

Section 12. Laboratory Considerations

12.1 Overview and General Guidance

This section contains information on the laboratory procedures performed in MTN 001.

As transmission of HIV and other infectious agents can occur through contact with contaminated needles, blood, blood products, and vaginal secretions, all study staff must take appropriate precautions when collecting and handling biological specimens. Sites must have appropriate written safety procedures in place before study initiation. Guidance on universal precautions available from the US Centers for Disease Control can be found at the following websites:

- http://www.cdc.gov/ncidod/dhqp/bp_universal_precautions.html

Some laboratory procedures will be performed in study site clinics or laboratories and others in the MTN Network Laboratory (NL) Johns Hopkins Laboratories or University of Washington. **Table 12-1 lists for each test the testing location, specimen type, specimen container and kit/method (if specified). Appendix 12-4 summarizes information for specimen requirements.**

Regardless of whether tests are performed in clinic or laboratory settings, study staff that performs the tests must be trained in associated QC procedures prior to performing the tests for study purposes; training documentation should be available for inspection at any time.

All site laboratories will be monitored by the MTN NL which will utilize information from DAIDS monitoring groups (PNL, IQA, VQA, etc...) to monitor and certify laboratories for testing. US Laboratories that are certified by CLIA (Clinical Laboratory Improvement Amendment) will be able to substitute this for some of the documentation requirements required of other labs. Valid CLIA certificates must be provided in these cases.

Only the U.S. sites will perform the Intensive PK sub study. Certain procedures in this section are limited to the intensive PK sub study and do not apply to the non-U.S. sites: Vaginal Biopsies and Cervical Cytobrushes. Some procedures for MTN 001 will be performed only at sites that have capacity: Flow Cytometry and PBMC for Intracellular tenofovir. Refer to section 7 of the protocol for details of laboratory testing for intensive PK and non-intensive PK sites.

**Table 12-1
Overview of Laboratory Testing Locations, Specimens,
and Methods for MTN 001**

Assay	Testing Location	Specimen Type	Tube/Container	Kit/Method
Urine pregnancy test	In clinic	Urine	Plastic screw top cup	Quidel Quick Vue
Urine SDA for gonorrhea and Chlamydia (neat method)	MTN Network Lab Regional or site lab	Urine	Plastic screw top Cup-Urine Preservative Tube for shipping	BD Probetec
Dipstick Urinalysis ¹	In Clinic	Urine	Plastic screw top cup	Bayer Multistix® 10 SG or Bayer Uristix 4
HIV antibody screen and Western Blot	Clinic/Local Lab	Plasma or whole blood (<i>serum acceptable</i>)	EDTA or plain tube	FDA approved tests ²
Complete blood count	Local Lab	Whole Blood	EDTA tube	Not specified
Chemistries (AST, ALT, Creatinine, Phosphorus)	Local Lab	Serum	Plain or serum separator	Not specified
Hepatitis B Surface Antigen	Local Lab	Serum	Plain or serum separator	Not specified
Flow Cytometry (CD38, CD3, CD4 and HLA-DR) ³	Local Lab	EDTA Whole Blood	EDTA Tube	Not Specified
Blood tenofovir level	Network Lab	Serum	Plain Tube	JHU method
Pap Smear	Local Lab	Ecto- and Endocervical cells	Slides	Not specified
Vaginal pH	In clinic	N/A	N/A	S/P pH Indicator Strips
Vaginal wet preparation	In clinic	Vaginal fluid swab	N/A	N/A
Syphilis Serology	Local Lab	Serum or Plasma	EDTA tube, plain or serum separator	Not specified
PBMC Isolation for Intracellular Tenofovir ³	Isolation and storage at local labs; testing at network lab	Whole Blood	CPT Tubes	JHU Protocol
Herpes Culture ⁴	Local Lab	Swab	Swab	Not Specified
Cervicovaginal Lavage (CVL) for vaginal flora proteomics, markers of inflammation and tenofovir level	Collected Locally, sent to network lab	Fluid recovered from CVL (saline used)	Conical Vial	Network Lab to provide SOP template
Cervical Cell lysate for intracellular tenofovir	Network Lab	Cervical Cytobrush	Screw top vial or test tube	JHU method
Vaginal Biopsy for tenofovir	Network Lab	Vaginal Biopsy	Cryovial	JHU method
Rectal Tenofovir Level ⁵	Network Lab	Rectal Sponge	5 ml cryovial	JHU Method

1 Perform Urine Culture as indicated if local standard of care. Dipstick tests are glucose, protein, leukocytes and nitrites.

2 If performing 2 rapid tests as part of the HIV algorithm, sites may use one non-FDA approved test with one FDA-approved test

3 To be performed only at sites with capacity

4 Only if local standard of care

5 Bronx Site only

**Table 12-2
Overview of Specimens for Storage and Shipment**

Specimen	Additive	Use LDMS?	Ship to:	Shipping schedule
PBMC for Intracellular Tenofovir ¹	CPT With Sodium Citrate	Yes	MTN Network Lab	Store at site until notified by MTN ²
CVL	Saline	Yes	MTN Network Lab	Store at site until notified by MTN ²
Plasma for storage	EDTA	Yes	MTN Network Lab	Store at site until notified by MTN ²
Serum for storage	None	Yes	MTN Network Lab	Store at site until notified by MTN
Urine for GC/CT testing (Only sites that do not have a validated Probetec)	BD Urine Preservation Tubes	No	MTN Network Lab or Regional Network lab	1-2 times per week, depending on volume
Cytology Brush ³	PBS	Yes	MTN Network Lab	Store at site until notified by MTN ²
Vaginal Biopsy ³	None	Yes	MTN Network Lab	Store at site until notified by MTN ²
Rectal Sponge ⁴	PBS	Yes	MTN Network Lab	Store at site until notified by MTN ²

1 To be performed only at sites with capacity

2 At the time of shipment, the MTN NL will furnish shipping instructions and addresses.

3 Intensive PK sub study sites only

4 Bronx Site only

Sites are responsible to ensure that specimen volumes collected do not exceed what is described in the informed consent process. The MTN NL may request details of collection containers and volumes for this purpose. These blood draws will vary by site. Ideally, one method, type of test kit, and/or combination of test kits will be used for each protocol specified test throughout the duration of the study. If for any reason a new or alternative method or kit must be used after study initiation, site laboratory staff must perform a validation study of the new method or test prior to changing methods. The MTN NL must be notified before the change (for non-CLIA certified labs) and can provide further guidance on validation requirements. Similarly, all labs (including CLIA certified labs) must contact the MTN NL in cases of changes to normal ranges.

Provided in the remainder of this section is information intended to standardize laboratory procedures across sites. Adherence to the specifications of this section is essential to ensure that data derived from laboratory testing will be considered acceptable to all regulatory authorities across study sites.

This section of the MTN 001 SSP manual gives basic guidance to the sites but is not an exhaustive procedure manual for all laboratory testing. This section must be supplemented with Standard Operating Procedures. SOP's will be provided by the MTN for local adaptation for PBMC for Intracellular Tenofovir and Cervicovaginal Lavage.

12.2 Specimen Labeling

All containers into which specimens are initially collected (e.g., urine collection cups, blood collection tubes) will be labeled with SCHARP-provided Participant ID (PTID) labels. Study sites will be provided with pre-printed labels or a template that can be used to generate labels. The date the specimens are collected should also be included on the label. If the date is handwritten, it should be in indelible ink (such as a Sharpie pen).

