Section 10. Laboratory Considerations

10.1 Overview and General Guidance

This section contains information on the laboratory procedures performed in MTN-015 and is intended to standardize laboratory procedures across sites.

All laboratory procedures should be performed in accordance with the DAIDS Guidelines For Good Clinical Laboratory Practice Standards, test kit/method package inserts (when applicable), and approved site standard operating procedures (SOPs).

All site laboratories will be monitored by the MTN Laboratory Center (LC) which will utilize information from DAIDS monitoring groups (PNL, IQA, VQA, etc) to monitor and certify laboratories for testing. MTN NL approval of site laboratory proficiency is required prior to site-specific study activation.

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 and Prevention can be found at the following websites:

http://www.cdc.gov/hai/

Some laboratory procedures will be performed in study site clinics or laboratories; others will be performed in Regional Laboratories and/or the MTN LC in Pittsburgh, PA. Table 10-1 lists for each test the testing location, specimen type, specimen container and kit/method (if mandated). Section Appendix 10-1 further summarizes specimen requirements.

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

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. Due to the duration of this study, this may not be feasible. 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 LC must be notified before the change and can provide further guidance on validation requirements. Similarly, all labs must contact the MTN LC in cases of changes to normal ranges.

Sites are responsible to ensure that specimen volumes do not exceed what is described in the informed consent process. The MTN LC will request details of collection containers and volumes for this purpose. These blood draws will vary by site.

Table 10-1
Overview of Laboratory Testing Locations, Specimens, and Methods for MTN-015

Test	Testing Location	Specimen Type	Tube/Container	Kit/Method
Pregnancy test	In clinic	Urine	Plastic screw top Cup	Quidel Quick Vue
NAAT for gonorrhea and chlamydia	Local Lab	Urine	Plastic screw top Cup	Cepheid GeneXpert or MTN LC approved methods,
HIV antibody Rapid Test	Local Lab	Plasma	EDTA tube	Validated MTN LC Approved Kits
Geenius	Local Lab	Plasma	EDTA tube	Bio-Rad Geenius
Plasma HIV-1 RNA Viral Load	Local Lab	Plasma	EDTA tube	FDA Approved Method
HIV-1 Genetic Resistance Test	Network Lab (Virology core)	Plasma	EDTA tube	Genotypic characterization
CD4+ T Cell Count	Local Lab	Whole Blood	EDTA tube	Not specified
Complete blood count	Local Lab	Whole Blood	EDTA tube	Not specified
Liver function (AST, ALT, Alk Phos, Total Bilirubin)	Local Lab	Serum	Plain or serum separator tube	Not specified
Renal function (Creatinine)	Local Lab	Serum	Plain or serum separator tube	Not specified
Pap Smear	Local Lab	Ecto- and endocervical cells	Slides	Not specified
Vaginal pH	In Clinic	Vaginal fluid	N/A	S/P pH Indicator Strips
Vaginal wet preparation	In Clinic or Local Lab	Vaginal fluid	N/A	Per Section 10.6.2 below
Rapid Trichomonas Test	In Clinic or Local Lab	Vaginal fluid	N/A	OSOM Rapid Trich
Syphilis Serology	Local Lab	Serum or Plasma	Plain or serum separator tube, or EDTA tube	Not specified
Vaginal Swabs	Network Lab	Vaginal fluid	Swab	Not Specified
PBMC	Network Lab	Whole Blood	EDTA tube	HVTN method
Plasma	Network Lab	Whole Blood	EDTA tube	Not Specified
CVL	Network Lab	Vaginal fluid/ Cell Pellet	Conical Vial/Cryovial	Not Specified

10.2 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. The date the specimens are collected should also be entered on the label. If the date is handwritten, it should be in indelible ink (such as a Sharpie pen). 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 Laboratory Data Management System (LDMS) and labeled with LDMS-generated labels, using the "LDMS1" format: plasma for storage, serum for storage, PMBCs, vaginal fluid swabs, and cervicovaginal lavage (CVL) fluids.

See Section 10.3 for more information on use of LDMS for this study.

10.3 Use of LDMS

Frontier Science Foundation (FSTRF) supports the LDMS program which is used to track storage and shipping of laboratory specimens. LDMS must be used at all sites to track the collection, storage, and shipment of the types of specimens listed in table 10.2.

All sites are required to maintain the current version of LDMS and monitor updates relating to use of the LDMS. Sites should update LDMS within 3 weeks of the version being available unless there extenuating circumstances. 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-015 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: +001 716-834-0900, ext 7311
Fax: +001 716-898-7711

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), check for errors in LDMS codes, and ensure storage information is entered for archive specimens. Any issues identified during the reconciliation are included in a monthly discrepancy report for each site. Sites are expected to resolve all issues within two weeks of receipt of the report. The MTN LC 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 reconciliation reports for critical samples (e.g., plasma needed for confirmatory HIV testing) that appear to be missing, and works with the LC 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 LC and SDMC will discuss and document any items that, although resolved, appear 'irresolvable in LDMS'.

