)</th>
<th>South Africa Cape Town (Orrell et al., 2009)</th>
<th>South Africa Johannesburg (Wallis et al., 2010)</th>
<th>South Africa Durban (Marconi et al., 2008)</th>
<th>South Africa CIPRA-SA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Switch Criteria</td>
<td>CD 4 cell count decrease &gt;30% or WHO staging</td>
<td>Viral load &gt;5000 RNA copies/ml</td>
<td>Viral load &gt;5000 or 1000 RNA copies/ml</td>
<td>Viral load &gt;1000 RNA copies/ml</td>
<td>Viral load &gt;1000 RNA copies/ml</td>
</tr>
<tr>
<td>Frequency of Monitoring</td>
<td>Irregular</td>
<td>6 monthly-viral load &amp; CD4+ T-cell</td>
<td>6 monthly-viral load &amp; CD4+ T-cell</td>
<td>6 monthly-viral load &amp; CD4+ T-cell</td>
<td>3 monthly-viral load &amp; CD4+ T-cell</td>
</tr>
<tr>
<td>Median Time on First-line (months)</td>
<td>36.5 (8-127)</td>
<td>Unknown</td>
<td>Unknown</td>
<td>10.8 (6.7-18.6)</td>
<td>15 (3-33)</td>
</tr>
<tr>
<td>% with failure &amp; resistance</td>
<td>95%</td>
<td>85%</td>
<td>83%</td>
<td>83.5%</td>
<td>82%</td>
</tr>
<tr>
<td>Subtype: C</td>
<td>100%</td>
<td>98%</td>
<td>96.5%</td>
<td>97.4%</td>
<td>100%</td>
</tr>
<tr>
<td>M184V</td>
<td>81%</td>
<td>78%</td>
<td>72%</td>
<td>64.3%</td>
<td>67.2%</td>
</tr>
<tr>
<td>NNRTI</td>
<td>93%</td>
<td>86%</td>
<td>78%</td>
<td>Unknown</td>
<td>75%</td>
</tr>
<tr>
<td>K103N</td>
<td>28%</td>
<td>55%</td>
<td>38%</td>
<td>51%</td>
<td>50%</td>
</tr>
<tr>
<td>V106M</td>
<td>6%</td>
<td>31%</td>
<td>17%</td>
<td>19%</td>
<td>14%</td>
</tr>
<tr>
<td>TAM5 &gt;3</td>
<td>56%</td>
<td>23%</td>
<td>11%</td>
<td>13%</td>
<td>1.5%</td>
</tr>
<tr>
<td>K25E</td>
<td>12%</td>
<td>8%</td>
<td>4.5%</td>
<td>2.6%</td>
<td>2%</td>
</tr>
</tbody>
</table>
HIV-1 drug resistance in antiretroviral-naïve individuals in sub-Saharan Africa after rollout of antiretroviral therapy: a multicentre observational study

Raph L Hamers, Carole L Wallis, Cissy Kityo, Margaret Siwale, Kishor Mandaliya, Francesca Conradie, Mariette E Botes, Maureen Wellington, Akin Osibogun, Kim C E Sigaloff, Immaculate Nankya, Rob Schuurman, Ferdinand W Wit, Wendy S Stevens, Michèle van Vugt, Tobias F Rinke de Wit, for PharmAccess African Studies to Evaluate Resistance (PASER)*

- 2436 (94%) of 2590, 57% women, CD4 median: 133 CD4 cells/ul; >18 years
- Sample weighted drug prevalence of resistance was: 5.6%: ranged from 1.1% in Pretoria (SA) to 12.3% in Kampala (Uganda)
- Pooled prevalence for 3 sites in Uganda was 11.6% compared to 3.5% for all other sites
- 2.5% NRTI, 3.3% for NNRTis, 1.3% for PI’s and 1.1% for dual NRTI and NNRTI
- Odds ratio for drug resistance- associated with each additional year since ART rollout was 1.3 (95% CI: 1.13-1.68)

Interpretation: The higher prevalence of primary drug resistance in Uganda than in other African countries is probably related to the earlier start of ART roll-out in Uganda. Resistance surveillance and prevention should be prioritised in settings where ART programmes are scaled up.

PASER project
WHO Surveys of Transmitted HIVDR

- In select LMIC countries: 6.6% in 2009 (95% CI 5.1-8.3%)
- In Africa NNRTI resistance: 3.4% (95% CI: 1.8-5.2%)
- Of 72 surveys: 20% classified as moderate (between 5-15%): increase from 18% in 2004-2006 period to 32% in 2007-2010

**Figure 3.5** Relationship between antiretroviral therapy coverage and prevalence of transmitted NNRTI drug resistance mutations

**Table 3.4** Results of WHO transmitted HIV drug resistance surveys

- Low prevalence (<5%): Any 52 (72.8%)
- Moderate prevalence (5%-15%): Only NNRTI 8 (11.9%)
- High prevalence (>15%): None
- Total number of surveys: 72
Evolution of resistance

- Retrospective study: Rate of DRM accumulation among South-African patients with continued virological failure.
- 43 patients, 38 (88.4%) harbored ≥ 1 DRM; multiple TAMs (23.3%), K65R (7.0%), Q151M (2.3%)
- TAMs accumulated at a high rate of 0.07 mutation per month of drug exposure after first detection of viremia, resulting in 1 new TAM for every 14.6 months of continued drug exposure. (confirmed in DART study: 2: 24-48 weeks)
- Patients who had acquired ≥ 1 TAM at time of treatment switch (T0): 0.15 additional TAM per month, i.e. 1 TAM for every 6.5 months of continued drug exposure
- The median time between two sequential resistance tests was 5 months (IQR: 3 to 10), median time on treatment was 22 months
- 56% patients achieved virological suppression despite mutations

(Sigaloff, Wallis, Stevens, Interest meeting 2011)
Impact in early detection of VF

Viral load at 3 months after initiation of antiretroviral therapy is associated with better virological and treatment outcomes than at 6 months.

