



# MTN 009 Laboratory Logistics



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# Testing Menu

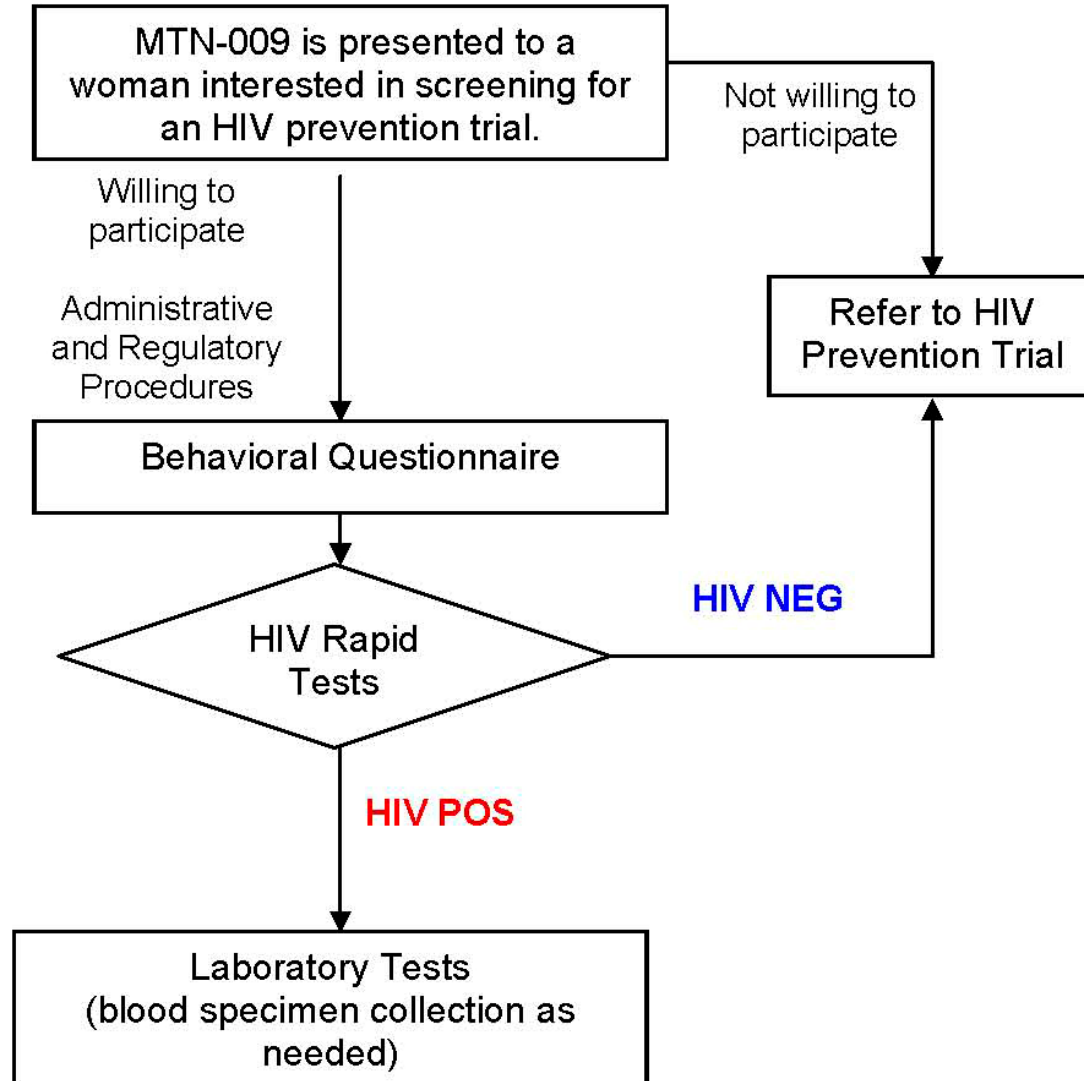
- Finger stick Blood Samples
    - HIV Rapid Tests\*
  - Venous Blood Samples
    - HIV Western Blot\*
    - CD4 Positive T Cell Count\*
    - HIV RNA Viral Load
    - HIV Resistance Testing
    - Test for Recent Infection
- \*Done locally in Durban*



