

MTN 015 SPECIMEN REVIEW

Gabriel Banda

Core Lab Supervisor

UNC Project, Lilongwe

Review of MTN 015 Study

- **MTN 015 is a multi-site observational cohort study whose study population are women who sero-converted during the HPTN 035 Microbicide Study. There are no investigational products.**
- **Primary Objective-**
To compare the plasma HIV-1 RNA level twelve months after HIV-1 sero-conversion among ART naïve participants assigned to an active microbicidal agent as compared to the control participants.

MTN 015 Specimen Collection

- Specimen collection from study participants is usually necessary to achieve the objectives of the study.
- All specimen collection, handling, testing, storage and shipment is conducted adhering to GCLP, MTN 015 SSP Manual, HPTN-MTN Lab Manual and the site SOPs.
- Specimen containers must be labeled with SCHARP provided participant ID labels. Specimen collection dates are handwritten in indelible ink.

Specimen Collection

- All specimens collected from MTN 015 participants in the clinic are documented on the site Lab Request Form.
- Specimens requiring storage are documented on the LDMS Tracking Sheet.
- Proper chain of custody is followed from sample collection, transportation, delivery, processing, testing, result reporting and sample storage.

UNC Project Lab Request Form

UNC
P.O. Box 7590
Chapel Hill, NC 27599-7590

UNC Project Lab Request Form
Form 1000, 12/15/99, 1000-1 (7/7/00)
Fax: 919/759-2531

LABORATORY REQUEST FORM No 148038

REQUESTING CLINICIAN _____

STUDY _____ PID _____ VISIT CODE _____

GENDER MALE FEMALE DOB _____ AGE _____

HEMATOLOGY <input type="checkbox"/> CBC/DIR <input type="checkbox"/> Hemoglobin <input type="checkbox"/> Manual Differential	CHEMISTRY <input type="checkbox"/> Alanine Aminotransferase (ALT) <input type="checkbox"/> Albumin <input type="checkbox"/> Alkaline Phosphatase <input type="checkbox"/> Amylase <input type="checkbox"/> Aspartate Transaminase (AST) <input type="checkbox"/> Bilirubin (Direct) <input type="checkbox"/> Bilirubin (Total) <input type="checkbox"/> Blood Urea Nitrogen (BUN) <input type="checkbox"/> Calcium <input type="checkbox"/> Carbon Dioxide <input type="checkbox"/> Cholesterol <input type="checkbox"/> Cholesterol (Total) <input type="checkbox"/> Creatinine Kinase <input type="checkbox"/> Creatinine <input type="checkbox"/> Glucose <input type="checkbox"/> Glutaryl Transferrase <input type="checkbox"/> HDL Cholesterol <input type="checkbox"/> Lactate <input type="checkbox"/> Lactate Dehydrogenase <input type="checkbox"/> Lipase <input type="checkbox"/> LIL Cholesterol <input type="checkbox"/> Phosphorus <input type="checkbox"/> Potassium <input type="checkbox"/> Sodium <input type="checkbox"/> Total Protein <input type="checkbox"/> Triglycerides <input type="checkbox"/> Urea Nitrogen (BUN) <input type="checkbox"/> Protein (CBF) <input type="checkbox"/> Other: _____	MICROBIOLOGY <input type="checkbox"/> APB Smear <input type="checkbox"/> Blood Culture (Bac/Ana) <input type="checkbox"/> Blood Culture (FB) <input type="checkbox"/> CBF Culture <input type="checkbox"/> CBF Cell Count <input type="checkbox"/> Gram Stain <input type="checkbox"/> Intra IFA <input type="checkbox"/> InPouch TV Test <input type="checkbox"/> Malaria Parasites (ASAP) <input type="checkbox"/> OIE Malaria Smear <input type="checkbox"/> Stool Culture <input type="checkbox"/> Stool Microscopy <input type="checkbox"/> Trichome Stain <input type="checkbox"/> Urine Culture <input type="checkbox"/> Urine Microscopy <input type="checkbox"/> Wet Mount <input type="checkbox"/> Culture (Other) _____	IMMUNOLOGY <input type="checkbox"/> CD4/CD8 (Including Percentages) <input type="checkbox"/> CD4/CD8/CD3 FACSCOUNT $\times 10^6$ <input type="checkbox"/> CD4/8 FACSCOUNT ACTG Method <input type="checkbox"/> CD4/8 FACSCOUNT IPTM Method <input type="checkbox"/> Cryptococcal Antigen <input type="checkbox"/> HIV (ELISA) <input type="checkbox"/> HIV (Rapid Test) <input type="checkbox"/> HIV (Western Blot) <input type="checkbox"/> HSV ELISA <input type="checkbox"/> HepB Ab <input type="checkbox"/> HepB Ag <input type="checkbox"/> HepG <input type="checkbox"/> Syphilis RPR <input type="checkbox"/> Syphilis Rapid <input type="checkbox"/> Syphilis TPHA <input type="checkbox"/> Toxoplasma IgG <input type="checkbox"/> Other: _____	MOLECULAR <input type="checkbox"/> CMV PCR <input type="checkbox"/> CT/NG DNA <input type="checkbox"/> HIV DNA PCR <input type="checkbox"/> HIV RNA PCR (Standard) <input type="checkbox"/> HIV RNA PCR (UltraSensitivity) <input type="checkbox"/> Other: _____	STORAGE <input type="checkbox"/> Serum Storage <input type="checkbox"/> Plasma Storage <input type="checkbox"/> Stem Cells 300k Storage <input type="checkbox"/> Serum Storage <input type="checkbox"/> Tear-Flu Storage <input type="checkbox"/> Occlusal Urinal Swab Storage <input type="checkbox"/> PBMC Storage <input type="checkbox"/> ECV Storage <input type="checkbox"/> DBS Storage <input type="checkbox"/> Whole Blood Pallet Storage <input type="checkbox"/> Storage Storage <input type="checkbox"/> Pap Smear Storage <input type="checkbox"/> Other Storage: _____	OTHER <input type="checkbox"/> CDAG - APTT <input type="checkbox"/> CDAG - INR <input type="checkbox"/> CDAG - PT <input type="checkbox"/> Pregnancy Test (urine) <input type="checkbox"/> Pregnancy Test (serum) <input type="checkbox"/> Urine Dipstick <input type="checkbox"/> Other: _____
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COMMENTS: _____