VL3 group: 22% less likely to experience subsequent virological failure, 27% less likely to be later switched to second line regimen.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cHR*  95% CI  p</td>
<td>aHR** 95% CI  p</td>
</tr>
<tr>
<td>Virological failure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3 months</td>
<td>1.10  0.93-1.29  0.257</td>
<td>0.78  0.64-0.95  0.016</td>
</tr>
<tr>
<td>Treatment switching</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3 months</td>
<td>1.02  0.85-1.23  0.803</td>
<td>0.73  0.58-0.92  0.008</td>
</tr>
</tbody>
</table>

* cHR, crude hazard ratio  ** aHR, adjusted hazard ratio

Presentation by Eric Goemaere

Viral load at 3 months after initiation of antiretroviral therapy is associated with better virological and treatment outcomes than at 6 months.

B. Kerschberger¹, A. M. Boulle², K. Kranzer³, K. Hilderbrand¹², M. Schomaker², D. Coetzee², E. Goemaere⁴, N. Ford²⁵, G. Van Cutsem¹²
Global Trends in Viral Load testing

At present
• Viral load testing is almost exclusively laboratory-based, highly centralized
• Most testing done on sophisticated, high-throughput instruments
• However, with improved automation, skill set required not as sophisticated, less user intervention, lower margin of error
• Unless leasing agreements, most remain relatively expensive
• Sample transport is required for patients not near central laboratory facilities.
• Current viral load testing market is dominated by four companies: Abbott, Roche, Biomeriuex and Siemens.
• Move to real-time PCR technologies for large suppliers
• The per-test cost of the assays on these instruments varies greatly with volumes. SA and Brazil have cheapest prices globally ($10-40)
• One open platform test (Biocentric) can accommodate the use of generic test reagents, particularly effective in identifying non-B HIV subgroups, especially HIV-2

Alternative to centralised testing
• POC testing options limited, although diagnostics pipeline developing.
• DBS is now a real option for some of the commercially-available platforms.
Challenges facing implementation of appropriate HIV diagnosis and monitoring on the continent:

- **Quantification of Needs and Volumes in Africa**
- **Substantial variation**: within and between countries in terms of size, population, HIV prevalence and resources
- **Lack of resources**: funding, facilities, equipment and skilled staff
- **HIV/AIDS plans** available in an increasing number of countries: not always a National Strategic Plan for laboratories
- **High levels of donor funding** supporting programs: often not-co-ordinated
- **Inappropriate validation, verification of instruments and reagents**
- **NRL present in many sites**: unlinked to rest of country program or private sector often with a focus on surveillance
- **Regular stock interruptions**: forecasting, procurement, supply and distribution
- **Maintenance difficulties, poor supplier support in network**
- **Poor Regulatory frameworks**: from laboratories to devices
- **Data Collection and M&E** generally poor
- **Poor Quality Management Systems**: No appropriate internal and external quality assurance (poor QMS)

Other challenges include:

- lack of consistent **power supply**,
- reliable **courier networks**, (WHO 2007: 57% unable to maintain required cold chain)
Realization and Calls to Action for Lab strengthening not a new initiative: yet Progress is slow!

- **Everybody's Business: Strengthening Health Systems to Improve Health Outcomes.** *Geneva, World Health Organization (WHO), 2007*
  - Service delivery, workforce, information, medical products (diagnostics)

- **Maputo declaration** on strengthening of laboratory systems; focus on lab challenges that limit scale-up for HIV, TB and malaria *(Mozambique, 2008)*
  - National strategic plans for laboratories, recognised a tiered laboratory system
  - Human resource agenda
  - Acceleration of the development of new tools
  - Integrate services across diseases

- **WHO 5th AFRO meeting** *(Senegal, 2008)*

- **WHO AFRO resolution AFR/RC58/6** *(Yaounde, June 2008)*

- **UNAIDS Declaration** *(2011)*

- **WHO 6th AFRO meeting** *(Coite d’voire, 2012)*

- **ASLM Ministerial call to action:** *(Dec 2012)*
Issues still being addressed

• Clinical needs assessment
  • The place for VL testing
  • Treatment guidelines changing

• Available platforms
  • Suitable for the volume of testing
  • Suitable for the environment

• Laboratory placement and scale
  • Throughput
  • Centralized vs POC

• Connectivity, data management and quality monitoring
1: Quantifying Needs:
VL: Qualitative Analysis

Figure 28: Proportion of Countries Performing Viral Load Test in 2003, 2005, 2007, 2011

Figure 29: Proportion of Laboratories Conducting Viral Load Testing by Sector (2005-2007-2011)

Figure 30: Frequency Distribution of HIV Viral Load Assays (2005-2007-2011)
Quantitating needs: Global VL Market Need: Donor perspective

Based on current guidelines, global viral load need is expected to grow as countries scale up treatment. Current CHAI country estimates suggest **16 million by 2020**, depending on algorithm may reach **40 million**.