# Testing Menu

- Urine Samples-None
- Pelvic Samples-None

### Summary Schema for MTN-009 Enrollment in the Context of Screening for HIV Prevention Trials





# Fingerprick

**Fingerprick blood: Fingerprick blood is collected in a SCHARP labelled 2ml Microtainer which contains EDTA,**

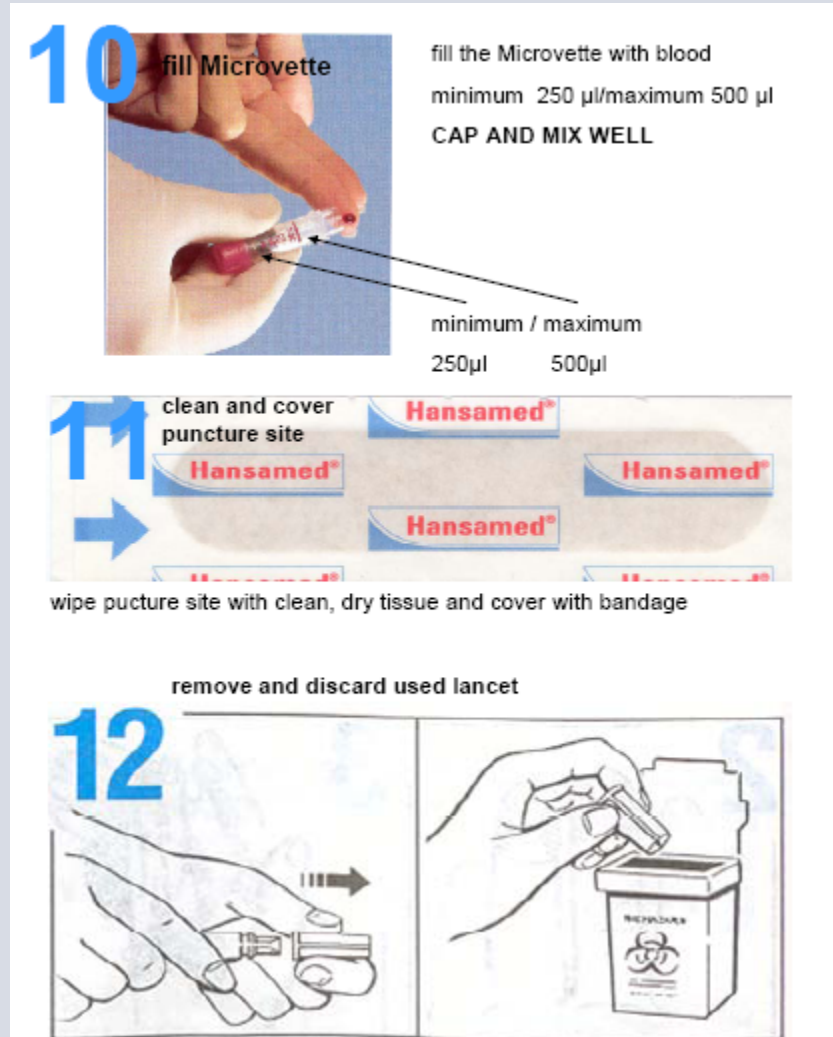
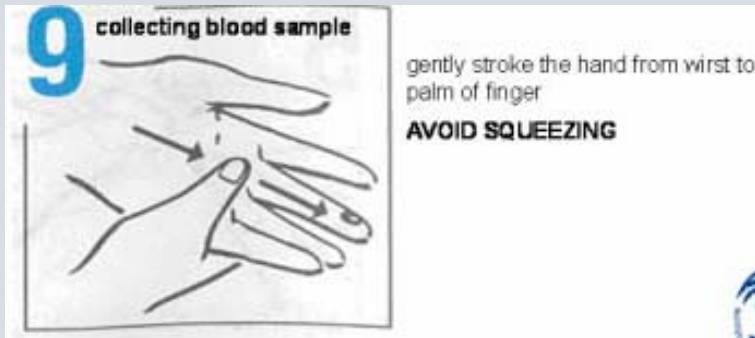
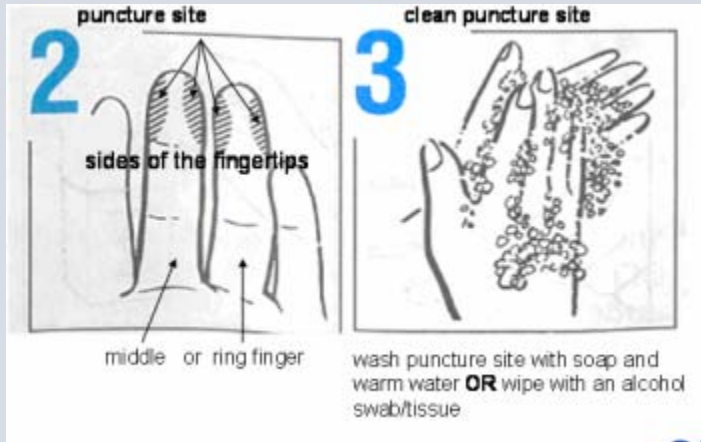
- It is very important for the validity of the HIV rapid test, that at least 250  $\mu$ l of fingerprick blood is collected and very well mixed.