LIS ACCESSION NUMBER _____

SPECIMEN COLLECTED BY: ID [] [] [] DATE / / Time

RECEIVING TECH: ID [] [] [] DATE / / Time

MTN 015 LDMS Tracking Sheet

MTN 015 Non-DataFax LDMS Specimen Tracking Sheet
For logs of MTN 015 stored specimens into LDMS

Participant ID: [] [] [] [] [] [] [] [] [] [] [] [] [] [] []
 Visit Code: [] [] [] [] []
 Specimen Collection Date: [] [] [] [] [] []
dM YYY

# of TUBES or SPECIMENS	PRIMARY SPECIMEN	PRIMARY ADDITIVE	ALBUQUIN DERIVATIVE	ALBUQUIN SUB ADDITIVE/DERIVATIVE	NOTES FOR LAB
<input type="checkbox"/>	Blood (BLD) Plasma	EDT <small>(purple vac)</small>	PL 12	N/A	For retrospective testing, allow 5x1.0 ml aliquots. For all other storage, store all specimens plasma in 0.5 ml aliquots (at least 6 aliquots). Contact the MTN 015, if there is insufficient plasma.
<input type="checkbox"/>	Blood (BLD) Serum	NON <small>(no additive) or SST</small>	SER	N/A	Package as many 0.5 ml aliquots as available to store. If less than 1.5 ml of serum is available, store that serum and inform the MTN 015.
<input type="checkbox"/>	Blood (BLD) PBMC	EDT <small>(purple vac)</small>	CEL	DMS	PBMC cell count for final cell suspension is entered on an excel file per vial. Enter collection date into LDMS.
<input type="checkbox"/>	Vaginal fluid (VAG)	PBS <small>(transport buffered saline)</small>	SWB	N/A	Place exsorb to separate crystals.
<input type="checkbox"/>	Cervicovaginal Leucage (CVL)	SAL <small>(Saline)</small>	CVL	N/A	Store as much fluid as is recovered. If less than 0.5 ml is recovered after processing, notify the MTN Network Laboratory.