CHAI, 2012, Trevor Peter personal communication
- **155 automated systems in sSA**
- EID Amplicor labs were not counted in this (additional sites)
- doubling of sites since 2010

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**Abbott HIV Viral Load Presence in Africa 2010:**
- With 154 m2000RT and 77 m2000sp instruments
<table>
<thead>
<tr>
<th>Country</th>
<th>Total volume of tests</th>
<th>Instrumentation</th>
<th>Coverage</th>
<th>VL performed, As part of treatment program</th>
<th>Assays used</th>
<th>Number of centers</th>
<th>Current Assay numbers</th>
<th>Funders</th>
<th>Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kenya</td>
<td>160,000</td>
<td>Automated</td>
<td>24%</td>
<td>Kenya</td>
<td>Yes</td>
<td>Roche and abbott</td>
<td>7, with 3 planned</td>
<td>Kenya govt and donors</td>
<td>Kenya govt and donors</td>
</tr>
<tr>
<td>Tanzania</td>
<td>6,000</td>
<td>Semi-automated</td>
<td>2%</td>
<td>Tanzania</td>
<td>No, mostly research</td>
<td>Roche Taqman v2</td>
<td>5</td>
<td>Donors</td>
<td>Donors</td>
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<tr>
<td>Ethiopia</td>
<td>18,000</td>
<td>Semi-automated</td>
<td>5%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Botswana</td>
<td>350,000</td>
<td>Automated</td>
<td>74%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Donors</td>
<td></td>
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<tr>
<td>Ghana</td>
<td>30,000</td>
<td>Automated</td>
<td>50%</td>
<td></td>
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<td></td>
<td></td>
<td>Donors</td>
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<tr>
<td>Nigeria</td>
<td>100,000</td>
<td>Semi-automated</td>
<td>19%</td>
<td></td>
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<tr>
<td>Zambia</td>
<td>10,000</td>
<td>Automated</td>
<td>2%</td>
<td>Zimbabwe</td>
<td>For resistance survey, patient care from April 2013</td>
<td>Roche Taqman 48/96, DBS on cioMerieux</td>
<td>1</td>
<td>Donors</td>
<td>Dr Zinyowera</td>
</tr>
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<td>Mozambique</td>
<td>5,000</td>
<td>Semi-automated</td>
<td>2%</td>
<td></td>
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<tr>
<td>Uganda</td>
<td>36,000</td>
<td>Automated</td>
<td>10%</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Rwanda</td>
<td>35,000</td>
<td>Automated</td>
<td>29%</td>
<td></td>
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<tr>
<td>Swaziland</td>
<td>15,000</td>
<td>Automated</td>
<td>21%</td>
<td></td>
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<tr>
<td>Namibia</td>
<td>85,000</td>
<td>Automated</td>
<td>60%</td>
<td></td>
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</tr>
<tr>
<td>Zimbabwe</td>
<td>10,000</td>
<td>Automated</td>
<td>2%</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Lesotho</td>
<td>2,000</td>
<td>Automated</td>
<td>3%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Malawi</td>
<td>60,000</td>
<td>Automated</td>
<td>18%</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cape Verde</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cavidi Exavir</td>
<td>1</td>
<td>1800-20000</td>
<td>Global Fund</td>
<td>Jose Roche, lab director central hospital</td>
</tr>
<tr>
<td>Senegal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Roche, Biocentric, NucliSEND</td>
<td>1, plans for 5 in place</td>
<td>6000/year</td>
<td>National govt and donors (Global Fund)</td>
<td>Comba Toure Kane</td>
</tr>
<tr>
<td>Guinea Bissau</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Abbott, Roche at 1 site, Nuclisens 1 site-decommissioned, NucliSENS</td>
<td>8 sites, 1 more in planning (n=1 NucliSENS)</td>
<td></td>
<td>National government, Esther AID, Global Fund, V</td>
<td>Abdelaye keita, Inacio Avarenga</td>
</tr>
<tr>
<td>South Africa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Roche Taqman v2</td>
<td>17</td>
<td>1,600 000</td>
<td>National govt</td>
<td>Wendy Stevens</td>
</tr>
<tr>
<td>Burkino Faso</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Abbott RealTime</td>
<td>6 in 2013</td>
<td>13 000</td>
<td></td>
<td>Dr Lassana sangare</td>
</tr>
<tr>
<td>Liberia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Nuclisens</td>
<td>6</td>
<td>16000 by 2014</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>Biocentric</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Abbott Realtime</td>
<td></td>
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</tr>
</tbody>
</table>
2. Addressing substantial variation: With Technology and selection of appropriate technology

Selection based on volumes and level of healthcare, technical skill and cost
Overview of current technologies for VL on plasma or DBS

<table>
<thead>
<tr>
<th>HIV RNA PCR</th>
<th>Versant HIV-1 RNA (bDNA) (Siemans), VERSANT KPCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NucliSENS EasyQ (bioMerieux)</td>
</tr>
<tr>
<td></td>
<td>Cobas Ampliprep / Cobas Taqman v2.0 (Roche)</td>
</tr>
<tr>
<td></td>
<td>RealTime HIV-1 (Abbott)</td>
</tr>
<tr>
<td></td>
<td>Generic Viral Load kit (Biocentric) – w/ any real-time platform</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Non-molecular</th>
<th>Ultrasensitive p24 Ag (Perkin Elmer) – not commercialised*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>*(research use only, not for diagnostic purposes); plasma only</td>
</tr>
<tr>
<td></td>
<td>ExaVir Load Reverse transcriptase ELISA (Cavidi) – plasma only</td>
</tr>
</tbody>
</table>