- The blood will be collected as follows:**

the puncture must be on the palmar surface of the distal phalanx . The middle finger and ring finger are preferred. Holding the hand into warm water (+- 37°C) for a few minutes before puncturing the finger will stimulate the bleeding. If no warm water is available the finger should be first massaged very well. The skin-puncture site must be cleansed with a 70% aqueous solution of isopropanol (70% v/v). The skin is punctured with the use of a disposable lancet device, and is performed according to the operational instructions provided by the manufacturer. After the chosen site has been punctured, the first drop of blood should be wiped away with a gauze pad. A second drop will form over the puncture site. Hold the finger with the nail side upward, this will avoid that the blood runs under the nail. When the tip of the 'Microtainer' touches this drop, blood will flow into the tube. Collecting the blood is easier if you rub with the finger on the tip of the microtainer. Gently massage the patient finger to establish the flow of blood. Do NOT squeeze the patients' finger. Blood flow from the puncture is enhanced by holding the puncture site downward and gently applying intermittent pressure proximal to the puncture site. After collecting a minimum of 250  $\mu$ l of blood, the collection device is immediately capped and the blood is mixed well. **PLEASE SEND TO ON SITE LAB FOR TESTING!**



# Fingerprick





# HIV Rapid Testing

- A validation of finger stick HIV testing procedures will be done before study activation.
- This is to be compliant with DAIDS guidelines and to ensure that results from finger stick specimens will be comparable to venous samples.



## HIV Rapid Testing-Cont.

- **Validation Scheme:**
  - **Population will be 20 HIV positive Individuals and 20 HIV negative individuals.**
  - **All 40 people will be given fingerstick HIV rapid tests.**
  - **For people with documented HIV status, no further testing required.**
  - **For others, a venous sample will be drawn for confirmatory testing. [1 X 4ml EDTA]**





## HIV Rapid Testing-Cont.

- **Validation Scheme:**
  - **The Finger stick results will be compared to known results or confirmatory test results.**
  - **If any results are discrepant, they will be investigated.**



## HIV Rapid Testing-Cont.

- The validation will also provide an opportunity to:
  - Train and asses competency of the staff
  - Set up work areas to accommodate Finger Stick HIV testing
  - Develop work flows and identify trouble spots



# Lab Workflow

- *No lab tests* during screening
- Lab tests commence after enrollment and administration of behavioral questionnaire.



## Lab Workflow-cont.

- **Step 1.** Two rapid HIV tests: Determine and FDA approved Unigold (using blood from fingerstick)
  - If both are negative, no further lab testing.
  - If either rapid test or both are positive proceed to step 2.





## Lab Workflow-cont.

- **Step 2.** Draw venous two 10 ml and plus one 2 ml EDTA tubes.
- **Step 3.** 2 ml tube is sent to BARC for CD4 T-Cell count for px that are pos or discordant  
Discordants: HIV Western Blot is sent to HPRU Central Routine Lab.
- **Step 4.** Separate and store all plasma from the two 10 ML EDTA tubes.

# MTN 009 LAB WORKFLOW

## ENROLLMENT

### SPECIMENS AND LABORATORY PROCEDURES

BLOOD

2 X RAPID HIV TESTS FINGER PRICK  
1 X 2ML EDTA MICROTAINER

NEG/NEG

POS/POS  
OR POS/NEG

POS/NEG

REFER TO  
HIV PREVENTION  
TRIAL AT CLINIC

CD4 COUNT  
OUTSOURCED LAB  
1 X 2ML EDTA  
VACUTAINER

VIRAL LOAD/  
GENOTYPE  
RESISTANCE/BED  
ON SITE/LDMS  
2 X 10ML EDTA

USE SAME 1 X 2ML EDTA  
SEND TO  
HPRU CENTRAL  
ROUTINE LAB  
FOR WESTERN BLOT

POS

NEG

INDETERMINATE

STORAGE

HIV VIRAL LOAD/GENOTYPE  
RESISTANCE & BED SAMPLE  
PREPARATION

ON SITE

PLASMA  
STORAGE

2 x 10ml EDTA  
Aliquot 10 x 1 ml ,  
Ship to LDMS who  
will store and ship  
to Network Core  
Virology Lab for  
HIV RNA,  
Genotype  
Resistance and  
BED testing