Microscope slides used for evaluation of vaginal/cervical fluids also will be labeled with SCHARP designed PTID labels. PTIDs are pre-printed on these labels; however study staff must write the specimen collection date on each label. The visit code also may be written on the label.

When specimens are tested at the local lab, any additional labeling required for on-site specimen management and chain of custody will be performed in accordance with site SOPs. The following specimens will be entered into LDMS and labeled with LDMS-generated labels: stored plasma specimens, stored serum specimens, PMBC, and cervical lavage specimens.

Specimen Labeling for PK specimens

In addition to standard specimen labels, there will be special labels for PK specimens. These will be for visit codes 4.0, 7.0 and 10.0. There will be specific labels for each time point to be drawn. On each PK specimen label, write in the specimen collection time and the date.

Non Intensive PK Sites Only (Uganda and South Africa):

Use the following conventions for labeling the Post dosing PK Time points (see Table 10 Page 57 in the MTN 001 Protocol)

- 1-3 Hours label as 1 hour timepoint
- 3-5 Hours label as 3 hour timepoint
- 5-7 Hours label as 5 hour timepoint

12.3 Procedures for Specimens that can not be evaluated

Specimens will be redrawn or recollected if it is found that they can not be evaluated per site SOP's. Sites will monitor specimen management problems as part of ongoing Quality Assurance. In cases where additional specimens need to be recollected either due to a laboratory error (lost or broken specimen or clerical error) or clinic error (clerical error), a protocol event form may be required.

12.4 Use of LDMS

The Laboratory Data and Management System (LDMS) is a program used for the storage and shipping of laboratory specimens. It is supported by the Frontier Science Foundation (FSTRF). LDMS must be used at all sites to track the collection, storage, and shipment of seven types of specimens in MTN 001: plasma, serum, PMBC, vaginal biopsies, cervical cytobrushes, rectal sponges and CVL.

Detailed instructions for use of LDMS are provided at: <https://www.fstrf.org/ldms> (may require a password).

All sites will be required to maintain the current version of LDMS and monitor updates relating to use of the LDMS. It is crucial to be aware of proper label formats to ensure that specimens are correctly labeled. Sites will be responsible to back up their LDMS data (frequency determined by site) locally and to export their data to FSTRF (at least weekly).

Questions related to use of LDMS in MTN 001 may be directed to Edward Livant or LDMS Technical (User) Support. Usual business hours for LDMS User Support are 7:30 am - 6:00 pm (ET) on Monday and Fridays and 7:30 am - 8:00 pm (ET) on Tuesdays, Wednesdays, and Thursdays. During business hours, please contact LDMS User Support as follows:

Email: ldmshelp@fstrf.org
Phone: +716-834-0900, ext 7311
Fax: +716-898-7711

LDMS User Support can be paged during off business hours if you are locked out of LDMS or experience errors that prevent you from completing LDMS lab work. To page LDMS User Support, email LDMS pager 1 (address shown in table below) and include the following information in the body of your email:

- LDMS lab number (this is a three-digit number that is different from your network assigned clinical site number)
- The full telephone number at which you can be reached, including the country code and city code if you are outside the United States
- A short description of the problem

If a response is not received within 15 minutes after emailing LDMS 1, try emailing LDMS 2, then finally, LDMS 3. The pagers also can be reached via telephone. When paging via telephone, after dialing you will hear a voice greeting followed by three quick beeps that indicate you are connected to the paging service. Please include the full telephone number at which you can be reached, including the country and city codes if you are outside the United States. Please call LDMS pager 1 first (telephone number shown in table below). If you do not receive a response within 15 minutes after calling LDMS 1, please try LDMS 2, then finally, LDMS 3.

**Table 12-3
LDMS User Support Paging Details**

Pager	Email Address	Telephone Number
LDMS 1	ldmspager1@fstrf.org	716-556-0583
LDMS 2	ldmspager2@fstrf.org	716-556-0584
LDMS 3	ldmspager3@fstrf.org	716-556-0585

Each site must export its LDMS data to Frontier Science (FSTRF) on a weekly basis. Exported data are used by the MTN SDMC to generate a monthly specimen repository report and to reconcile data entered in LDMS with data entered on study case report forms. Any discrepancies identified during the reconciliation are included in a monthly discrepancy report for each site. Sites are expected to resolve all discrepancies within two weeks of receipt of the report. The MTN NL is responsible for reminding sites to adhere to the two week timeframe and for following up with sites that do not resolve discrepancies within two weeks. The MTN SDMC reviews the discrepancy reports for critical samples (e.g., plasma needed for confirmatory HIV testing) that appear to be missing, and works with the NL and site staff to undertake appropriate corrective action. All corrective action should be documented in paper-based clinic and/or laboratory records as appropriate, and entered in the details section of LDMS. The NL and SDMC will discuss and document any items that, although resolved, appear 'irresolvable' in LDMS.

**Table 12-4
LDMS Specimen Management Guide to Logging in MTN 001 Specimens**

Test	Primary	Additive	Derivative	Sub Add/Derv	Primary Volume	Aliquot Volume	Units
Vaginal Biopsies	VGL	NON	TIS	N/A	Variable	1	N/A
PBMC for Intracellular Tenofovir	BLD	CPS	CEL	MET	Variable	1	ml
Cervicovaginal Lavage	CVL	NSL	CVL	N/A	Variable	1	ml
Plasma for storage	BLD	EDT	PL1/2	N/A	Variable	1-2	ml
Serum for Tenofovir	BLD	NON	SER	N/A	Variable	1-2	ml
Cytology Brushes	CER	PBS	CTB	N/A	Variable	1	N/A
Rectal sponge for PK (Bronx Site only)	REC	PBS	SPG	N/A	Variable	1	Ea

