Section 13. Laboratory Considerations

This section contains information on the laboratory procedures performed in MTN-003.

13.1 Overview and General Guidance

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 website:

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

Section Appendix 13-1 provides an overview of the laboratory testing locations, specimens, and methods for MTN-003. Laboratory procedures will be performed in study site clinics or laboratories, approved commercial laboratories and in the MTN Network Laboratory (NL), including the MTN Pharmacology Core (at Johns Hopkins University) and MTN Virology Core (at the University of Pittsburgh). Regardless of testing location, all study staff performing testing must be trained proper testing methods and associated quality control (QC) 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 (pSMILE, IQA, VQA, etc.) to monitor and certify laboratories for testing. Please refer all questions related to laboratory testing to the MTN NL using the following email address: mtnnetworklab@mtnstopshiv.org.

In addition to the specimen guidelines provided in Section Appendix 13-1, laboratory processing guidelines are provided in Section Appendix 13-2. Although specimen collection volumes may vary somewhat across sites, all sites must ensure that collection volumes collected do not exceed the specifications of their study informed consent forms. The MTN NL may request details of specimen collection containers and volumes for purposes of assisting sites in meeting this requirement.

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 and can provide further guidance on validation requirements. Similarly, the MTN NL must be notified when normal ranges are changed.

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. It should be noted however that this section is not intended to serve as an exhaustive procedures manual for all laboratory testing. This section must be supplemented with site standard operating procedures (SOPs) for specimen management, processing, and testing.

13.2 Specimen Labeling

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

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, which are stored for later off-site testing, will be entered into the Laboratory Data and Management System (LDMS) and labeled with LDMS-generated labels:

- Vaginal fluid slides for Gram stain evaluation at the MTN NL
- Vaginal fluid swabs for biomarker evaluation at the MTN NL
- Endocervical swabs biomarker evaluation at the MTN NL
- Plasma for storage (may be used for HIV testing and testing of study drug levels at the MTN NL, may be used for future research if consent provided by the participant).

Specimens that are tested locally do not need to be logged into LDMS or labeled with LDMS-generated labels.

13.3 Procedures for Specimens That Cannot be Evaluated

Specimen collection will be repeated (whenever possible) if it is found that specimens cannot be evaluated per site SOPs. Site clinic and laboratory staff will monitor specimen collection, processing, and management as part of ongoing quality assurance (QA) procedures and take action as needed to address any issues or problems.

13.4 Use of LDMS

LDMS is a program used to track 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 the four types of specimens listed in Section 13.2. Additional specimens also must be tracked in LDMS at sites conducting MTN-003B, as described in Section 18 of this manual. Section Appendix 13-3 provides a guide for logging MTN-003 specimens into LDMS. Detailed instructions for use of LDMS are provided at https://www.fstrf.org/ldms (may require a password).

All sites are 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. All sites must routinely back up their LDMS data locally (frequency determined by site) and export their data to FSTRF at least weekly.

Questions related to use of LDMS in MTN-003 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 (US ET) on Monday and Fridays and 7:30 am - 8:00 pm (US 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

FSTRF no longer supports the use of pagers. The email addresses in Table 13-1 can still be used as needed.

Table 13-1
LDMS User Support Paging Details

Pager	Email Address
LDMS 1	ldmspager1@fstrf.org
LDMS 2	ldmspager2@fstrf.org
LDMS 3	ldmspager3@fstrf.org

Each site must export its LDMS data to Frontier Science (FSTRF) on a weekly basis. Exported data are used by the MTN Statistical and Data Management Center (SDMC) to generate a monthly specimen repository report and to reconcile data entered in LDMS with data entered on study case report forms (CRFs). 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.

13.5 Urine Testing

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, an aliquot of urine should first be obtained for pregnancy testing and/or dipstick urinalysis and the remaining specimen should be reserved for chlamydia and gonorrhea testing. Collect urine specimens before collecting any pelvic specimens. Heavy menses may interfere with dipstick and pregnancy test-sites should use discretion and contact the NL in case of question.

13.5.1 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 to:
  - Not clean the labia prior to specimen collection.
  - Collect the first 15 to 60 mL of voided urine (not mid-stream).
  - Screw the lid tightly onto the cup after collection.
- At visits when pregnancy testing and/or dipstick urinalysis is required, aliquot 5 to 10 mL for these tests and store the remaining urine at 2°C to 8°C or transfer the urine immediately into the Urine Preservation Tube (UPT) for subsequent chlamydia and gonorrhea testing.