Amplification from whole blood (e.g. DBS) using real-time techniques will result in the co-amplification of cellular pro-viral DNA along with viral RNA, resulting in falsely elevated viral loads below 5000 copies/ml. Isothermal techniques (e.g. NASBA) do not amplify DNA as the reaction does not get hot enough to cleave double stranded DNA.
<table>
<thead>
<tr>
<th>Assay</th>
<th>Company</th>
<th>Method</th>
<th>Diagnostic Markers</th>
<th>Subtype/group</th>
<th>Sample volume (mL)</th>
<th>Linear range (copies/mL)</th>
<th>Polyvalency</th>
<th>Caveats</th>
</tr>
</thead>
<tbody>
<tr>
<td>COBAS AmpliPrep/COBAS Taqman assay (v2)</td>
<td>Roche</td>
<td>RT-qPCR</td>
<td>RNA (gag) and HIV-1 LTR region</td>
<td>Group M: A-H and Group O</td>
<td>0.85 (plasma) 6hrs</td>
<td>20 to 10 000 000</td>
<td>HIV DNA, Hepatitis B+C, MTB, Clamydia, CMV VL</td>
<td>High resources required; co-amplifies DNA from whole blood, large lab footprint, reliable power</td>
</tr>
<tr>
<td>RealTime HIV-1 Assay (v2)</td>
<td>Abbott</td>
<td>RT-qPCR</td>
<td>RNA (pol)</td>
<td>Group M: A-H Groups N &amp; O</td>
<td>Four options: 6hrs</td>
<td>40 to 10 000 000 (0.6 and 1.0 mL)</td>
<td>Hepatitis B+C, HPV, CT/NG, CMV, MRSA</td>
<td>High resources required; co-amplifies DNA from whole blood, large lab footprint, reliable power</td>
</tr>
<tr>
<td>Nuclisens EASY Q</td>
<td>Biomerieux</td>
<td>NASBA</td>
<td>gag</td>
<td>All?</td>
<td>50-3million</td>
<td></td>
<td></td>
<td>Plasma and DBS</td>
</tr>
<tr>
<td>Versant HIV-1 RNA 3.0</td>
<td>Siemens</td>
<td>bDNA</td>
<td>RNA (gag)</td>
<td>Group M: A-G</td>
<td>1.0 6hrs</td>
<td>75 to 500 0000</td>
<td>Hepatitis B+C</td>
<td>Cannot use DBS, large lab footprint, reliable power</td>
</tr>
<tr>
<td>VERSANT kPCR 1.0</td>
<td></td>
<td>RT-PCR</td>
<td>RNA (gag)</td>
<td></td>
<td>0.5 ml 6hrs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ExaVir RT Assay</td>
<td>Cavidi</td>
<td>Reverse transcriptase assay</td>
<td>RT</td>
<td>All</td>
<td>1.0 48-72hrs</td>
<td>200 upwards</td>
<td>None</td>
<td>Cannot use DBS; controls cannot be supplied</td>
</tr>
<tr>
<td>LTR-based HIV-1 RNA RT-PCR</td>
<td>Biocentric</td>
<td>RT-qPCR</td>
<td>RNA (LTR)</td>
<td>All</td>
<td>0.2 4hrs</td>
<td>300 to 10 000 000</td>
<td>HIV DNA, M. tuberculosis (with Hain test)</td>
<td>Co-amplifies DNA from whole blood, reliable power</td>
</tr>
</tbody>
</table>
Addressing variation with selection of appropriate technology and placement: The tiered, integrated laboratory network for HIV testing

Level IV: **National/multi-country reference lab**
- **Staff**: Senior Health Specialist / lab management
- **Dx**: HIV resistance testing, HIV viral load, EID PCR, ELISA, WB, CD4, GXP, AFB, culture & susceptibility testing

Level III: **Regional provincial Lab**
- **Staff**: Lab specialists, senior techs, Programme officer
- **Dx**: Quantitative HIV (PCR other), qualitative/quantitative EID PCR, ELISA, GXP, AFB, culture

Level II: **District lab**
- **Staff**: Lab specialists, senior techs, Programme officer
- **Dx**: HIV serology by ELISA, other ELISA, CD4 count, ? Viral load (SA setting)

Level I: **Primary Lab**
- **Staff**: Doctors, Nurses, lab or Medical assistants, phlebotomists
- **Dx**: HIV rapid tests, other point-of-care tests* and DBS collection

*Malaria RDT, Glucometer, HemoCue Hb, Pregnancy test, Urine strip, Syphilis RDT, Lactate Accutrend, Reflotron, PIMA basic chemistry, haematology & microbiology, at all levels potentially

Ref: http://www.who.int/hiv/amds/amds_cons_tech_oper_lab_test.pdf
HIV Genotypes in Africa: Diversity creates assay design issues: Molecular Surveillance no longer at the forefront and needs to be revitalized

3. Addressing Resources

- Sustainability needs to be assured beyond donor funding
- HIV/AIDS Plan needs to be included in National Laboratory Strategic plan
- Costing, cost-effectiveness and modelling of intervention is needed
- Packaged integrated services
- Workforce development
- Rational use through guidelines
- Leadership: governance, policy guidance, accountability
3. Equipment Capacity – Overview

In 9 example countries\(^3\), based on an existing 73 automated VL instruments, **current available capacity is larger than the testing needs** based on the existing VL algorithm, hence is currently largely underutilized.