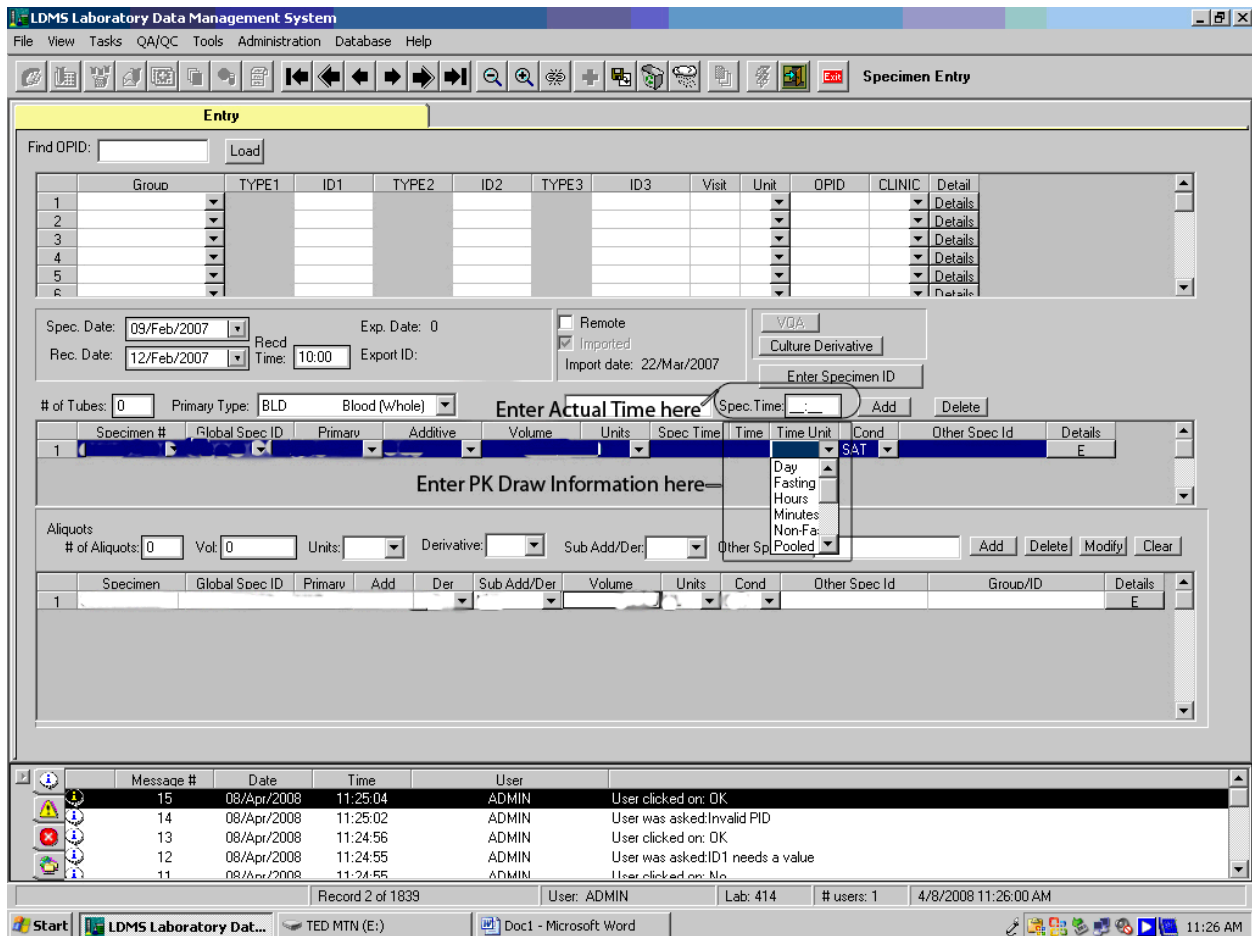
The table above should be used as a guide when logging in MTN 001 specimens. Please use the LDMS codes listed above when logging in specimens for each test

listed. Tests that are listed as local do not require that a sample be logged into the LDMS. See Appendix 12-1 for a copy of the LDMS tracking sheet.

Logging in PK Samples

- Enter the actual time in the Specimen Time area (See Image 1)
- Enter the PK timepoint information in Time and Time Unit area (See Image 1)
 - For Pre dose samples
 - The time should be 0:00
 - Select “Pre-dose” from the drop menu for units
 - For Post dose samples
 - Enter the number that corresponds to the PK timepoint (“1” for 1-Hour, “2” for 2-Hour, etc...)
 - Select “Hours” from the drop menu for units

IMAGE 1: LDMS Entry Screen



12.5 Urine Testing for Pregnancy, Dipstick, Chlamydia, and Gonorrhea

The urine tests performed at each study visit will depend on the time point of the visit and the clinical presentation of the participant. In general, at study visits when urine testing is required, a single specimen will be collected and then aliquots will be made for each test when possible. When doing multiple tests from one specimen, the correct order is separation of urine for the Chlamydia and Gonorrhea first, pregnancy test next, then the urine dipstick last. Collect urine specimens before collecting any pelvic specimens.

12.5.1 Specimen Collection

- The participant should not have urinated within one hour prior to urine collection.
- Provide the participant with a sterile, plastic, preservative-free screw-top urine collection cup labeled with a SCHARP-provided PTID label.
- Instruct the participant not to clean the labia prior to specimen collection.
- Collect the first 15-60 ml of voided urine in a sterile collection cup. (Not mid-stream).
- Instruct the participant to screw the lid tightly onto the cup after collection.
- At visits when pregnancy testing and/or dipstick urinalysis is required, aliquot 5-10 ml for these tests and store the remaining urine at 2-8°C or introduce the urine immediately into the Urine Preservation Tube (UPT) for subsequent chlamydia and gonorrhea testing.

12.5.2 Dipstick Urinalysis

Dip the urinalysis test strip into an aliquot of urine. At visits when both pregnancy testing and dipstick urinalysis are required, the same aliquot should be used for both tests, but the urinalysis should be performed after urine has been pipetted from the aliquot for the pregnancy test.

Either the Bayer/Siemens Multistix 9, Bayer Uristix 4 urine test strips or other Bayer/Siemens dipstix with the necessary tests must be used at all sites. Perform this test according to site SOPs and the package insert. Assess and record results for protein, leukocytes and nitrites. If leukocytes or nitrites are positive, perform a urine culture (may omit if not standard of care for UTI diagnosis). To avoid overgrowth of bacteria, refrigerate specimen before and during transport to laboratory.

Inventory should be monitored closely and re-supply orders placed at least 8-12 weeks in advance of actual need (or longer if needed per site procurement policies and procedures). Notify the NL immediately if any kit inventory or quality control problems are identified, so that appropriate action can be taken.

When entering results onto a CRF, a dipstick result of 1+ or greater is considered a “positive” result.

12.5.3 Pregnancy Testing

At visits when pregnancy testing is required, aliquot approximately 5-10 ml of urine from the specimen collection cup and pipette from this aliquot for pregnancy testing. If the urine is too dark to read the pregnancy test, another urine sample will need to be collected.

Note: Protocol-specified pregnancy testing is not discontinued during pregnancy.

The Quidel QuickVue One-Step hCG urine pregnancy test must be used at all sites. Perform the test according to site SOPs and the package insert. Do not perform any other urine pregnancy tests for confirmatory purposes.

12.5.4 Chlamydia and Gonorrhea Testing

This testing will be done at the MTN NL (U.S. sites) and site laboratories using the BD Probe Tec Method. Sites that have an approved Probetec will do the testing themselves using urine collected in any sterile plastic, preservative-free screw-top urine container. Sites which have met NL validation requirements will perform the neat procedure (no Urine Preservative Pouch). When shipping is required, send samples using the BD Urine Preservation Tubes (UPT). Following are shipping instructions:

Instructions for transferring urine into the UPT

- Collect urine as noted above.
- Open the UPT kit and remove the UPT and transfer pipette. Label the UPT with the participants PTID number and date.
- Hold the UPT upright and firmly tap the bottom of the tube on a flat surface to dislodge any large drops from inside the cap.
- Uncap the UPT and use the transfer pipette to transfer enough urine to fill the tube to the level indicated on the tube between the black lines. Do not under fill or overfill the tube.
- Cap tightly and invert the tube 3-4 times to ensure that the specimen and reagent are mixed.
- The specimen can now remain at 2-30°C for 30 days.

Below are shipping instructions for urine samples to Magee-Womens Research Institute for US sites. Shipping instructions for other site laboratories will be provided as needed for non-US sites.

- Urine specimens are stable for 30 days in the UPT therefore specimens can be batched and sent once a week if your turn around time is 8-9 days.
- Fill out a shipping manifest with the information listed in the example located in appendix 12-2 (Do not use LDMS for urine specimens).
- Package the specimens according to the IATA packing instructions 650 for non-refrigerated specimens.

- Place the tubes in a biohazard zip-lock bag.
- Enclose the tubes in the small Styrofoam container without ice packs.
- Place the Styrofoam container inside the cardboard box.
- Insert the box and the shipping manifest in a FedEx Diagnostic envelope.
- Check 2 day delivery on the FedEx air bill when shipping only urines (2 day delivery will save shipping costs)
- The day of shipment, send Lorna Rabe an e-mail at rabelk@upmc.edu with the FedEx tracking number.