13.5.2 Pregnancy Testing

At visits when pregnancy testing is required, aliquot approximately 5 to 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.

Either the Quidel QuickVue One-Step urine hCG or Quidel Quick Vue Combo urine and serum hCG 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.

The urine only kit and the combo kit are different kits and have different CAP method codes for EQA panels. If sites are running both kits, they must run CAP EQA panels on both kits. In most cases, the CAP results forms will only allow for entry of one kit. Sites can generally submit results to CAP for one kit and do a self evaluation for the other kit. Consult SMILE, MTN NL or your PNL in case of questions.

In all situations where using pregnancy tests in VOICE where there may potentially be interference from vaginal products, make sure to pay special attention to internal control bands.

13.5.3 Dipstick Urinalysis

At visits when dipstick urinalysis is required, 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.

Bayer/Siemens urine test strips must be used at all sites. Sites may choose test strips that have all tests necessary at a specified visit. Perform this test according to site SOPs and the package insert. Assess and record results for glucose, protein, leukocytes and/or nitrites as specified by the protocol.

Inventory should be monitored closely and re-supply orders placed at least 8 to 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.

In cases where the urine dipstick glucose grading does not correspond exactly to the data entry fields on the CRF, use this system:

Dipstick Glucose Result	**CRF Result**
Negative	Negative
100 mg/dl	Trace
250 mg/dl	1+
500 mg/dl	2+
1000 mg/dl	3+
2000 mg/dl	4+

13.5.4 Chlamydia and Gonorrhea Testing

This testing will be done using the Becton Dickenson (BD) Probe Tec strand displacement assay (SDA). Sites will perform the testing per site SOPs and the package insert.

Specimen Stability:

- Neat:
  - 2 to 30°C: 30 hours
  - 2 to 8°C: 7 days
  - ≤ -20°C: 2 months
- In Urine Preservation Tubes at 2-30°C:
  - 30 days (used generally when shipping required)
- Lysed Specimens:
  - 18 to 30°C: 6 hours
  - 2 to 8°C: 5 days (must re-vortex and re-lyse)
  - ≤ -20°C: 98 days (must be thawed to room temperature, re-vortexed and re-lysed)

13.6 Blood Testing

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

13.6.1 Specimen Collection and Initial Processing

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

- Allow plain tubes (non additive tubes or serum separator tubes are used) to clot, then centrifuge per site SOPs to yield serum.
- Gently invert EDTA tubes at least eight times after specimen collection to prevent clotting. If whole blood and plasma are to be taken from the same tube, the whole blood testing 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.

13.6.2 HIV Testing

Plasma, whole blood and/or serum will be tested for HIV using tests that have been validated at the study site. At all sites, HIV infection status will be assessed per the testing algorithms in protocol Appendices II and III; these algorithms are also provided in Section Appendix 13-4.

Before starting MTN-003, sites will be required to complete a review of HIV testing: methods/kits to be used, validations performed, testing locations, specimen types, QA and QC. This review must be approved by the MTN NL as part of the study activation process. When performing Western blot (WB) testing, all sites must use the FDA-approved Genetic Systems WB test manufactured by Bio-Rad Laboratories.

All HIV tests will be performed according to test kit package inserts and site SOPs. All tests, and associated QC procedures, must be documented on local laboratory log sheets or other laboratory source documents. These documents must capture the start and end/read times of each test. 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 specified timeframes for the tests and prior to disclosure of results to participants. In addition to initialing or signing the testing logs to document review and verification of the results, the second staff member must also record the time at which the results were reviewed and verified.

<u>SCREENING</u>

Sites will use two rapid HIV tests at screening. At least one of the rapid tests must be FDA approved.

If both rapids are negative, the participant will be considered HIV-uninfected. If both are positive, the participant will be considered HIV-infected.

If the rapid tests are discordant, i.e., one rapid test is positive and one is negative, the Genetic Systems FDA-approved WB will be performed. Inform the MTN NL whenever discordant results are obtained. The NL may provide technical guidance at this time if needed; however WB testing at the local lab should proceed immediately upon identification of at least one positive rapid test result.