**Equipment Capacity** vs Market Need -

<table>
<thead>
<tr>
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<th>Current Market Need</th>
<th>Expected Market Need</th>
<th>Total capacity</th>
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<tr>
<td># tests</td>
<td>808,000</td>
<td>3,900,000</td>
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**Utilization of Equipment Capacity**\(^4\) -

- **Total capacity**: 3,285,000
- **Capacity Utilized**: 466,000
- **86% Spare Capacity**\(^*\)

On average, only 14% of capacity is being used

---

3: The total capacity in 9 countries (Ethiopia, Kenya, Tanzania, Mozambique, Malawi, Swaziland, Zimbabwe, Zambia, Uganda) is a multiple of instruments and automated equipment capacity (supplier approved) per year - Roche CAP/CTM and Abbott M2000 system are 45,000 samples/year and bioMérieux NucliSENS EasyQ is 25,000.

4: The capacity in 9 countries (Ethiopia, Kenya, Tanzania, Mozambique, Malawi, Swaziland, Zimbabwe, Zambia, Uganda) which is currently utilized is the sum of existing viral load and EID testing volumes.

*Courtesy Trevor Peter, Chai*
4. Re-direction of resources for unnecessary investigations towards increased quality viral load testing?

• Questions around volumes of CD4 testing even in high income countries?
  • Gale et al. 2013: Is frequent monitoring of CD4 T-lymphocyte count monitoring necessary for persons with Counts greater than 300 cells/µl and HIV-1 suppression? CID Advance Access 2013)

• Move towards Universal Access:
  • See editorial: When to start in Africa? in NEJM Perspective by Kevin de Kock) suggesting movement towards universal treatment. This editorial addresses the problems of that 350-500 cell/ul group who are at risk of TB and bacterial infection.
  • An interesting study conducted in San Francisco (2245 adults) demonstrated the increased rate of viral suppression when patients were initiated with a CD4 count >500 cells/ul (Geng et al. CID 2012:55(12): 1690-7). It should be noted that 500 cells/ul is the lower limit of normal for the South African population. Are we moving towards Test and Treat?

• Monitoring of chemistry parameters in symptomatic patients only
Steps to be taken to ensure sustainability

Coordinate efforts, define priorities
Develop implementation plan, documents
Prepare sites
Build capacity for training (lab + clinic), support resources
Support data collection and impact evaluation

SA processes

NDoH/NHLS engage
• National plan
• Testing algorithm
• Align with treatment guidelines

Discuss with partners
• Funders
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• Laboratory
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Laboratory functions
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Approach
- Allows for the lab to be remotely monitored, managed, and service intelligent devices, deployed around the world, allowing them to be optimised

Key Deliverables
- Remote monitoring
- Preventive maintenance
- QC monitoring
- Remote results reviewing by pathologist (off-site)

Source: www.axeda.com
Strong Case for LIS/Connectivity solutions (also for POC)
CCMT M&E reporting

- M&E Reports have been developed with the support of CDW lead by Sue Candy in conjunction with:
  - Prof Gayle Sherman (PI) supported by UNICEF funding
  - Prof Wendy Steven (PI) UNITAID/RTC grant for M&E CCMT SA
- Phase 1: produce CD4 and VL monthly reports
- Phase 2: extending more comprehensive data analysis

Acknowledgements: Sue Candy (CDW), Gayle Sherman (NPP – EID stream)
Viral Load Monthly report

Viral Load Testing in SA for the Month of Jan 2012 vs Jan 2011

Total by Province VL > 1,000

By Province vs Last Year (LY)

Results by Range by Province vs Last Year (LY)

Acknowledgements: Sue Candy (CDW), Gayle Sherman (NPP – EID stream)
Though the advantages of virological monitoring for patients on anti-retroviral therapy have been established, cost and complexity limit its full implementation and scale up.

- Key challenges of VL testing programs implementation and scale up -

Currently available VL platforms require a high level of technical skill and laboratory infrastructure and need regular maintenance. These factors make them only suited to national or reference laboratories.

Sample transportation systems are normally informal and fragmented. This negatively impacts on TAT and efficiency.

Low testing volume and lack of transparency on market demand result in high cost per test.

Whole venous blood must be drawn by a health professionals and plasma must be separated from the whole blood within six hours of blood draw.
Global partners and countries must collaborate to implement key practices that would make VL testing programs effective and sustainable.

The development of simpler laboratory-based tests and/or point-of-care devices could go a long way in solving access problems.

The use of Dried Blood Spot and pooled sample testing could represent valid solutions that need to be further assessed.

More organized and strong sample transportation and results delivery systems would result in reduced turnaround time and reduced LTFU.