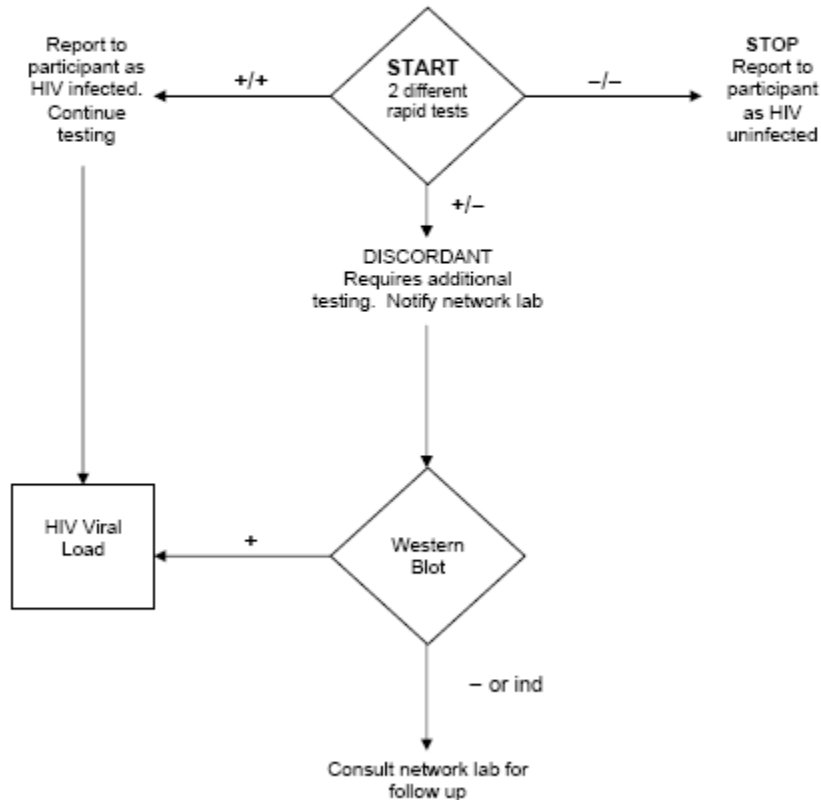
OUTCOME OF RESULTS  
NL TO ADVISE

P.S PROCESS WITHIN 6  
HOURS OF COLLECTION!!!



# HIV ALGORITHM

## APPENDIX II: MTN-009 Algorithm for Determination of HIV Infection





## Testing done locally

- HIV Western Blot done at HPRU Central Routine Lab
- CD4 Count will be performed at BARC Durban





# Testing at Network Lab

- The following tests will be done at Network Lab
  - **Viral load**
  - **Standard Resistance Testing**
  - **Sensitive Resistance Testing**
  - **Test for Recency of Infection**



# Specimens for Shipment

- All done from EDTA plasma
- Stored at MRC within 8 hours of collection
- Stored in LDMS
- Shipments will be coordinated with MTN NL



## Assays Used at Network Lab

Test	Assay Used	Results Sent?
<b>Viral Load</b>	Abbott M2000	YES
<b>Standard Resistance Test</b>	Celera ViroSeq	YES
<b>Sensitive Resistance Test</b>	In house method (Allele-Specific Real-time PCR)	NO – for research only
<b>BED</b>	Sedia BED HIV-1 Incidence EIA	NO – for research only



# Viral Load and Resistance Tests

- **Viral load**
  - **Linear Range: 40 copies/ml – 10,000,000 copies/ml**
  - **Detects Subtypes A-H, Group O, Group N**
- **Standard Resistance Test**
  - **Sequence based**
  - **Reports all major mutations in the protease and reverse transcriptase genes in HIV**
  - **Detection limit: 25%**
- **Sensitive Resistance Test**
  - **Real time PCR based**
  - **Gives % of an individual mutation**
  - **Detection limit: 0.1%**