If the WB is negative, the participant will be considered HIV-uninfected. If the WB is positive, the participant will be considered HIV-infected. If the WB is indeterminate, the participant will be asked to return to the study site in approximately one month for re-testing. At that time, the testing will be repeated per algorithm from the beginning. Sites may choose to inform the MTN NL whenever indeterminate results are obtained but this is not required.

FOLLOW UP

During follow up, sites will use one FDA approved rapid HIV test. If the rapid test is negative, the participant will be considered HIV-uninfected. If the rapid test is positive, the Genetic Systems FDA-approved WB will be performed. If additional blood must be drawn for the WB, the specimen is still considered sample 1 per the testing algorithm.

In the first step of the testing algorithm, sites may add an additional rapid HIV test if required by local HIV testing policies or guidelines. In this case, WB testing should be done if either of the two rapid tests is positive. Please inform the MTN NL if discordant rapid tests are encountered at follow up. The NL may provide technical guidance at this time if needed; however WB testing at the local lab should proceed immediately upon identification of at least one positive rapid test result.

If the sample 1 WB is negative or indeterminate, an HIV RNA viral load will be performed. This is true for sites using either one or two rapid tests at follow up; you do not need to wait for MTN approval to proceed to viral load. A viral load result above the limit of detection will be considered positive for the algorithm (requiring additional WB testing of sample 2). A viral load result below the limit of detection will be considered negative for the algorithm; based on this result, the participant will be considered HIV-uninfected.

If the sample 1 WB is positive, or if the sample 1 HIV viral load is positive, a second FDA-approved Genetic Systems WB must be performed on a second blood sample collected from the participant (sample 2). In addition to being used for WB testing, sample 2 includes blood that will be used for CD4+ cell count, HIV viral load, and plasma archive at the local laboratory.

If the sample 2 WB is positive, HIV infection is considered confirmed for study purposes per the algorithm. If the sample 2 WB is negative or indeterminate, additional testing must be performed, possibly requiring additional sample collection. In this case, inform the MTN NL of all sample 1 and sample 2 test results and request NL input on next steps and timeframes for additional specimen collection and testing. Always contact the MTN NL as soon as possible after obtaining the participant's sample 2 test results, so that adequate time is available for consultation before the participant returns to receive her sample 2 results.

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

Results from MTN 009 HIV rapid tests may be used for MTN 003 provided:

- They are done the same day as the MTN 003 visit
- They are the same kits as used for MTN 003
- Prior approval has been obtained from the MTN NL

13.6.3 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.

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 semi-annual following syphilis diagnosis to confirm treatment effectiveness. If the RPR titer does not decrease four-fold or revert to sero-negative within six months after treatment, treatment should be repeated. Please consult the MTN-003 Protocol Safety Review Team (PSRT) 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 also be directed to the PSRT.

13.6.4 Hepatitis B Surface Antigen and Surface Antibody

This testing will be done on serum per local SOPs. These tests may be done locally or, if approved by the MTN NL, shipped to a regional or network laboratory. For surface antibody testing, a qualitative result is required at screening for purposes of eligibility determination. If surface antibody testing is done during follow-up to confirm immune response to Hepatitis B vaccination, a quantitative result is required.

13.6.5 Hematology Testing

Complete blood counts with five-part differentials will be performed at all sites. Each of the following must be analyzed and reported:

- Hemoglobin
- Hematocrit
- Mean Corpuscular Volume
- Platelets
- White blood cell count with differential
  - Absolute neutrophil count
  - Percent neutrophils
  - Absolute lymphocyte count
  - Absolute monocyte count
  - Absolute eosinophil count
  - Absolute basophil count

These tests will be performed on EDTA whole blood per local site SOPs.

13.6.6 Serum Chemistries

The following chemistry tests will be performed on serum per local SOPs:

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

Each time serum creatinine is tested, the participant's creatinine clearance rate will be calculated, per the Cockcroft-Gault formula listed in protocol Section 5.3. Worksheets that may be used to calculate creatinine clearance rates are available in the Study Implementation Materials section of the MTN-003 web page.

13.6.7 Plasma Archive

For plasma archive, use whole blood collected in EDTA tubes. If the blood is held at room temperature, plasma must be processed and frozen within 4 hours of collection. If the blood is kept refrigerated or on ice, plasma must be processed and frozen within 24 hours of collection. Plasma should be stored frozen on site ≤ -70°C until requested for shipping and/or testing by the MTN NL.