Incentives to new manufacturers to enter a growing market for viral load testing and increased volumes need to be reflected in a much lower and sustainable price per test and better Service & Maintenance contracts.
UNITAID HIV Diagnostic Activities & Investments

Discovery > Development > Evaluation > Registration > Market

Landscape Analyses

CHAI/UNICEF (Mar 2012): Catalyze Market; Scale-up

MSF (Mar 2012): Operational Research

New Investment Area

Market Entry (Dec 2012): Address Developers’ Market Barriers

Market Intelligence Systems: monitoring, estimate impact

Collaboration with World Health Organization and others
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<tr>
<th>Category</th>
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<td><strong>In early clinical validation</strong></td>
<td>LIAT (IQUUM)</td>
<td>Multiplex real-time PCR</td>
</tr>
<tr>
<td></td>
<td>SAMBA (DRW)</td>
<td>Isothermal amplification, Dipstick readout</td>
</tr>
<tr>
<td></td>
<td>HDA (Biohelix)</td>
<td>Helicase dependent amplification</td>
</tr>
<tr>
<td><strong>NAT technology that could be adapted</strong></td>
<td>GeneXpert Cepheid</td>
<td>Multiple targets, real-time TB, MRSA</td>
</tr>
<tr>
<td></td>
<td>BD Max (Becton Dickinson)</td>
<td>Real-time microfluidic PCR Influenza</td>
</tr>
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<td></td>
<td>Enigma ML system (Enigma diagnostics)</td>
<td></td>
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<td></td>
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</tr>
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<td>Cavidi Amp</td>
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<td>PanNAT (Micronics)</td>
<td></td>
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Overview of poc options: Future solutions?

LIAT (Lab-in-a-tube) IQUUM
- Automated sample preparation, amplification and detection
  - silica magnetic bead extraction
  - multiplex real-time PCR detection
- Reagents pre-packed in unit doses separated by peelable seals
- Internal control, LOD: 81 cp/ml in plasma.
- Dynamic Range: $10^2$ - $1.5 \times 10^6$ copies/ml
- Sample volume: Plasma: 150 ul, Blood: 75 ul
- Assay time: Plasma: 30 mins, Blood: 35 mins

SAMBA Qualitative (SAMBA-Q – proviral DNA) and Semi-Quantitative (SAMBA-SQ- RNA)
- Test use isothermal amplification and visual detection by dipstick.
  - Two step process: sample preparation and amplification/ detection.
- RNA/DNA target is extracted by binding to a silica membrane in a column, washed and eluted sample added to a SAMBAmph cartridge.
- Cartridge is a closed system.
Acknowledgements

- National Department of Health
- National Health Laboratory Service and
- National Priority Program (Leigh Berrie)
- University of the Witwatersrand
- Sergio Carmona and PCR team
- R&D team (Lesley Scott, Natasha Gous, Brad Cunningham, Pam Horsfield)
- IQUUM
- Grand Challenges Canada
- USAID, PEPFAR
- Clinical partners (Ian Sanne, Francois Venter, Andrew Black, Regina Osih, Johan Potgieter)
- Cambridge Health Tech Institute

UNDETECTABLE
HOW VIRAL LOAD MONITORING CAN IMPROVE HIV TREATMENT IN DEVELOPING COUNTRIES

www.muslimaccess.org
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By Province vs Last Year (LY)

Results by Range by Province vs Last Year (LY)

<table>
<thead>
<tr>
<th>Province</th>
<th>Total</th>
<th>&lt;= 1,000 (log 3)</th>
<th>&gt; 1,000 (log 3) &lt;= 10,000 (log 4)</th>
<th>&gt; 10,000 (log 4)</th>
<th>Average (log)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Current</td>
<td>% LY</td>
<td>Current</td>
<td>% LY</td>
</tr>
<tr>
<td>Eastern Cape</td>
<td>EC</td>
<td>12,649</td>
<td>6,882</td>
<td>5,168</td>
<td>41.2</td>
</tr>
<tr>
<td>Free State</td>
<td>FS</td>
<td>8,109</td>
<td>6,064</td>
<td>3,109</td>
<td>38.3</td>
</tr>
<tr>
<td>Gauteng</td>
<td>GP</td>
<td>29,755</td>
<td>24,286</td>
<td>13,667</td>
<td>46.9</td>
</tr>
<tr>
<td>KwaZulu-Natal</td>
<td>KZN</td>
<td>43,348</td>
<td>31,199</td>
<td>32,835</td>
<td>57.7</td>
</tr>
<tr>
<td>Limpopo</td>
<td>LP</td>
<td>8,529</td>
<td>6,108</td>
<td>6,049</td>
<td>70.9</td>
</tr>
<tr>
<td>Mpumalanga</td>
<td>MP*</td>
<td>10,151</td>
<td>17,532</td>
<td>7,270</td>
<td>71.6</td>
</tr>
<tr>
<td>North West</td>
<td>NW</td>
<td>8,006</td>
<td>6,822</td>
<td>3,028</td>
<td>37.8</td>
</tr>
<tr>
<td>Northern Cape</td>
<td>NC</td>
<td>2,666</td>
<td>1,652</td>
<td>766</td>
<td>36.5</td>
</tr>
<tr>
<td>Western Cape</td>
<td>WC</td>
<td>10,336</td>
<td>8,058</td>
<td>3,282</td>
<td>31.8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>132,879</td>
<td>102,645</td>
<td>75,194</td>
<td>58.5</td>
</tr>
</tbody>
</table>

Viral Load (VL) is represented as RNA copies per ml

Acknowledgements: Sue Candy (CDW), Gayle Sherman (NPP – EID stream)
VL Scale up – Key Challenges

Though the advantages of virological monitoring for patients on anti-retroviral therapy have been established, **cost and complexity limit its full implementation and scale up**

- Key challenges of VL testing programs implementation and scale up -

Currently available VL platforms require a **high level of technical skill** and **laboratory infrastructure** and need **regular maintenance**. These factors make them only suited to national or reference laboratories.