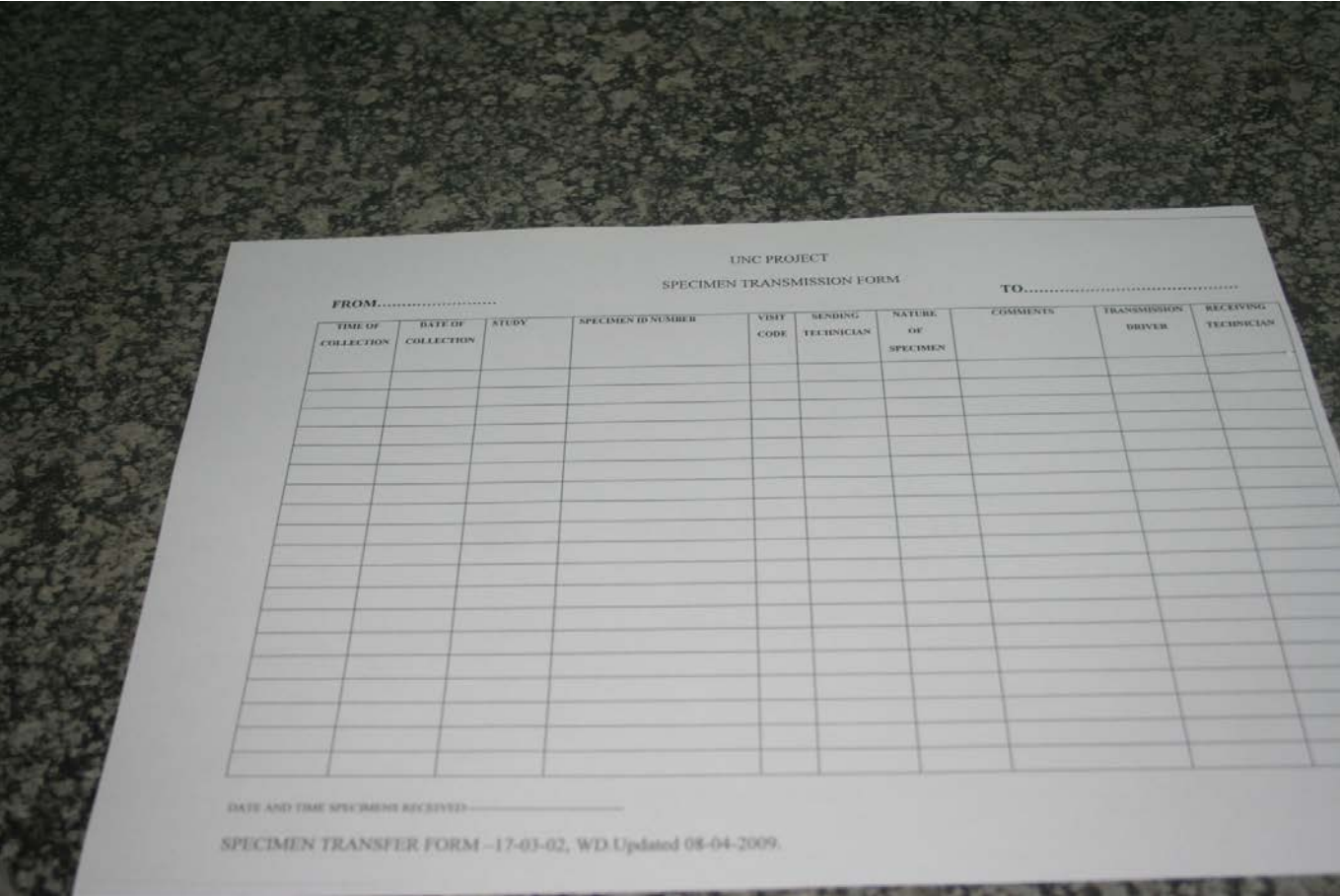
Comments: _____

Initialed: [] [] [] [] [] [] [] [] [] [] [] [] [] [] [] [] []
Working Staff Tracking Staff

