HIV-1 Viral Load Testing and the use of Dried Blood Spot (DBS) in Resource-Limited Settings

Dr. Laura Trivino Duran (MedCo, MSF Malawi) 
June 2012
Key Facts for Providers and Patients

- VL is the best measure for the level of progression of HIV infection.
- VL = number of HIV copies in a milliliter (copies/mL) of plasma.
- More virus $\Rightarrow$ Faster destruction of CD4 cells $\Rightarrow$ More severe immunosuppression.
- VL is done using an advanced lab method (RNA-PCR) on a blood sample.
- VL are costly.
- VL can be done from:
  1. Blood (plasma): Transport in cooler box to lab within 24 hours.
  2. Dried blood spot (DBS): Transport in plastic bag with desiccant at ambient temperature, sample viable for 3 months or more.
MALAWI RECOMENDATIONS (July 2011)

**ROUTINE VL**
- Patients harbouring drug-resistant HIV when starting ART will be found with a VL after 6 months on ART > Important early sign for **poor adherence**.
- After that, patients who are adherent and clinically well have a low risk of ART failure. Therefore, routine follow-up VLs are done at 2 years, 4 years, 6 years, etc. after ART initiation.

**TARGET VL**
- Do additional VLs outside of this schedule for patients with suspected ART failure

VL > 5000 copies x ml switch to 2nd line treatment
THYOLO - Malawi

- MSF in Thyolo since 1997
- Thyolo HIV prevalence = 14% (N= 600,000).
- 34,730 people had ever initiated on ARVs by March, 2012.
- 27,446 people are alive and on ART
- <1% 2nd line ART

CAN WE USE VL IN A RURAL DISTRICT?

- VL laboratory in Thyolo as a pilot to validate the use of DBS finger prick compared to plasma and DBS from EDTA tubes
Choosing the Platform
NucliSSENS EasyQ HIV-1 v2.0 Assay

- Use of DBS from EDTA venous blood validated and good correlation with plasma
- Cost = 27.7 euros
- Technical support from bioMerieux South Africa
- Small size of the equipment
- Technically simple to operate
- Fast, results available in a few hours
- Low chances of cross-contamination
- Maintenance: daily / weekly/monthly → simple
Infrastructure

- 2 separate rooms (extraction/amplification)
- AC to maintain temperature 18-25°C
- Sink with running water

DETAILS:
- Sealed windows, protective film
- Separate benches for easyMAG and EasyQ analyzer from other "vibrating" equipment (centrifuge, vortex, etc)
- Common area: Refrigerator and freezer for reagents (2 if samples to be stored) – good quality!!!
Amplification/Detection Room
Equipment capacity
Number of tests

- easyMAG = 24 samples
- EasyQ = 48 samples
- Feasible → 72-96 tests/day = 1440 (DBS) - 1920 (plasma) tests/month

Considerations:
- Controls = 1 Positive and 1 Negative control / run → 22 samples / extraction
- 2 easyMAG + 1 EasyQ (?)
Human Resources

- HR needs:
  - 2 lab techs full time → 1 in each room
  - 1 lab manager
  - (72/96 tests/day = 1440/1920 tests/mth)
- bioMerieux training = 3 days → need a molecular biologist to ensure transfer of knowledge to untrained lab staff
- bioMerieux technical support
HIV VL in Resource-Limited Settings

Challenges > Lessons Learned
MSF Operational Research

Validation of VL Testing Using DBS Samples Collected from Finger Prick

- Time frame:

MSF study started end of April 2011 - April 2012
Study design:

265 HIV-1 infected patients

EDTA whole blood

- 50 μL spotted on 5 Whatman circles with pipette
- Centrifugation within 3 hours
- Stored at room temperature

EDTA venous blood DBS

Finger-prick

- 50 μL spotted on 5 Whatman circles with Microsafe
- Stored at -20° C
- Stored at room temperature

Finger-prick DBS

Plasma

- Stored at -20° C

Plasma viral load

V vs. Finger-prick DBS viral load

All patients were asked to provide written informed consent
## Population

<table>
<thead>
<tr>
<th>n = 265 with both FP + Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender (n = 265)</strong></td>
</tr>
<tr>
<td><strong>Mean Age (n = 265)</strong></td>
</tr>
<tr>
<td><strong>Months on ART</strong></td>
</tr>
<tr>
<td><strong>Routine monitoring vs. Treatment failure</strong></td>
</tr>
<tr>
<td><strong>Viral Load range</strong></td>
</tr>
</tbody>
</table>