**Sample transportation** systems are normally **informal and fragmented**. This negatively impacts on TAT and efficiency.

**Low testing volume and lack of transparency** on market demand result in high cost per test.

**Whole venous blood must be drawn** by a health professionals and **plasma must be separated** from the whole blood within six hours of blood draw.
Global partners and countries must collaborate to implement key practices that would make VL testing programs effective and sustainable.

- Possible interventions to facilitate VL testing program implementation and scale up -

The development of **simpler laboratory-based tests** and/or **point-of-care devices** could go a long way in solving access problems.

- **Incentives to new manufacturers to enter a growing market** for viral load testing and **increased volumes** need to be reflected in a much lower and sustainable price per test and better Service & Maintenance contracts.

- **The use of Dried Blood Spot** and **pooled sample testing** could represent valid solutions that needs to be further assessed.

More **organized and strong sample transportation and results delivery systems** would result in reduced turnaround time and reduced LTFU.
UNITAID HIV Diagnostic Activities & Investments

Discovery → Development → Evaluation → Registration → Market

Landscape Analyses

CHAI/UNICEF (Mar 2012): Catalyze Market; Scale-up

MSF (Mar 2012): Operational Research

Market Entry (Dec 2012): Address Developers’ Market Barriers

Market Intelligence Systems: monitoring, estimate impact

New Investment Area

Collaboration with World Health Organization and others
<table>
<thead>
<tr>
<th>Category</th>
<th>Technology</th>
<th>General Principle</th>
</tr>
</thead>
<tbody>
<tr>
<td>In early clinical validation</td>
<td>LIAT (IQUUM)</td>
<td>Multiplex real-time PCR</td>
</tr>
<tr>
<td></td>
<td>SAMBA (DRW)</td>
<td>Isothermal amplification, Dipstick readout</td>
</tr>
<tr>
<td></td>
<td>HDA (Biohelix)</td>
<td>Helicase dependent amplification</td>
</tr>
<tr>
<td>NAT technology that could be adapted</td>
<td>GeneXpert Cepheid</td>
<td>Multiple targets, real-time TB, MRSA</td>
</tr>
<tr>
<td></td>
<td>BD Max (Becton Dickinson)</td>
<td>Real-time microfluidic PCR</td>
</tr>
<tr>
<td></td>
<td>Enigma ML system (Enigma diagnostics)</td>
<td>Influenza</td>
</tr>
<tr>
<td></td>
<td>TwistDx (Alere)</td>
<td>Recombinase polymerase amplification (RPA)</td>
</tr>
<tr>
<td>Early development</td>
<td>EO-NAT HIV (wave 80)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Semi-Bio</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ultrasensitive p24 (North-western University)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Eiken chemical</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alere Nat system</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BART (Lumora)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Advanced Liquid Logic</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cavidi Amp</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PanNAT (Micronics)</td>
<td></td>
</tr>
</tbody>
</table>
Overview of poc options: Future solutions?

**LIAT (Lab-in-a-tube) IQUUM**
- Automated sample preparation, amplification and detection
  - silica magnetic bead extraction
  - multiplex real-time PCR detection
- Reagents pre-packed in unit doses separated by peelable seals
- Internal control, LOD: 81 cp/ml in plasma.
- Dynamic Range: 10^2 - 1.5x10^6 copies/ml
- Sample volume: Plasma: 150 ul, Blood: 75 ul
- Assay time: Plasma: 30 mins, Blood: 35 mins

**SAMBA Qualitative (SAMBA-Q – proviral DNA) and Semi-Quantitative (SAMBA-SQ- RNA)**
- Test use isothermal amplification and visual detection by dipstick.
  - Two step process: sample preparation and amplification/ detection.
- RNA/DNA target is extracted by binding to a silica membrane in a column, washed and eluted sample added to a SAMBAmp cartridge.
- Cartridge is a closed system.
Acknowledgements

- National Department of Health
- National Health Laboratory Service and
- National Priority Program (Leigh Berrie)
- University of the Witwatersrand
- Sergio Carmona and PCR team
- R&D team (Lesley Scott, Natasha Gous, Brad Cunningham, Pam Horsfield)
- IQUUM
- Grand Challenges Canada
- USAID, PEPFAR
- Clinical partners (Ian Sanne, Francois Venter, Andrew Black, Regina Osih, Johan Potgieter)
- Cambridge Health Tech Institute
COUNTRY EXPERIENCE
SOUTH AFRICA
Centralized CD4, HIV VL, HIV EID sites

Red: CD4, HIV VL & EID
Blue: CD4
Purple: CD4 & EID
Yellow: CD4 & HIV VL
Green: HIV VL & EID
Roche CAP/CTM vs Abbott RealTime HIV-1

Bland-Altman overlay scatter plots

A. CAP-CTM HIV-1 v2.0 versus CAP-CTM HIV-1 and versus Real-Time HIV-1 (n = 62). The vertical axis is the difference (CAP-CTM HIV-1 v2.0 minus other assays), and the horizontal axis is the CAP-CTM HIV-1 v2.0 log VL copies/ml

B. CAP-CA versus CAP-CTM HIV-1 v1.0 and CAP-CTM HIV-1 v2.0. The vertical axis is CAP-CA minus other assays, and the horizontal axis is CAP-CA log VL copies/ml. The symbol keys indicate the assays for each plotted point.