LDMS Data Entry Date: [] [] [] [] [] [] [] []
dM YYY

Version 2.6, 14-OCT-08

Specimen Transmission Form



MTN 015 Specimens

- **Three Types of Specimens are collected from MTN 015 participants.**
- **Urine**
- **Blood**
- **Pelvic Samples**

Urine Samples

- Collection- 15-60mls is collected in a sterile plastic urine cup labeled with PTID label. Urine should not be mid-stream and participant should not have urinated 1hour prior to collection.
- Testing- pregnancy test and chlamydia and gonorrhea. At visits when both tests are required, 5-10ml of urine is aliquoted to a secondary container for pregnancy testing (in the clinic) the rest is sent to lab for GC/CT testing.
- Storage- for sites without a validated SDA test.

Blood Samples

- Collection-Different blood volumes are collected in EDTA or plain tubes depending on test/storage requirement at each visit.
- Testing-
 - EDTA blood – HIV RNA, CD4 T cell ct, CBC, PBMC processing.
 - Plain tube blood – Syphilis, liver (ALT, AST, ALP, T.Bili) and kidney (creatinine) function tests.

Blood Samples

- Storage-

Serum- for HIV-1 ELISA and WB confirmation at MTN NL.

Plasma – for genotypic resistant testing, PCR for drug resistant codons at NL and HSV2 at regional Lab.

PBMC- testing for markers of cell mediated immunity at the HVTN lab.

Pelvic Samples

- Collection- pelvic samples are collected per the site's clinic SOPs.
- Testing- vaginal pH testing (in clinic), wet mount testing (in lab), pap smears at selected sites.
- Storage
 - Vaginal swabs – for vaginal flora proteomics and markers of inflammation at NL.
 - Cervicalvaginal lavage- for HIV-1 viral load, HIV-1 genotypic resistant shedding and molecular analysis of vaginal flora.

Storage of MTN 015 Specimens

Plasma, serum, PBMCs, Vaginal Swabs, CVL and urine samples must be entered into the LDMS and labeled with the LDMS generated labels before storage.

Weekly, the MTN designated technician reconciles LDMS stored samples against the clinic MTN 015 sample collection log. The study coordinator and lab manager are notified of any discrepancies.

Shipment of Stored Samples

Samples are shipped to MTN NL when the site is notified.

All personnel handling the shipments must participate in IATA training every two years and must have IATA certification on file.

Test Methodology

<u>Test</u>	<u>Location</u>	<u>Kit/Method</u>
• Pregnancy	Clinic	QuidelQuickVue
• GC/CT	Site Lab	BD ProbeTec
• WB	Site Lab	FDA approved test
• HIV-1 RNA	Site Lab	Roche Amplicor
• HIV-1 Resistant	NL	Genotyp. Characterization
• CD4+ Cell	Site Lab	Not specified
• CBC	Site Lab	Not specified
• Liver function	Site Lab	Not specified
• PBMC	Site Lab	HANC CN SOP

Test Methodology

- **It's important to adhere to the test methodology approved by the MTN NL for the duration of the study; however if its necessary to switch to an alternate method or kit after study initiation, the site must inform the MTN NL and perform a validation study.**

EQA Testing

- CAP
Hematology, chemistry, hCG, syphilis, SDA GC/CT
- UKNEQAS
CD4+ T Cell Count
- VQA
HIV-1 RNA PCR
- DUKE IQA
PBMC
- MTN NL
Wet Mount

Challenges in MTN 015 Study

- **Clinic Problems**
- **Incorrect documentation on requisition forms- e.g. forget to mark Alk phos test.**
- **Insufficient blood collection.**
- **Missed sample collection e.g. vaginal swabs**
- **Mismatch of information on sample container against the Lab Request Form.**

Challenges in MTN 015 Study

- **Lab-**
- **Samples forgotten at 2 – 8C until following day.**
- **Samples lost in the lab-missed storage.**
- **Hemolyzed sample for PBMCs are not reported to the clinic immediately for recollection.**
- **Missed test requisition in LIS.**
- **Clerical errors when logging samples in LDMS/LIS.**

Resolutions to Challenges

- **Retraining of nurses in blood collection procedures and filing of Lab Request Forms.**
- **Checklist in reception area –used at end of shift.**
- **Hired a lab QC assistant to double check the LIS/LDMS entries.**
- **Weekly reconciliation of LDMS storage samples.**