If sending Monday through Thursday, send to:

Lorna Rabe

Magee-Womens Research Institute

204 Craft Ave, Room 530

Pittsburgh, PA 15213

Phone # 412-641-6042

(If sending on Friday, do not check Saturday delivery)

12.6 Blood Specimens for HIV testing, Syphilis, Hematology, Chemistries, PBMC for Intracellular Tenofovir, Blood Tenofovir, Hepatitis B Surface Antigen, Flow Cytometry and Plasma Archive

The blood tests performed at each study visit vary depending on the time point of the visit and potentially the clinical presentation of the participant. Perform all tests according to site SOPs and package inserts.

12.6.1 Specimen Collection and Initial Processing

Label all required tubes with a SCHARP-provided PTID label at the time of collection.

After collection:

- Allow plain tubes (no additive or serum separator tubes) to clot, then centrifuge per site SOPs to yield serum for syphilis, liver function, renal function testing, HBsAG and tenofovir level (do not use serum separator for tenofovir).
- EDTA Tubes should be gently inverted at least eight times after specimen collection to prevent clotting. EDTA tubes are used for hematology, HIV testing and plasma archive. If whole blood for hematology testing and plasma are to be taken from the same tube, the hematology must be completed before the tube is centrifuged and aliquotted. If whole blood is to be used for multiple tests, ensure that the tube is well mixed before removing any specimen.
- Cell Preparation Tubes (CPT) with Sodium Citrate are used for PBMC for intracellular Tenofovir. These should be mixed gently after collection.

12.6.2 HIV Testing

Plasma (whole blood and serum are also acceptable) will be tested for HIV using tests that have been validated at the study site. US sites must perform testing in laboratories certified by the Clinical Laboratory Improvement Amendment (CLIA) for HIV testing. At all sites, all tests and associated QC procedures must be documented on local

laboratory log sheets or other laboratory source documents. Also for all HIV testing done in non-CLIA certified labs or clinics, a second independent clinic or laboratory staff member trained in proper HIV testing and result recording procedures must review, verify, and sign-off on test results within the timeframe of the tests and prior to disclosure of results to participants. At all sites, HIV infection status will be assessed per the MTN 001 HIV testing algorithm (see appendix 12-3 in this section of the MTN 001 SSP or appendix III in the current version of the MTN 001 protocol).

SCREENING

Sites will use either one non rapid ELISA or two rapid tests at screening. When using rapid tests, at least one of the rapid tests must be FDA approved. If the ELISA or both rapid tests are non-reactive, the participant will be considered HIV-uninfected.

If the non rapid ELISA is reactive, or one rapid test is positive and one is negative, an FDA-approved Western Blot (WB) will be performed. If additional blood must be drawn for the WB, this is still considered sample 1 per the algorithm. If the WB is negative, the participant will be considered HIV-uninfected; this situation is not anticipated-contact the MTN NL if this occurs for instruction before proceeding. If the WB is positive, the participant will be considered HIV-infected. If the WB is indeterminate, notify the MTN NL. The participant will be asked to present to the study site in approximately one month for re-testing. At that time, the testing will be repeated per algorithm from the beginning. A WB will only be performed if the EIA is reactive.

FOLLOW UP

For follow up, sites will use either one non rapid ELISA or one FDA approved rapid test. If the ELISA or rapid test is non-reactive, the participant will be considered HIV-uninfected.

If the ELISA or rapid test is positive, an FDA-approved Western Blot (WB) will be performed. (Counsel the participant per local guidelines at this point.) If additional blood must be drawn for the WB, this is still considered sample 1 per the algorithm. If the WB is negative, the participant will be considered HIV-uninfected; this situation is not anticipated-contact the MTN NL if this occurs for instruction before proceeding.

If the WB is positive, the participant will be instructed to come back for a second draw (sample 2). If this WB is positive, the participant is considered seroconverted for MTN 001. All seroconversions will be confirmed by Western Blot at a MTN Network Lab.

Contact the MTN NL in any cases of indeterminate WB results.

Plasma from the enrollment archives from all participants will be retested by the MTN NL for HIV for Quality Assurance purposes.

Kit inventories should be monitored closely and re-supply orders placed at least 8-12 weeks in advance of actual need (or longer if needed per site procurement policies and

procedures). Notify the NL immediately if any kit inventory or quality control problems are identified, so that appropriate action can be taken.

At all sites, all test results must be documented on local laboratory log sheets or other laboratory source documents. In addition to initialing or signing the testing logs to document review and verification of the results, a second staff member must also record the time at which the results were reviewed and verified.

12.6.3 Syphilis Testing

Syphilis testing will be performed using a rapid plasma reagin (RPR) screening test followed by a confirmatory microhemagglutinin assay for *Treponema pallidum* (MHA-TP) or *Treponema pallidum* haemagglutination assay (TPHA) for reactive samples. Sites may choose to eliminate the RPR and test all samples with the confirmatory assay.

Any RPR, MHA-TP, and TPHA test may be used at each study site; however titers must be obtained and reported for all positive RPR tests. RPR tests may be performed on either serum or plasma. MHA-TP and TPHA tests must be performed on serum. All testing and QC procedures must be performed and documented in accordance with study site SOPs.

For reactive RPR tests observed during screening, a confirmatory test result must be received and appropriate clinical management action taken, prior to enrollment in the study. Clinical management should include repeat RPR tests at quarterly intervals following syphilis diagnosis to confirm treatment effectiveness. If the RPR titer does not decrease four-fold or revert to sero-negative within three months after treatment, treatment should be repeated.

Please consult the MTN NL with any questions related to Syphilis testing to confirm treatment effectiveness and/or interpretation of unusual test results.

Questions related to result interpretation vis-à-vis eligibility and enrollment in the study should be directed to the MTN 001 Protocol Safety Review Team.

Sites may perform only the confirmatory microhemagglutinin assay for *Treponema pallidum* (MHA-TP) or *Treponema pallidum* haemagglutination assay (TPHA) in lieu of performing first the RPR if this is consistent with local treatment standards. This must be approved before study initiation by the MTN NL.

12.6.4 Hematology Testing

Complete blood counts will be performed at all sites, per protocol .at the Screening, Enrollment, Week 7, Week 14, and Week 21 Visits. In addition, at the intensive PK sites, CBC testing *with differential* is required at each of the end-of-study period visits (Weeks 6, 13, and 20) to obtain the lymphocyte counts needed for flow cytometry.

Each of the following must be analyzed and reported:

- Hemoglobin
- Hematocrit
- Platelets
- White blood cell count with differential (differential required only for flow cytometry calculations at the Week 6, 13, and 20 Visits)
- Red blood cell count

These tests will be performed on EDTA whole blood per local site SOP's.

12.6.5 Serum Chemistries

Liver Function

- Aspartate aminotransferase (AST)
- Alanine transaminase (ALT)

Renal Function

- Creatinine (Calculated creatinine clearance is done each time creatinine is done
Formula: mL/min = (140 - age in years) x (weight in kg) x 0.85/72 x (serum creatinine in mg/dL)

Other

- Phosphorus

These chemistry tests will be performed on serum per local SOP's.

12.6.6 Plasma archive

For plasma archive, use EDTA. These will be stored at $\leq -70^{\circ}\text{C}$ and batched onsite until the MTN 001 study team requests shipping and/or testing.