Source: Scott et al. 2009
Why are laboratory services for HIV difficult to manage in South Africa?

### Testing Volumes 2010/2012 period

<table>
<thead>
<tr>
<th>Period</th>
<th>&lt;=200</th>
<th>&gt;200</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apr 2012-Jan 2013</td>
<td>731 086</td>
<td>2 434 734</td>
<td>3 165 820</td>
</tr>
<tr>
<td>Apr 2011-Jan 2012</td>
<td>821 535</td>
<td>2 338 544</td>
<td>3 160 079</td>
</tr>
<tr>
<td>April 2010- Jan 2011</td>
<td>781 545</td>
<td>2 045 552</td>
<td>2 827 097</td>
</tr>
</tbody>
</table>

**CD4 targets test volumes: 2013/14 = 7 060 572 tests**

### HIV EID PCR

<table>
<thead>
<tr>
<th>Period</th>
<th>Positive</th>
<th>Negative</th>
<th>Other</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>FY: April 2011-Mar 2012</td>
<td>16,340</td>
<td>278,514</td>
<td>3,710</td>
<td>298,564</td>
</tr>
<tr>
<td>FY: April 2010-Mar 2011</td>
<td>21,358</td>
<td>265,733</td>
<td>2,770</td>
<td>289,861</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>37,698</td>
<td>544,247</td>
<td>6,480</td>
<td>581,945</td>
</tr>
</tbody>
</table>

**Projections for 2013/14 = 361 800 tests**

### VL

<table>
<thead>
<tr>
<th>Period</th>
<th>&lt;= 400</th>
<th>&gt; 400  &lt;= 50,000</th>
<th>&gt; 50,000  &lt;= 100,000</th>
<th>&gt; 100,000</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apr 2012-Jan 2013</td>
<td>1 165 605</td>
<td>288 327</td>
<td>44 042</td>
<td>121 266</td>
<td>1 619 240</td>
</tr>
<tr>
<td>Apr 2011-Jan 2012</td>
<td>885 761</td>
<td>257 312</td>
<td>36 338</td>
<td>103 630</td>
<td>1 283 041</td>
</tr>
</tbody>
</table>

**Projection for 2013/14 – 2.9 million tests. Again, significant scale-up is needed**

*NHLS will need to significantly scale up testing volumes*
Tiered laboratory approach – Modeling and place for POC?

- Two independent studies were conducted to develop mathematical models to identify/verify current gaps in service delivery.

  - The LTS study used population density and HIV/TB prevalence to establish the need for Point-of-Care placement. The study proposed 52 NHLS CD4 laboratories with 49 POC sites to provide national coverage with a four hour travel time (Google drive time used).

  - The Southampton model (John Smith) included test volumes and distance to nearest testing facility. Using different cut off times in hours from nearest testing facility, 4 different scenarios were proposed:
    - Total centralisation (4 hour), i.e. 15 centralised labs with no POC
    - 3-Tiered model (3 hours), i.e. 41 laboratories of different sizes + 2 POC sites
    - 4-Tiered model (2 hours), i.e. 61 laboratories of different sizes + 20 POC sites
    - Total decentralisation (1 hour): i.e. 127 laboratories of different sizes + 190 POC sites
Totally Centralised model (4hr)

Solution:
15 Labs
No POC
Decentralised model (1hr)

Solution:
127 Labs
190 POC
Summary of initial findings

- **Centralise**
- **Decentralise**

- **Current Running Costs**
  - Centralised
  - 3Tier
  - 4Tier
  - Decentralised

- **Causes:**
  - Poor sample integrity
SMS printers

- 2096 new SMS Printers including barcode scanners

- Project commencement February 2013 – December 2013. A district at a time with the district Managers and DoH ARV managers full involvement

- Project plan:
  - Phase 1 – Pilot (NHI District - NW). Kenneth Kaunda
  - Phase 2 – NHI Districts (10)
  - Phase 3 – ART/TB Initiating Clinics
  - Phase 4 – dashboard monitoring development

- To distribute SOP, paper request form, Facility load form and terms of reference, questionnaire
Plasma Preparation Tubes

- Transport of blood samples without loss of RNA integrity
- PPT centrifuged to separate cellular components from RNA.
- The mean difference between EDTA and PPT prepared samples (n = 261) was acceptable (log 0.04 copies/ml, percentage similarity CV 3.53%).
- PPT can be used for viral load testing on the CAP/CTM HIV-1 v2.0.
Drug resistance refers to a **reduction** in the **ability** of a particular **drug** or combination of drugs to **cure a disease** or **block replication** of pathogens.

The timing is right: Treatment guidelines need revision, ARV coverage is increased, and signs of possible increased transmitted drug resistance emerging!
Conclusions

• The purpose of global ART: effective long-term treatment for chronic patients (including paediatrics).
• VL testing becoming more important for detecting VL failure and a move towards routine VL testing.
• Level of access required for VL testing is most likely mixed model:
  • Centralised (super labs): easier to manage but requires good efficient logistics around specimen transport (ppt, DBS) and result reporting (sms printers)
  • Decentralized (POC): increase access, manage LTFU, consider: demand/ location/ throughput/ operator, must have connectivity
• Implementation:
  • Clinical & Lab Service mapping including volumes critical
  • Equipment standardisation for costing
  • Pre-analytical and post-analytical remain problematic for different reasons
  • Careful costing, modelling, monitoring and evaluation of impact needed
  • Connectivity for national reporting critical