Specimen collection in Clinic



Specimen Receipt in Lab



Test Requisition in LIS



Labeling Specimen with Accession



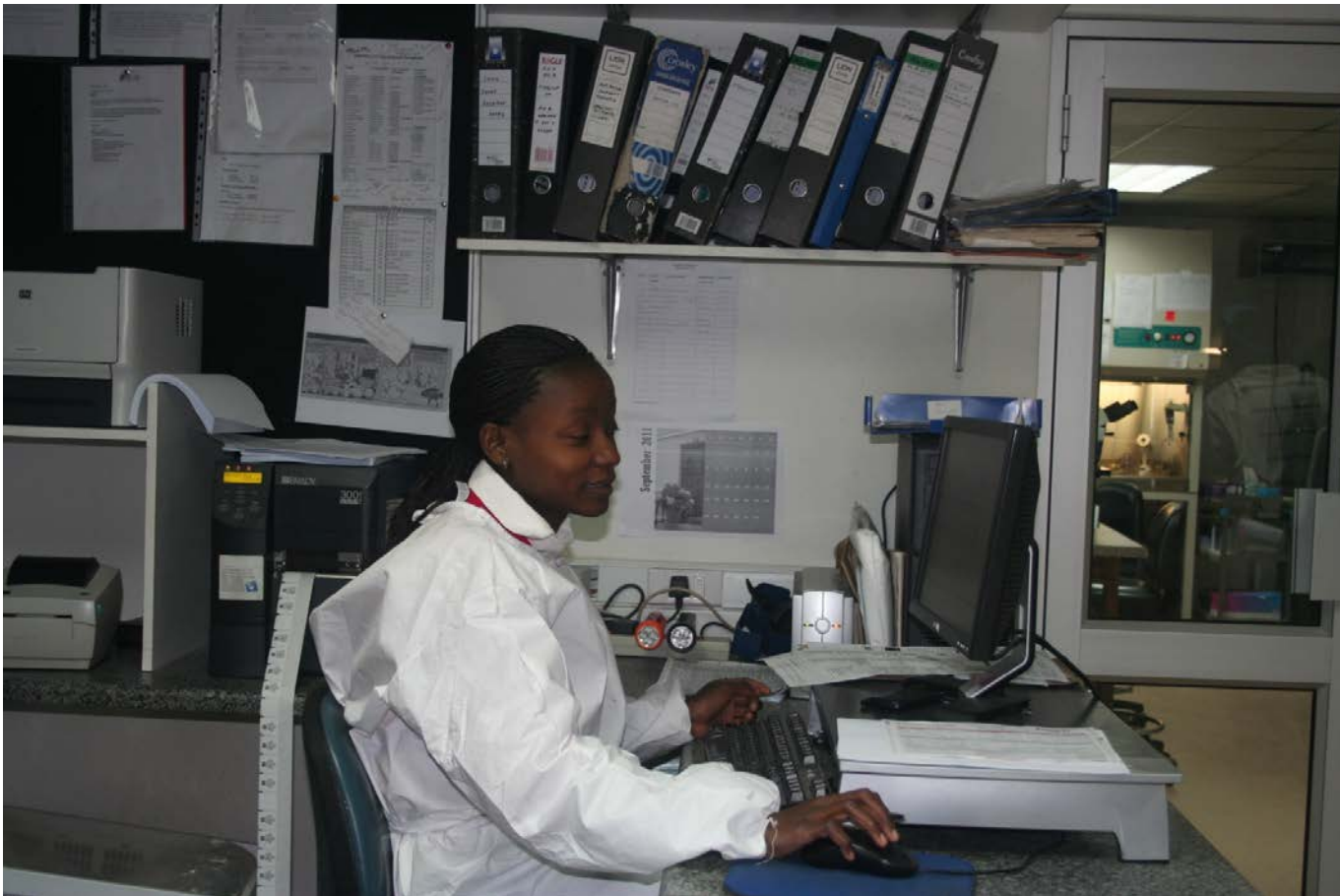
Processing of Specimens



Specimen Testing



Specimen entry into LDMS



Specimen Storage



▫ QUESTIONS???