There are three situations that require plasma archive:

Table 13-2
Volume Guide for Plasma Archive

Plasma Archive	Draw volume	Minimum Plasma Required
Routine (enrollment, quarterly, semi-annual, annual, PUEV, Termination Visit)	~10 mL	4 mL
Sample 2 in follow-up HIV testing algorithm	~15 mL	6 mL
Post-seroconversion Months 1, 3, 6 and every 6 months thereafter	~15 mL	6 mL

Note: the minimum plasma volume required for archive does not exceed 6 mL for any one participant visit.

Routine plasma samples may be used confirmatory HIV testing at the MTN NL, testing for study drug levels at the MTN NL, and possible future research testing (if consent provided by the participant). Sample 2 and post-seroconversion plasma samples will additionally be used for HIV genotypic resistance testing.

For all three types of plasma listed in Table 13-2:

- If the minimum volume specified in Table 13-2 is not obtained, notify the MTN NL.
- Use LDMS to label and track all aliquots.
- Store all aliquots frozen on site ≤ -70°C.
- The MTN NL will send instructions when shipping and/or testing is required.
- If samples are hemolysed, store the aliquots as per normal and enter comments in LDMS.

For routine plasma archive, standard processing per site SOPs should be performed. Prepare as many 1 to 2 mL aliquots as possible.

For sample 2 and post-seroconversion plasma archive, because the plasma will be used for HIV genotypic resistance testing, the plasma must be spun (approximately 3000 RPM) and frozen in 1 mL aliquots.

Plasma archive is allowable in the protocol at any visit after enrollment "as indicated". Any positive HIV test results after enrollment may be considered an indication for plasma archive. Sites are encouraged to store a plasma archive for any post enrollment positive HIV result even if not required by the protocol.

In cases where a mandatory archive is not stored or is less then 50% of the required minimum volume, sites may recall the participant to collect the plasma archive without prior approval from the MTN NL. Sites must always notify the MTN NL in these situations.

Leftover Specimens: Leftover specimens may be temporarily stored for site QA purposes and problem resolution. This process must be described in an SOP or policy onsite which indicates how long the samples will be stored. Local guidelines and regulations must be followed in these situations.

13.6.8 CD4+ T Cell Count

CD4+ T cell counts are only performed for participants at sample 2 in the follow-up HIV testing algorithm and during post-seroconversion follow up, if applicable, per protocol Section 7.6.1.

Site laboratories will test EDTA whole blood by flow cytometry for absolute CD4+ T cell counts per local SOPs. Testing will be performed on FDA approved instruments per site SOPs and package inserts. Sites must participate in United Kingdom External Quality Assurance (UKNEQAS) programs and be approved by the Immunology Quality Assurance (IQA) group to perform this testing.

13.6.9 HIV RNA PCR

HIV RNA PCR (viral load) testing is only performed for participants at sample 2 in the follow-up HIV testing algorithm, and during post-seroconversion follow up, if applicable, per protocol Section 7.6.1.

All sites will participate in the Viral Quality Assurance (VQA) program. HIV RNA viral loads will be performed on EDTA plasma using the Roche AMPLICOR™ v 1.5 Standard Assay and the ultra-sensitive method as needed. Use of other assays is allowable but must be approved by the MTN NL. All testing will be performed according to site SOPs and package inserts.

When performing viral load testing per the follow-up HIV testing algorithm, refer to Section 13.6.2 above. For this purpose, the standard assay, which has a lower limit of detection of 400 copies, is recommended. Use of the ultra-sensitive method is not required for this purpose but is allowable without prior approval.

When performing viral load testing during post-seroconversion follow-up, sites may first perform the standard assay. For samples with results below the limit of detection on the standard assay, the ultra-sensitive method is required. For sites that do not have the capacity to do the ultra-sensitive method, stored plasma specimens will shipped to the MTN NL or approved regional laboratory for testing. Follow plasma archive specimen requirements. Alternatively, sites with capacity to perform the ultra-sensitive method may initially choose to perform the ultra sensitive method on all samples.

13.7 Testing of Vaginal and Cervical Specimens

Collect urine specimens before pelvic specimens. Refer to the current Screening and Follow-up Pelvic Exam checklists in Section 7 of this manual for further information on the required sequence of specimen collection and diagnostic procedures to be performed during study pelvic exams.