MSF-OCB NucliSENS VL Testing  
Thyolo – Malawi - 2012
Sample collection Using Finger Prick

Our fears

- RNAse contamination of samples (fingers, etc) → powder-free gloves, avoid touching circles with patient's finger
- Quantitative test: use of exact sample volume → 50µl/circle (Microsafe pipette)?
- Squeezing/milking the finger → anti-inflammatory cytokines, ratio plasma/cells
- Amount of virus in capillary X venous blood
- EDTA X non-anti-coagulated blood
Results

Positive Predictive Value (PPV) using 5,000 copies/ml as the cutpoint

Results:

<table>
<thead>
<tr>
<th>Proportion of plasma samples above cutpoint</th>
<th>PPV-FP</th>
<th>PPV-VB</th>
</tr>
</thead>
<tbody>
<tr>
<td>5%</td>
<td>90%</td>
<td>100%</td>
</tr>
<tr>
<td>10%</td>
<td>95%</td>
<td>100%</td>
</tr>
<tr>
<td>15%</td>
<td>97%</td>
<td>100%</td>
</tr>
<tr>
<td>20%</td>
<td>98%</td>
<td>100%</td>
</tr>
<tr>
<td>25%</td>
<td>98%</td>
<td>100%</td>
</tr>
</tbody>
</table>
Positive Predictive Value (PPV) using 1,000 copies/ml as the cutpoint

Proportion of plasma samples above cutpoint

PPV-FP

PPV-VB

<table>
<thead>
<tr>
<th>Proportion</th>
<th>PPV-FP</th>
<th>PPV-VB</th>
</tr>
</thead>
<tbody>
<tr>
<td>10%</td>
<td>76%</td>
<td>76%</td>
</tr>
<tr>
<td>15%</td>
<td>83%</td>
<td>83%</td>
</tr>
<tr>
<td>20%</td>
<td>88%</td>
<td>88%</td>
</tr>
<tr>
<td>25%</td>
<td>90%</td>
<td>90%</td>
</tr>
<tr>
<td>30%</td>
<td>92%</td>
<td>92%</td>
</tr>
</tbody>
</table>
Negative predictive value (NPV) of DBS using 5,000 copies/ml as the cutpoint

Proportion of plasma samples above cutpoint

NPV-FP

NPV-WB
Negative predictive value using 1,000 copies/ml as the cutpoint

NPV-FP
NPV-WB

Proportion of plasma samples above cutpoint

0% 10% 15% 20% 25% 30%

99% 99% 98% 97% 97% 96% 97%
Conclusion:

- Viral load measured using **fingerprick DBS** and viral load measured using **EDTA DBS** had comparable levels of agreement with plasma viral load results, demonstrating that, for measuring viral load, **fingerprick DBS** performs **as well as** **EDTA DBS** as an alternative to plasma.
Recommendations:

- The choice of sample type should be based on **practical considerations** and the prevalence of an elevated viral load specific to the setting.

- Task shifting for FP needs to be validated before general roll-out (Phase 2).

**Research Q:**
"*Can FP DBS and EDTA DBS be task shifted to other cadre at Health Center level and result's correlation be equally optimal?*

- Use of 5000 copies x ml threshold for **switching** vs. 1000 copies x ml threshold for **enhance adherence** sessions for all types of collection sample system.
Algorithm: To be used for whole blood, EDTA DBS or fingerprick DBS

Viral load to be taken:

1. **Routine VL**: At Mth 6, 24, 48 then 2 yearly
2. **Target VL**: For confirmation on clinical or immunological failure at any time point
3. **For patients more than 6 months on D4T switching to TDF**

---

**VL <1000 copies x ml**
- Groups 1 and 2 continue first line regimen
- Group 3 switch to TDF

**VL >1000 copies x ml**
- All groups start enhanced adherence
- Repeat viral load with same method 3 **months** after first viral load

**VL <1000 copies x ml**
- Groups 1 and 2 continue first line regimen
- Group 3 switch to TDF

**VL >1000 ≤ 5000 copies x ml**
- Groups 1 and 2 continue first line regimen
- Group 3 switch to AZT and repeat
- For all repeat VL after **6 months**

**VL > 5000 copies x ml**
- All groups consider switch to second line
- If > 0.5 log drop repeat VL after 3 months
THANKS A LOT

Malawi MSF Team