- LDMS will be used to label and track the specimens.
- If at room temp, freeze within 4 hours. If refrigerated or on ice after collection, freeze within 24 hours.
- Prepare as many 1-2 mL aliquots as available to store. If less than 5 mLs of plasma are available, store that plasma and inform the MTN NL for instruction.
- The MTN NL will send instructions to the site when shipping and/or testing is required.

12.6.8 Serum Tenofovir

Serum will be frozen at $\leq -70^{\circ}\text{C}$ onsite and tracked in LDMS for Tenofovir levels within 8 hours of collection. A minimum of 2 mls serum is required. The MTN NL will notify sites for batch shipping details.

12.6.8 Hepatitis B Surface Antigen

This testing will be done on serum per local SOP's.

12.6.9 Peripheral Blood Mononuclear Cells for Intracellular Tenofovir

To be performed at all intensive PK sites and non intensive sites with capacity. Draw 16 mLs into Cell Preparation Tubes (CPT) with Sodium Citrate (BD Cat# 362761 is recommended). Specimens must be processed within 8 hours. Refer to Johns Hopkins SOP for details.

12.6.10 Flow Cytometry for CD38 and HLA-DR

To be performed at all intensive PK sites and non intensive sites with capacity. EDTA whole blood is analyzed for CD38 and HLA-DR by methods defined in local SOP's. Report total cells, percent positive cells, and mean fluorescent intensity (MFI) results. A complete blood count to obtain absolute lymphocyte count may be required for these calculations; this is not a scheduled test in the protocol at the PK visits and should be done as part of flow cytometry testing.

12.7 Testing of Vaginal and Cervical Specimens

Refer to the Screening and Follow-up Pelvic Exam checklists in other sections of this manual for further information of the required sequence of specimen collection and diagnostic procedures to be performed during study pelvic exams.

Pelvic specimen collection sequence:

1. Collect any urine specimens before taking pelvic specimens.
2. Vaginal pH and Wetmount (Herpes Culture if indicated and local standard of care)
3. Cervicovaginal Lavage
4. Cervical Cytobrush (Pap Smear if Indicated)
5. Vaginal Biopsy

12.7.1 Vaginal pH

Vaginal pH will be assessed as part of on-site evaluations for bacterial vaginosis. pH Indicator Strips (pH range 3.6 to 6.1) from Machery-Nagel (92130), Baker (4394-01) or SP (P1119-22) must be used unless other strips are approved by the MTN NL.

- During pelvic examination, vaginal fluids are collected via swab and then swabbed onto the pH strip. Avoid contact with cervical mucus, which has a high pH. Avoid contact with cervical mucus, which has a high pH.
- Match the resulting color of the indicator strip to the color scale provided with the strips to determine the pH value.
- Record the pH value directly onto the appropriate case report form. It is not necessary to record pH values onto laboratory log sheets or other source documents prior to recording values onto case report forms.

12.7.2 Vaginal Fluid Wet Mount Testing

Wet mount procedures for this study consist of two different preparations —saline prep and potassium hydroxide (KOH) prep —for diagnosis of bacterial vaginosis, trichomoniasis, and candidiasis, as summarized in Table 12-5.

If wet prep slides are read in-clinic by clinical staff, results may be recorded directly onto appropriate case report forms. If slides are read by lab staff (either in the local laboratory or a designated in-clinic lab area), results must be recorded onto laboratory log sheets or other laboratory source documents and then transcribed onto appropriate case report form.

Prior to study initiation, the MTN NL will conduct on-site training and proficiency testing for clinic and laboratory staff designated to perform wet mounts. CLIA regulations require semi-annual proficiency testing; therefore the MTN NL will administer a web-based proficiency testing approximately every six months. The MTN NL will post wet mount slides on the MTN web pages for this purpose every 6 months; results will be entered directly on the website (contact: Lorna Rabe: rabelk@upmc.edu). The MTN NL will report results back to the Laboratory Manager and also specify any corrective action that may be needed based on the results. Contact the MTN NL for additional information and guidance on performing and documenting the proficiency testing. Also contact the MTN NL when new laboratory staff is hired, so that appropriate training can take place prior to such staff performing wet mounts for study purposes.

**Table 12-5
Summary of Wet Prep Assessments and Diagnostic Criteria**

Assessment	Saline Prep	KOH Prep
Whiff Test	Not applicable	Positive if fishy amine odor detected
Clue Cells	Individual cells rather than clusters of cells should be examined. Positive if at least 20% clue cells observed. Cells must be completely covered with bacteria (<i>Gardnerella vaginalis</i> and/or anaerobic GNR) to be counted as clue cells.	Not applicable (clue cells are lysed by KOH)

Trichomonads	Positive if at least one motile trichomonad is observed. Actively motile organisms are easily seen upon low power (10X). High power (40X) may be needed to detect less vigorously motile organisms when only the flagella may be moving.	Not applicable (organisms are lysed by KOH)
Yeast	Positive if pseudohyphae and/or budding yeast are observed. Pseudohyphae and budding yeast may be obscured by epithelial cells. These cells will be lysed by KOH, thus pseudohyphae and budding yeast not observed in saline prep may be observed in KOH prep.	Positive if pseudohyphae or budding yeast are observed.

Note: Bacterial vaginosis will be diagnosed based on the presence of any three of the following Amsel's criteria: homogenous vaginal discharge, vaginal pH greater than 4.5, positive whiff test, at least 20% clue cells

Prepare and examine wet prep slides according to study site SOPs as follows:

- Use a pencil to write the PTID and specimen collection date on one side of the frosted end of two microscope slides. Affix a SCHARP-provided PTID label to the other side of the slides (on the frosted end, under the pencil markings) and write the specimen collection date in indelible ink (e.g. Sharpie pen) on each label.
- Immediately following collection from the lateral vaginal wall via swab, smear vaginal fluid specimens onto each slide. Alternatively, the swab may be placed in a glass or plastic tube with approximately six drops (100 µL) sterile physiologic saline to allow for non-immediate slide preparation. In this case, vaginal fluid specimens should be smeared onto the two slides upon receipt from the collecting clinician.
- Apply one drop of 10% KOH to one slide and immediately perform whiff test for a “fishy” amine odor. Then apply cover slip.
- Apply one drop of sterile physiologic saline to the second slide, emulsify with the vaginal fluid specimen, and then apply coverslip. Examine immediately at 10X magnification for epithelial cells, motile trichomonads, budding yeast, and pseudohyphae. Examine at 40X magnification to determine whether observed epithelial cells are clue cells and quantitate the cells. Clue cells are irregularly bordered squamous epithelial cells that are completely covered with bacteria (*Gardnerella vaginalis*). Clue cells must comprise at least 20% of the observed epithelial cells for the saline prep to be considered positive for clue cells.
- Examine the KOH slide at both 10X and 40X magnification for yeast and pseudohyphae.

12.7.3 Cytobrush Collection and Storage

Cytobrush: use the Medscand Sample Collection Kit, reference # 02500. Company Phone Number (at the time of printing) 800-243-2974. If sites have trouble obtaining this item, contact the MTN NL.