13.7.1 Vaginal pH

Note that pH Indicator Strips (pH range 3.6 to 6.1) from Machery-Nagel (92130), Baker (4394-01), or SP/Cardinal Health (P1119-22) must be used unless other strips are approved by the MTN NL.

- During all pelvic examinations, vaginal fluids are collected via swab and then swabbed onto the pH strip. Avoid contact with cervical mucus, which has a higher 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.

13.7.2 Wet Mount for Candidiasis

Wet mount (KOH) testing for candidiasis is only done when clinically indicated.

If wet mount 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.

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

Immediate examination of wet mount in clinic:

- Affix a SCHARP-provided PTID label to the frosted end of the slide and write the specimen collection date in indelible ink (e.g. Sharpie pen) on the label.
- Immediately following collection from the lateral vaginal wall via swab, smear vaginal fluid onto the labeled slide. Immediately add a drop of 10% KOH onto the smear and place a coverslip over the specimen.
- Wait 2 to10 minutes for the bacterial and epithelial cells to lyse and then exam the slide microscopically at 100X and 400X for yeast and pseudohyphae.
- The test is positive if pseudohyphae and/or budding yeast are observed.

Non-immediate wet mount examination in laboratory:

- Immediately following collection of vaginal fluid from the lateral vaginal wall via swab, place the swab in a glass or plastic tube with approximately six drops (100 μL) sterile physiologic saline. Snap off the shaft of the swab and cap the tube.
- Deliver the tube to the laboratory for testing as described above for immediate examination. Testing must take place within 4 hours.

13.7.3 Rapid Test for Bacterial Vaginosis (BV)

This testing is only done when clinically indicated.

This testing will be done using the BV Blue test with vaginal swabs per site SOPs approved by the MTN NL.

- Affix a SCHARP-provided PTID label to a clean glass or plastic tube with a cap.
- Collect specimen using a cotton swab from the lateral vaginal wall (fluids also may be collected from the posterior fornix; avoid collecting specimens from the cervix).
- Immediately place the swab in the labeled tube, break off the shaft of the swab, and cap the tube.
- Testing is expected to be performed during the participant visit. However, specimens may be stored at room temperature for up to 48 hours or refrigerated for 7 days before testing.

13.7.4 Rapid Test for Trichomoniasis

This testing will be done using the Genzyme Rapid Trichomonas test with vaginal swabs per site SOPs approved by the MTN NL. The kit provides Dacron swabs for this test.

- Affix a SCHARP-provided PTID label to a clean glass or plastic tube with a cap.
- Collect specimen using kit-provided swab from the lateral vaginal wall (fluids also may be collected from the posterior fornix; avoid collecting specimens from the cervix).
- Immediately place the swab in the labeled tube, break off the shaft of the swab, and cap the tube.
- Testing is expected to be performed during the participant visit. However, specimens may be stored at room temperature for 24 hours or refrigerated for 36 hours before testing.

13.7.5 Vaginal Gram Stain

Dried vaginal fluid smears will be prepared for Gram staining and assessment for bacterial vaginosis at the MTN NL. Two slides will be prepared at each required time point and both will be entered into LDMS. One will be shipped to the MTN NL and the other will be archived on site until written notification is received from the MTN SDMC that the slide may be discarded. Instructions for slide preparation and shipping are provided below.

- Use a pencil to write the PTID and specimen collection date on one side of the frosted end of one microscope slide. 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 specimen collection from the lateral vaginal wall via swab, roll the swab across each of the slide. Do not place the swab in saline, transport medium, or any transport container prior to slide preparation.

- Allow the specimens to air-dry on the slides. Do not heat-fix.

- Deliver the slides and an LDMS Specimen Tracking Sheet to the local LDMS laboratory.

- Using the LDMS Specimen Tracking Sheet, log the slides into LDMS (specimen type = VAG) and label the slides with LDMS labels. Place the LDMS label on the frosted end of the slide, on the opposite side of the slide from the SCHARP-provided label, on top of the pencil markings.

- Store both slides in the slide box locations assigned in LDMS at room temperature.

- The laboratory will be notified by the MTN NL to ship one of the two slides collected for each participant and visit.

- The duplicate slide will be archived on site until written notification is received from the MTN SDMC that the slide may be discarded.