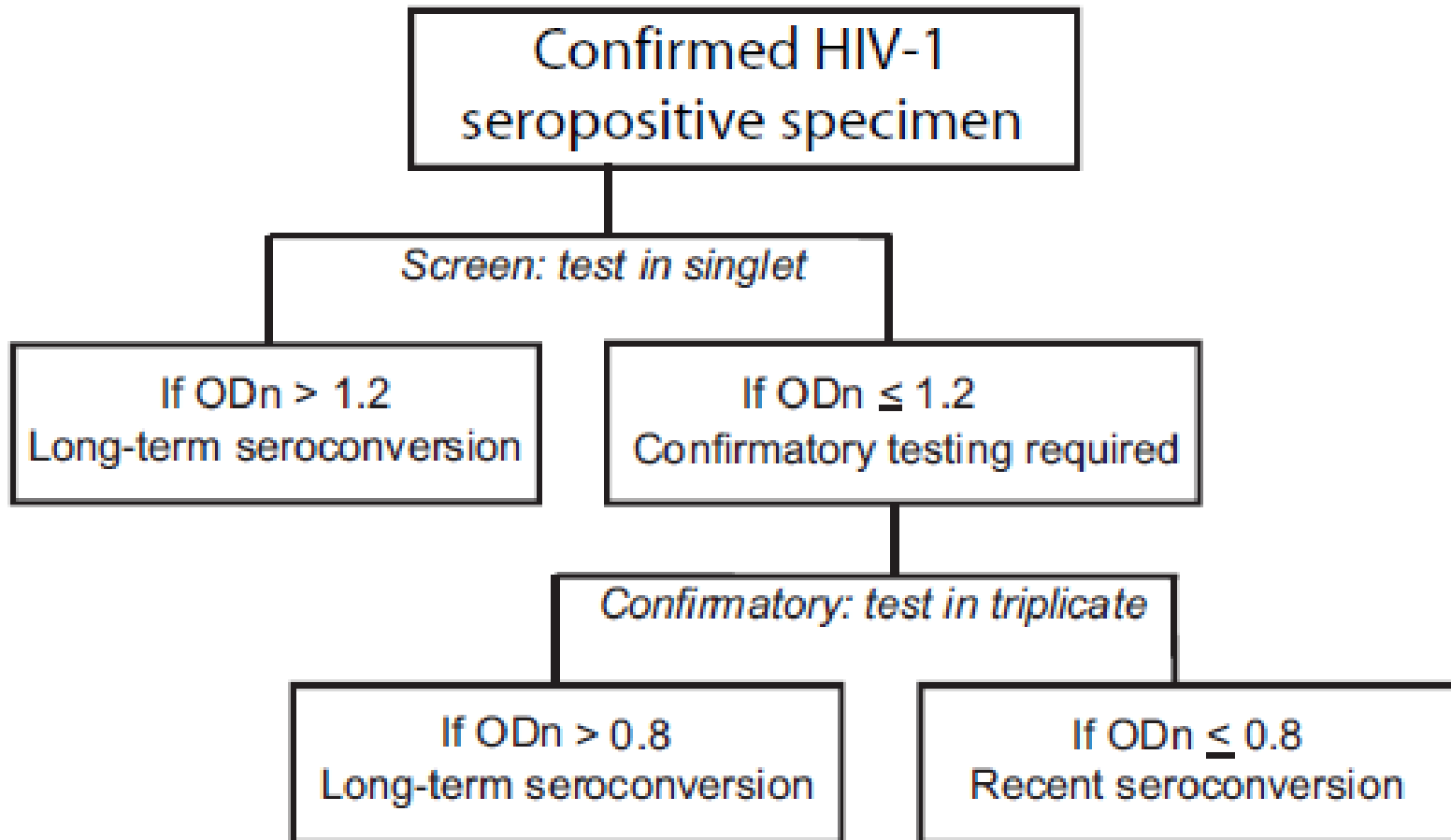


## Test for recency of infection (BED)

- In vitro quantitative enzyme immunoassay (EIA)
- Distinguishes recent HIV-1 infections from those that are long term by measuring HIV-1 specific IgG to total IgG
  - **IgG is a kind of antibody**
- Research use only for surveillance studies
- Cannot be used for diagnosis or determining clinical outcome or treatment



# BED Test Algorithm





## Results Dissemination

- Results from resistance testing and Viral load will be sent back to MRC when complete from NL
- Results from sensitive resistance testing and BED recency assay are for research only and will not be sent back to MRC
- VL=2 DAYS 96/BATCH
- RT=6-8 WEEKS 10/BATCH=3MONTHS



# Questions?