1. Place cytobrush in the cervical os and collect sample using 2 - 360° turns.
2. Add 3.5 mL of 1x PBS to a 5 mL screw cap vial or test tube labeled with a SCHARP-provided PK specimen label. The shaft of the cytobrush can be broken off at this step to cap the sample. Keep on ice or refrigerate until processing for storage.
3. Elute the cervical mononuclear cells into the PBS by agitation and rolling against the side of the tube - pulse vortex on medium 1-2 seconds approximately 4x
4. Clip off the cytobrush head from the support and centrifuge the tube at 400xg for 10 minutes
5. Carefully remove the cytobrush head and vortex on a medium setting for 2 seconds.
6. Remove 50 µL aliquot to count cells using a hemocytometer with trypan blue exclusion.
 - a. Record the total number of cells (including squamous cells*) and percent viable.
 - b. The MTN NL will provide an excel sheet to record these results.
7. Centrifuge tube at 400xg for 10 minutes
8. Pour off supernatant
9. Add 1mL 70% ice cold methanol and lyse cells on ice for 15 minutes.
10. Centrifuge tube at 800g x 10 minutes
11. Pour off supernatant into cryovial and store at -70°C.

*Note: squamous cells are expected to be rare on this specimen and will appear similar to squamous cells in urine. They will be larger than cervical mononuclear cells and will have a “fried egg” appearance. These should be counted in the same fashion as cervical mononuclear cells.

12.7.4 Cervicovaginal Lavage (CVL)

CVL specimens are collected and processed following approved site SOP's. Saline is used for MTN 001 CVL-the speculum may be warmed with warm saline for the comfort of the participant. The MTN NL will provide the sites with an SOP template. The sites will adapt this SOP for their site; these SOP's will be approved by the MTN NL.

CVL specimens are kept on ice or refrigerated after collection until they processed. All collected liquid will be spun for 10 minutes at ~800xg. Remove the supernatant from the cell pellet and re-spin at 10 minutes at ~800xg. Store all supernatant in as many 1-2 mL aliquots as possible. Store aliquots at -70°C within 8 hours of collection and track in LDMS. The MTN NL will send instructions to the site when shipping is required. If less than 6 mls of supernatant are recovered, contact the MTN NL. Discard Cell pellets.

Study sites will schedule PK visits to avoid menses. If a participant is menstruating when CVL is scheduled, collect the CVL and include a comment on the LDMS tracking sheet and then when the sample information is entered electronically in LDMS.

12.7.5 Vaginal Biopsy

The vaginal biopsy should be the last part of the exam performed using standard procedures. Two biopsy specimens are collected from different areas of the vagina. The biopsy should be taken using cervical or vaginal forceps. The forceps may be used to apply pressure to the site of the biopsy to slow bleeding. Place the biopsy specimen into a cryovial with no additive and label. Place the cryovial on ice and freeze at -70°C until shipment is requested by the MTN. Track the specimens in LDMS. More information can be found in the MTN 001 SSP Section 10.8.2.2.

12.7.6 Herpes Culture

This testing will only be performed if it is the local standard of care. Perform as indicated per local SOP's.

12.7.7 Papanicolaou (Pap) Test

Pap smears will be performed at selected sites. At visits when Pap smears are required, ecto- and endocervical cells will be collected after all tissues have been visually inspected and all other required specimens have been collected. Specimen collection, slide preparation, slide interpretation, and QC procedures must be performed and documented in accordance with study site SOPs.

- At some study sites, Pap smear results may include notations of findings associated with certain STIs (e.g., trichomoniasis). Because Pap smear methods are not adequately sensitive and specific for STIs (including HPV), Pap smear findings associated with STIs should not be used to diagnose of any STI's during follow up.

12.8 Rectal Specimens (Bronx Site Only)

Materials

Sponge: Available at Fisher Scientific Cat # NC9830573

5ml cryovial: Fischer Scientific Cat # 10-500-27 or equivalent.

Scale able to measure to .01 Grams

Disposable Transfer Pipettes

PBS

PRE personal lubricant

Preparation of Materials (45 minutes prior to procedure):

1. Prepare insertion tube using transfer pipette by cutting off the end approximately 1 inch from the tip. Make sure the stem of the sponge will fit the pipette snugly and will not dislodge during insertion or extraction from the rectal cavity.
2. Weigh the sponge, insertion tube and 5 ml cryovial. Record the total dry weight on CRF (Item 2a). Note that the CRF does not mention the cryovial.
3. Add 50 μ L of sterile PBS to cryovial and insert the sponge into the cryovial to moisten. The PBS may be added up to 2 weeks in advance as long as the cryovials are kept tightly capped.
4. Weigh the cryovial containing 50 μ L of PBS, insertion tube and sponge for tenofovir and record the total wet weight on the CRF (Item 2b). Note that the CRF does not mention the cryovial.
5. When removing the sponge from the cryovial before insertion in the anus, expel PBS by pushing it against the inside wall of the cryovial. Recap the cryovial and ensure that no PBS is spilled.

Collection Procedure:

1. Use the PRE personal lubricant to lubricate the anoscope.
2. Introduce the 1 sponge (attached to the pipette extensions) through the anoscope into the rectum.
3. Hold (or leave) sponge in place for 5 minutes.
4. Remove the sponge.
5. Slowly remove anoscope.
6. Disengage sponge from plastic pipettes and place the sponge in the same cryovial from the initial weighings (steps 1 and 3 from preparation of materials above) and cap.
7. Weigh the cryovial, insertion tube and sponge after collection and record the total weight on CRF (Item 2c). Note that the CRF does not mention the cryovial.
8. Record the time the sponge was collected on the CRF. Use the time the sponge was removed.
9. Place on ice for transport to the study site laboratory within 4 hours.
10. The laboratory will freeze at $< -70^{\circ}\text{C}$.

Appendix 12-1 LDMS Tracking Sheets

MTN 001 Africa Sites - LDMS Specimen Tracking Sheet

For login of MTN 001 stored specimens into LDMS

Participant ID		Visit Code	Specimen Collection Date	
[][]-[][][][]-[][]	[][][][]-[][][][]-[][]	[][][]-[][]	[][][] [][][][] [][][]	[][][] [][][][] [][][]
Site Number	Participant Number	Chk	<i>dd</i>	<i>MMM</i> <i>yy</i>
SPECIMEN TYPE/ TIMEPOINT	PRIMARY SPECIMEN TYPE	TIME COLLECTED hh:mm <i>24-hr clock</i>	NUMBER of TUBES or SPECIMENS COLLECTED (Primary additive)	INSTRUCTIONS FOR PROCESSING LAB
Enrollment Visit	Cervico-vaginal Lavage (CVL)	<i>Not applicable</i>	<input type="checkbox"/> NSL (saline)	Keep on ice or refrigerate until specimen is frozen long term. Centrifuge and Freeze supernatant within 8 hours of collection. Store with derivative CVL.
Plasma for Storage	Blood (BLD)	<i>Not applicable</i>	<input type="checkbox"/> EDT (Purple top)	Store as plasma with derivative PL 1/2.
PK Specimens				
Mid-period Visit	Blood (BLD) Tenofovir Level		<input type="checkbox"/> Non (red top)	Transport to lab and process within eight hours. Freeze immediately after centrifugation. Store with derivative SER.
Pre-dose	Blood (BLD) PBMC		<input type="checkbox"/> CPS (CPT Tube)	At sites with Capacity. The time from blood draw to centrifugation and lysis should be eight hours or less. Enter "pre dose" in comments field. Store with derivative CEL.
	Blood (BLD) Tenofovir Level		<input type="checkbox"/> Non (red top)	Transport to lab and process within eight hours. Freeze immediately after centrifugation. Store with derivative SER.
Post-dose <i>Circle correct time point</i>	Blood (BLD) PBMC		<input type="checkbox"/> CPS (CPT Tube)	At sites with Capacity. The time from blood draw to centrifugation and lysis should be eight hours or less. Store with derivative CEL.
	1-3 Hour	Blood (BLD) Tenofovir Level	<input type="checkbox"/> Non (red top)	Transport to lab and process within eight hours. Freeze immediately after centrifugation. Store with derivative SER.
	3-5 Hour			
	5-7 Hour	Cervico-vaginal Lavage (CVL)	<input type="checkbox"/> NSL (saline)	Keep on ice or refrigerate until specimen is frozen long term. Centrifuge and Freeze supernatant within 8 hours of collection. Store with derivative CVL.