Instructions for shipping slides to MTN NL
Prepare a LDMS shipping manifest.

Ship to:
Lorna Rabe
Magee-Womens Research Institute
204 Craft Ave, Room A530
Pittsburgh, PA, 15213
USA
Phone: 412-641-6042
e-mail address: lrabe@mwri.magee.edu

13.7.6 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 any STIs.

13.7.7 Vaginal Swabs for Biomarker Analysis

At each pelvic exam, vaginal fluids are collected from the posterior fornix using a Dacron swab with a plastic shaft for biomarker analysis at the MTN NL.

- Collect vaginal fluid using a Dacron swab from the posterior fornix.
- Place the swab in a labeled cryovial containing 400 uL PBS (1X Concentration) and cap the vial.
- Deliver the tube and an LDMS Specimen Tracking Sheet to the local LDMS laboratory within 8 hours.
- Using the LDMS Tracking Sheet, log the cryovial into LDMS (specimen type = VAG. See Section Appendix 13-3 for LDMS for additive codes) and label the vial with a LDMS label.
- Freeze at -70^{o}C.

13.7.8 Endocervical Swabs for Biomarker Analysis

At each pelvic exam, endocervical cells will be collected using a Dacron swab with plastic shaft for biomarker analysis at the MTN NL.

- Remove cervical mucus with a large swab to expose the cell layer (discard swab).
- Collect endocervical cells by inserting a Dacron swab approximately 1 cm into the endocervical canal and rotating two full turns.
- Withdraw the swab, place it in a labeled cryovial containing 400 uL PBS (1X Concentration), and cap the vial.
- Deliver the tube and an LDMS Specimen Tracking Sheet to the local LDMS laboratory within 8 hours.
- Using the LDMS Tracking Sheet, log the cryovial into LDMS (specimen type = CXS. See Section Appendix 13-3 for LDMS for additive and derivative codes) and label the vial with a LDMS label.
- Freeze at -70^{o}C.

Section Appendix 13-1
Overview of Laboratory Testing Locations, Specimens, and Methods for MTN-003

Assay	Testing Location	Specimen Type	Tube/Container	Kit/Method
Urine Pregnancy Test	Clinic/Local Lab	Urine	Plastic screw top cup	Quidel Quick Vue
Urine SDA for Gonorrhea and Chlamydia (neat method)	Local, Regional, or MTN Network Lab	Urine	Plastic screw top cup	BD Probetec
Dipstick Urinalysis	Clinic/Local Lab	Urine	Plastic screw top cup	Bayer/Siemens urine dipsticks
HIV Rapid Tests	Clinic/Local Lab	Plasma, Whole Blood, Or Serum	EDTA or plain tube	At least one FDA approved test
HIV Western Blot	Local Lab	Plasma, Whole Blood, Or Serum	EDTA or plain tube	FDA approved Genetic Systems WB
Complete Blood Count	Local Lab	Whole Blood	EDTA tube	Not specified
Chemistries (AST, ALT, Creatinine, Phosphate)	Local Lab	Serum	Plain or serum separator tube	Not specified
Hepatitis B Surface Antigen and Surface Antibody	Local, Regional or MTN Network Lab	Serum	Plain or serum separator tube	Not specified
Syphilis Serology	Local Lab	Serum or Plasma	EDTA, plain or serum separator tube	Not specified
CD4+ T Cell Count*	Local Lab	Whole Blood	EDTA tube	Not specified
HIV-1 RNA PCR*	Local Lab	Plasma	EDTA tube	Roche AMPLICOR™ v 1.5 Standard or approved method
Plasma Archive	Stored at Local Lab	Plasma	EDTA tube	N/A
Pap Smear	Local Lab	Ecto- and Endocervical Cells	Slides	Not specified
Vaginal pH	Clinic	Vaginal Fluid Swab	Swab	S/P pH Indicator strips
KOH wet preparation	Clinic/Local Lab	Vaginal Fluid Swab	Slide	Microscopy
Trichomonas Rapid Test	Clinic/Local Lab	Vaginal Fluid Swab	Plastic Tube	OSOM Rapid Test
BV Rapid Test	Clinic/Local Lab	Vaginal Fluid Swab	Plastic Tube	BV Blue
Vaginal Gram Stain	Stored at Local Lab	Vaginal Fluid Swab	Slides	MTN NL procedure
Vaginal/Endocervical Swabs	Stored at Local Lab	Vaginal/Endocervical Swabs	Cryovial	MTN NL procedure

***** These tests are only done for participants who reach sample 2 in the follow-up HIV testing algorithm and for post seroconversion follow up when applicable.

