Cell Pellet Training

Laboratory Break-Out Session
MTN Regional Meeting
Cape Town, October 2010
DNA PCR for Infant Diagnosis

Rapid tests and Western blots cannot be used to test infants for HIV

- DNA PCR used for HIV testing in infants under 18 months of age
- Antibody based tests look for the body’s reaction to the virus
- Infants still have their mother’s antibodies
- All HIV-exposed infants will be “positive” on antibody tests.
# RNA vs DNA PCR

## RNA PCR (“Viral Load”)
- Quantitative
  - Copies/ml result
- Detects HIV-1 RNA from plasma virus
- Viral load can fluctuate from undetectable to high numbers

## DNA PCR
- Qualitative
  - pos/neg result
- Detects HIV-1 DNA in peripheral blood cells
- Infected cells will always give a positive result, even when viral load is undetectable
When should I do DNA PCR?

- **MTN-003 or MTN-009**
  - When NL requests it for ambiguous HIV status
  - Not part of the algorithm
  - Not a routine test in these protocols; Special cases only

- **MTN-015**
  - Not expected; only if NL requests it for participant safety

- **MTN-016**
  - For infant HIV diagnosis – part of algorithm
Algorithm for Infant HIV Testing in MTN-016

START
Sample 1 DNA PCR (cell pellet)
Negative
Positive
Sample 1 RNA PCR (plasma)
<400 copies
≥400 copies
Sample 2 RNA PCR
Confirmatory and resistance testing
<400 copies
≥400 copies
Infant is HIV infected. Schedule for counseling
Notify MTN NL
Notify MTN NL
STOP
If baby has continued exposure, redraw at a later date

MTN
Assays Available

- Roche
  - AMPLICOR HIV-1 DNA Test, v1.5 (MWP or COBAS®)*
  - COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test*,#
- Abbott \( m2000 \) HIV-1 DNA Qualitative#
- In-house

*For cell pellet
#For DBS and plasma
Sample Collection

- Blood should be collected in either Vacutainer Blood Collection Tubes, using EDTA (lavender-top) or ACD (yellow top) or equivalent tubes as the anticoagulant.
- Heel stick (infants) or venipuncture can be used.
- Samples anticoagulated with heparin are unsuitable for this test.
## Blood volumes required

<table>
<thead>
<tr>
<th>Participant</th>
<th>Total Blood Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant</td>
<td>EDTA tube 1.5 – 2 ml</td>
</tr>
<tr>
<td>Adult</td>
<td>Typical for the visit</td>
</tr>
</tbody>
</table>

E.g, EDTA microtainer tube for infants
Sample Storage

- Whole blood should be stored at 2-25°C; Do not freeze.

FOR BEST RESULTS IN DNA ASSAYS, USE FRESH BLOOD OR BLOOD STORED FOR ≤ 3 DAYS.
Overall Steps

- **PROTOCOL A:** Plasma processing
- **PROTOCOL B:** Cell pellet processing
Sidebar: What is blood made of?

- **Plasma** - 55% of Total Blood Volume
  - 91% Water
  - 7% Blood Proteins (fibrinogen, albumin, globulin)
  - 2% Nutrients (amino acids, sugars, lipids)
  - Hormones (erythropoietin, insulin, etc.)
  - Electrolytes (sodium, potassium, calcium, etc.)

- **Cellular Components** - 45% of Total Blood Volume
  - **Buffy Coat**
    - White Blood Cells (7000-9000 per mm³ of blood)
    - Platelets (250,000 per mm³ of blood)
  - **Red Blood Cells (RBCs)**
    - About 5,000,000 per mm³ of blood
Reminder: Use Universal Precaution

Work in a biosafety cabinet
Protocol A: Plasma Processing

1. Plasma
2. WBCs
3. RBCs
4. PBS
5. WBCs
6. RBCs
Protocol A: Plasma Processing

1. Mark level of whole blood with indelible ink on the outside of tube
2. Spin whole blood at 800-1600 x g for 20 min at room temp to separate plasma
3. Store a min of 0.5 ml/cryovial at -70°C
4. Bring specimen back up to original marked volume using PBS
5. Invert tube 10-15 times to mix
What is happening?

- Red blood cells lysed
- White blood cells pelleted
- Supernatant removed
- Pellet frozen
Materials Needed

- Transfer pipette
- Safety glasses
- Gauze pads
- 2 ml screw-cap tube
- gloves
- Biosafety cabinet
- Aerosol filter tips
- Pipette
- microcentrifuge
Reagent Needed: Cell Lysis Buffer

- Can use:
  - **BLD-WS**: Specimen Wash Solution From Roche Amplicor HIV-1 DNA Test v1.5 Kit
  - **RBC Lysis Buffer**: Available separately; Roche Cat 1 814 389 001

- How does it work?
  - Lysis buffer contains Ammonium chloride (NH₄Cl) or sodium azide which effectively lyses non-nucleated cells (red blood cells), but not cells with nuclei (white blood cells)
Procedure

1. For each sample that will be processed, add **1.0 ml cell lysis buffer**\(^*\) to a 2-ml screw-cap tubes

\[\text{More than 1 pellet can be made from each specimen if volume is adequate (500 } \mu\text{l whole blood per pellet.)}\]

\(^*\)Buffer BLD WS in Roche kit
Procedure

3. Invert tube of whole blood 10-15 times to mix thoroughly

4. Remove cap from tube with a gauze pad to avoid aerosol contamination

5. Pipette 500 μl of whole blood into the tube containing lysis buffer using a pipette with an aerosol barrier tip
Procedure

6. Incubate for 5 min at room temperature
   - Invert tubes 10-15 times to mix thoroughly
   - Incubate 5 additional min at room temperature

7. Microcentrifuge the tubes for 3 min at max speed
Procedure

8. Using a fine-tip transfer pipette, aspirate the supernatant, being careful to avoid disturbing the pellet

9. Add 1.0 ml lysis buffer to each tube, recap and vortex to re-suspend the pellet

10. Microcentrifuge the tubes for 3 min at max speed
Procedure

11. Aspirate the supernatant being careful to avoid disturbing the pellet

12. The dry pellet may be extracted immediately or stored at -70°C until extraction.

Next step: Use pellet for DNA PCR assay.
What is in the cell pellet?

White blood cells are pelleted from whole blood, and DNA from the cell is extracted to check for the presence of HIV using a DNA PCR kit.
If you will be doing HIV-1 DNA Testing:

- You must sign up for proficiency testing, similar to viral load
- VQA will send you whole blood every other month
- You must process the whole blood into cell pellets, and test using your DNA PCR assay
- Requires pre-qualification
- Contact Urvi or Ted (NL) or Cheryl Jennings (VQA) for further info
Final Note: LDMS

<table>
<thead>
<tr>
<th>Test</th>
<th>Primary</th>
<th>Additive</th>
<th>Derivative</th>
<th>Sub Add/Derv</th>
<th>Primary Volume</th>
<th>Aliquot Volume</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Blood for HIV DNA and RNA PCR testing</td>
<td>BLD</td>
<td>EDT</td>
<td>PL1/2</td>
<td>N/A</td>
<td>1 ml</td>
<td>Minimum of 0.5</td>
<td>ML</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EDT</td>
<td>CEL</td>
<td>PER</td>
<td>1 ml</td>
<td>5 x 10^6</td>
<td>CEL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EDT</td>
<td>DBS</td>
<td>N/A</td>
<td>1 ml</td>
<td>100</td>
<td>UL</td>
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</table>

Don’t forget to log your specimens into LDMS
QUESTIONS?