Initials: _____ / _____ LDMS Data Entry Date: [][][] [][][][] [][][][] / _____
Sending Staff Receiving Staff *dd* *MMM* *yy* LDMS Staff

Version 1.0, 03-SEP-08

MTN 001

US Sites - LDMS Specimen Tracking Sheet

For login of MTN 001 stored specimens into LDMS

Page 1 of 2

Participant ID		Visit Code		Specimen Collection Date	
[][]-[][][][]-[]	[][][][][]	[][]	[][]	[][]	[][][][] [][]
Site Number	Participant Number	Chk		<i>dd</i>	<i>MMM</i> <i>yy</i>

SPECIMEN TYPE/VISIT	PRIMARY SPECIMEN TYPE	TIME COLLECTED hh:mm 24-hr clock	NUMBER of TUBES or SPECIMENS COLLECTED (Primary additive)	INSTRUCTIONS FOR PROCESSING LAB
Enrollment	Cervicovaginal Lavage (CVL)	<i>Not applicable</i>	<input type="checkbox"/> NSL (saline)	Keep on ice or refrigerate until specimen is frozen long term. Centrifuge and Freeze supernatant within 8 hours of collection. Store with derivative CVL.
Plasma for storage	Blood (BLD)	<i>Not applicable</i>	<input type="checkbox"/> EDT (Purple top)	Store as plasma with derivative PL 1/2.
PK Specimens				
Mid-period Visit	Blood (BLD) Tenofovir Level		<input type="checkbox"/> Non (red top)	Transport to lab and process within eight hours. Freeze immediately after centrifugation. Store with derivative SER.
Pre-dose	Blood (BLD) Tenofovir Level		<input type="checkbox"/> Non (red top)	Transport to lab and process within eight hours. Freeze immediately after centrifugation. Store with derivative SER.
	Blood (BLD) PBMC		<input type="checkbox"/> CPS (CPT Tube)	The time from blood draw to centrifugation and lysis should be eight hours or less. Store with derivative CEL.
1 Hour	Blood (BLD) Tenofovir Level		<input type="checkbox"/> Non (red top)	Transport to lab and process within eight hours. Freeze immediately after centrifugation. Store with derivative SER.
	Blood (BLD) PBMC		<input type="checkbox"/> CPS (CPT Tube)	The time from blood draw to centrifugation and lysis should be eight hours or less. Store with derivative CEL.
2 Hour	Blood (BLD) Tenofovir Level		<input type="checkbox"/> Non (red top)	Transport to lab and process within eight hours. Freeze immediately after centrifugation. Store with derivative SER.
	Blood (BLD) PBMC		<input type="checkbox"/> CPS (CPT Tube)	The time from blood draw to centrifugation and lysis should be eight hours or less. Store with derivative CEL.

Initials: _____ LDMS Data Entry Date: [][] / [][][][] / [][][][]
Sending Staff Receiving Staff *dd* *MMM* *yy* LDMS Staff

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 Version 2.0, 10-OCT-08

**Appendix 12-2: Sample Shipping Manifest (Shipments for Probetec GC/CT Only:
Use LDMS for all other shipments)**

MTN 001

Site:

**Contact person: (fill in)
(Fill in address)**

Phone number:

Fax number:

E-mail address:

Shipment Date _____

Specimen type: Urine for GC/CT testing

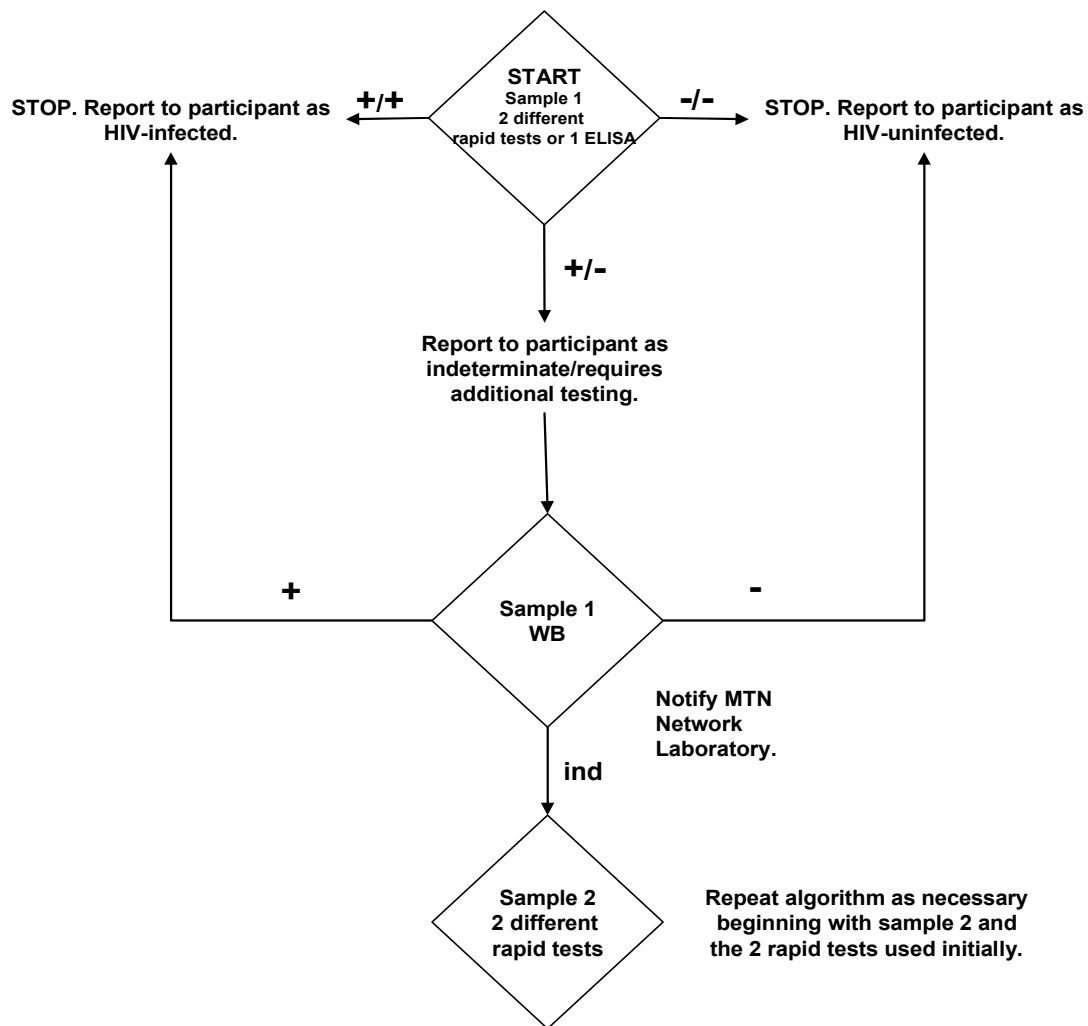
PTID	Collection Date	Visit Code

Comments _____

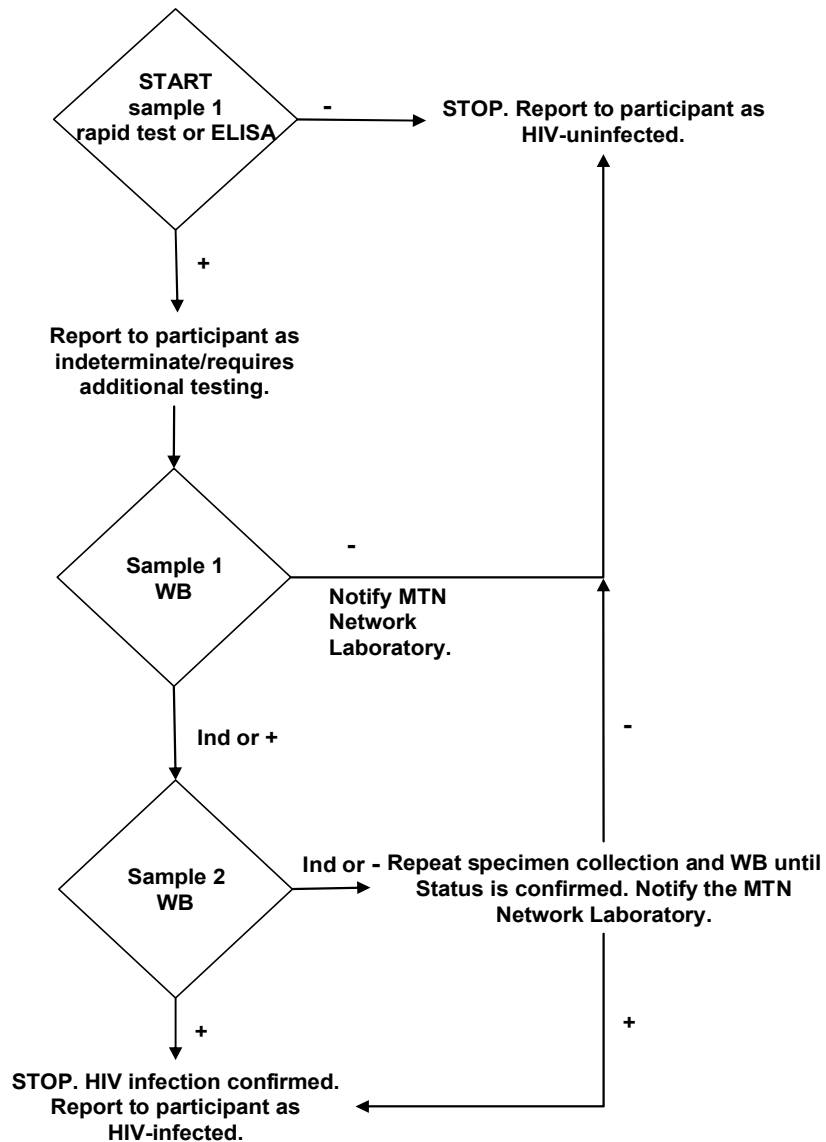
Ship to: Lorna Rabe Magee-Womens Research Institute 204 Craft Ave. Room 530 Pittsburgh, Pa. 15213 412-641-6041
--

On the day of shipment E-mail the FedEx tracking # to
rabelk@upmc.edu and cosentinola@upmc.edu

Algorithm for HIV Antibody Testing (Screening)



Algorithm for HIV Antibody Testing (Follow-up)



Appendix 12-4 Specimen Requirements Overview

MTN 001 LAB SPECIMEN PROCESSING GUIDELINES-PELVIC AND URINE SPECIMENS

Assay	Primary Specimen	Additive/Container	Minimum Volume	Testing Specifications	Handling Requirements
SDA for GC/CT	Urine	Local Testing: Urine Container- No additive	4 ml	Locally: batched 2-3 times per week	Performed Locally: 30 hours at room temp or 7 days refrigerated
		For Transport: Use Urine Preservative Tube (UPT)		Shipped: site dependent	Shipped in UPT: 30 days at room temperature
Dipstick Urinalysis	Urine	Urine Container- No additive	Enough to cover strip	Locally in real time	Room temp-analyze within 2 hours of collection
hCG	Urine	Urine Container- No additive	3 drops	Locally in real time	Room temp-test within 8 hours Refrigerate-test within 72 hours
Culture	Urine	Urine Container (Sterile) - No additive	N/A	Locally in real time	Locally Defined
Vaginal Biopsies	Vaginal Tissue	Plain Tube-No additive	N/A	Stored and shipped for analysis in batches.	Keep on ice or refrigerate until specimen is frozen long term. Freeze within 8 hours of collection.
Cytobrush	Cytobrush	PBS	N/A	Stored and shipped for analysis in batches.	Keep on ice or refrigerate until processing for storage. Vortex 1 minute, centrifuge and resuspend in buffer. Freeze within 8 hours of collection.
CVL	Saline	Conical Vial	10 cc's of saline used- recover all fluid. If less than 6 mls recovered, contact NL. Store at least 3 aliquots of 1-2 mls.	Stored and shipped for analysis in batches.	Keep on ice or refrigerate until specimen is frozen long term. Centrifuge and Freeze supernatant within 8 hours of collection.
Pap Smear	Cervical Cells	Slide	N/A	Locally in real time	Locally Defined
Herpes Culture	Swab from Lesion	Locally Defined	N/A	Locally in real time	Locally Defined
Wet Mount	Vaginal Fluid Swab	Variable	N/A	Locally in real time	Read within 30 minutes- If transported to lab: place swab in tube with 5 drops of saline
Vaginal pH	Vaginal Fluid	None-performed at bedside	N/A	Locally in real time	Done immediately at bedside

MTN 001 LAB SPECIMEN PROCESSING GUIDELINES-BLOOD SPECIMENS

Assay	Primary Specimen	Additive/Container	Minimum Volume	Testing Specifications	Handling Requirements
AST and ALT	Blood	Plain Tube-No additive or Serum separator tube (locally defined)	Locally defined	Locally in real time	Locally Defined
Phosphorus and Creatinine	Blood	Plain Tube-No additive or Serum separator tube (locally defined)	Locally defined	Locally in real time	Locally Defined
Syphilis Serology	Blood	Plain Tube-No additive or Serum separator tube (locally defined)	Locally defined	Locally in real time	Locally Defined
HBsAG	Blood	Plain Tube-No additive or Serum separator tube (locally defined)	Locally defined	Locally in real time	Locally Defined
Full Blood Count	Blood	EDTA Tube	Locally defined	Locally in real time	Locally Defined
HIV-1 Test	Blood	EDTA Tube	Locally defined	Locally in real time	Locally Defined
Flow Cytometry (CD38 and HLA-DR)	Blood	EDTA Tube	Locally defined	Locally in real time	Locally Defined
Tenofovir Level	Blood	Plain Tube-No additive	2 mls serum	Stored and shipped for analysis in batches.	Transport to lab and process within eight hours. Freeze immediately after centrifugation.
Plasma Archive	Blood	EDTA Tube	5 mls plasma	Stored and shipped for analysis in batches.	If at room temp, freeze within 4 hours. If refrigerated or on ice after collection, freeze within 24 hours.
PBMC for Intracellular Tenofovir	Blood	CPT Tube-Sodium Citrate	16 mls whole blood	Stored and shipped for analysis in batches.	The time from blood draw to centrifugation and lysis should be eight hours or less.