Section Appendix 13-2

MTN-003 LAB SPECIMEN PROCESSING GUIDELINES — PELVIC AND URINE SPECIMENS

Assay	Primary Specimen	Additive/Container	Minimum Volume	Testing Specifications	Handling Requirements
SDA for GC/CT	Urine	Sterile Urine Container- No additive	4 mL	Batched per local discretion	Specimen Stability: • Neat: o 2-30°C: 30 hours o 2-8°C: 7 days o ≤ -20°C: 2 months • Lysed Specimens: o 18-30°C: 6 hours o 2-8°C: 5 days (must re-vortex and re-lyse) o ≤ -20°C: 98 days (must be thawed to room temperature, re-vortexed and re-lysed) • UPT tubes: 30 days at 2-30°C
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
Pap Smear	Cervical Cells	Slide	N/A	Locally in real time	Locally Defined
Vaginal pH	Vaginal Fluid	None-performed at bedside	N/A	Locally in real time	Done immediately at bedside
KOH Wet Mount	Vaginal Fluid Swab	KOH if done immediately. Saline if testing delayed	N/A	Locally in real time	Done immediately if a microscope is in the clinic or within 4 hours if the specimen must be transported to the lab.
Trichomonas Rapid Test	Vaginal Fluid Swab	Plastic Tube	N/A	Locally in real time	Test within 48 hours at room temperature; 36 hours if refrigerated or frozen
BV Rapid Test	Vaginal Fluid Swab	Plastic Tube	N/A	Locally in real time	Test within 48 hours at room temperature; 7 days refrigerated
Vaginal Gram Stain	Vaginal Fluid Swab	Slide	N/A	Stored locally for shipment	Room temperature
Vaginal Swabs	Vaginal Fluid Swab	Cryovial with 400 uL PBS	N/A	Stored locally for shipment	Freeze within 8 hours
Endocervical swabs	Endocervical swabs	Cryovial with 400 uL PBS	N/A	Stored locally for shipment	Freeze within 8 hours

Section Appendix 13-2
MTN-003 LAB SPECIMEN PROCESSING GUIDELINES — BLOOD SPECIMENS

Assay	Additive/Container	Minimum Volume	Testing Specifications	Handling Requirements
AST, ALT, Phosphate and Creatinine	Plain Tube-No additive or SST	Locally defined	Locally in real time	Locally Defined
Syphilis Serology	Plain Tube-No additive or SST	Locally defined	Locally in real time	Locally Defined
HBsAG and HBsAB	Plain Tube-No additive or SST	Locally defined	Locally in real time	Locally Defined
Full Blood Count	EDTA Tube	Locally defined	Locally in real time	Locally Defined
HIV Rapid Tests and WB	EDTA or Plain Tube	Locally defined	Locally in real time	Locally Defined
CD4+ T Cell Count	EDTA Tube	Locally defined	Locally in real time	Locally Defined
HIV-1 RNA PCR	EDTA Tube	Locally defined	Locally in real time or shipped to the MTN NL	Locally Defined
Plasma Archive: Routine*	EDTA Tube	4 mL 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.
Plasma Archive: Sample 2*		6 mL plasma		
Plasma Archive: Post seroconversion*		6 mL plasma		

*Refer to Section 13.6.7 for more information on plasma archive requirements.

Section Appendix 13-3
LDMS Specimen Management Guide to Logging in MTN-003 Specimens

Test	Primary	Primary Additive	Primary Volume	Primary Units	Aliquot Derivative	Aliquot Sub Add/Derv	Aliquot Volume	Aliquot Units
Vaginal Swabs	VAG	PBS	2	EA	SWB	N/A	1	EA
Endocervical Swabs	CXS	PBS	2	EA	CXS	N/A	1	EA
Vaginal Gram Stain Slides	VAG	NON	2	EA	SLD	GRS	1	EA
Plasma for Storage	BLD	EDT	Variable	mL	PL 1/2	N/A	1-2	mL

Section Appendix 13-4
MTN-003 HIV Testing Algorithms