Sites are encouraged to have a weekly QC of LDMS data versus CRF data to correct discrepancies before they make it to the LDMS reconciliation reports.

Sites may use LDMS to track samples for local testing but these samples must be marked as "never store" in LDMS or they may appear on the LDMS reconciliation reports. This feature will be discontinued when LDMS becomes an online program. This guidance will be updated at that time.

The LC recommends you separate your specimens by sample type in the freezers. This may save time in the future when sorting.

Table 10-2
LDMS Specimen Management Guide to Logging in MTN-015 Specimens

Test	Primary	Primary Additive	Primary Volume	Primary Units	Aliquot Derivative	Aliquot Sub Add/Der	Aliquot Volume	Aliquot Units
Vaginal Swabs	VAG	PBS	2	Swabs	SWB	N/A	1	N/A
PBMC	BLD	EDT	~50	mL	CEL	DMS	XX.X*	CEL
Cervicovaginal Lavage (supernatant)	CVL	NSL	Variable	mL	CVL	N/A	1	mL
Cervicovaginal Lavage (Cell Pellet)	CVL	NSL	Variable	mL	CEN	NSL	0.5	mL
Plasma for storage	BLD	EDT	Variable	mL	PL 1/2	N/A	1.0	N/A

*PBMC cell count for final cell suspension is entered as xx.x million cells per milliliter

10.4 Urine Collection and Testing for Pregnancy, Chlamydia, and Gonorrhea

In general, at study visits when urine testing is required, a single specimen will be collected and then aliquots made for each test when possible. When doing multiple tests from one specimen, the correct order is separation of urine for chlamydia and gonorrhea first then pregnancy test.

10.4.1 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.
- Instruct the participant to collect the first 15-60 mL of voided urine (NOT mid-stream) in the labeled cup and to screw the lid tightly onto the cup after collection.
- At visits when pregnancy testing and urine testing for chlamydia and gonorrhea is required, aliquot 5-10 mL for the pregnancy test and transport the remaining urine to the local lab per site SOPs.

10.4.2 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.

Either the Quidel QuickVue One-Step urine hCG or Quidel QuickVue 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 serum hCG testing. 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 LC or your PNL in case of questions.

10.4.4 Chlamydia and Gonorrhea Testing

This testing will be done using the) NAAT methods: Gene Xpert or GenProbe Aptima. Sites will perform the testing per site SOPs and the package insert. The LC can approve other FDA approved NAAT methods. Contact the LC for guidance.

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10.5 Blood Collection, Processing, Testing, and Storage

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.

10.5.1 Specimen Collection and Initial Processing

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

- Allow plain (no additive) and serum separator tubes to clot, then centrifuge per site SOPs to yield serum for syphilis, liver function, and renal function testing.

- EDTA tubes should be gently inverted at least eight times after specimen collection to prevent clotting. EDTA tubes are used for hematology, peripheral blood mononuclear cell (PBMC) isolation, 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 aliquots made. If whole blood is to be used for multiple tests, ensure that the tube is well mixed before removing any specimen.

10.5.2 HIV RNA Viral Load

HIV-1 RNA PCR will be performed on EDTA plasma on an FDA-approved platform. Approved platforms include the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 (limit of detection [LOD] of 20 copies/ml) or the Abbott *m*2000 RealTi*m*e HIV-1 (LOD of 40 copies/ml). If using Abbott *m*2000, perform the 0.6 ml or 1.0 ml assay for a LOD of 40 copies/ml. All testing will be performed according to site SOPs and package inserts.

Contact the LC for use of the Abbott *m*2000 0.5 ml assay (LOD 75 copies/ml) or the 0.2 ml assay (LOD 150 copies/ml) in cases of insufficient plasma volume. Contact the LC to request use of older viral load platforms (e.g. Roche AMPLICOR™ v1.5 Standard or Ultrasensitive).

Please note that on automated platforms, "<40 copies/ml, detected" or "<20 copies/ml, detected" should be distinguished from "target not detected" and reported accordingly.

VQA Proficiency
Sites must maintain VQA proficiency for the platform(s) used. The VQA 200 copy control must be included with each run. Interchanging platforms when both are available is not recommended, even when both platforms are VQA-certified. A VQA-approved validation is required for switching platforms.

Two circumstances may necessitate specimen dilution:
(1) Insufficient volume of plasma is available for testing
(2) Plasma specimen has a result above the upper limit of detection of the assay.

Perform dilutions using Basematrix 53 (available from SeraCare). To report viral loads on diluted samples, sites must first contact the VQA for validation requirements.

10.5.3 PMBC Isolation and Storage

PBMCs will be isolated from whole blood collected in EDTA tubes following the HIV Vaccine Trials Network's validated PBMC isolation and storage procedure. Sites must demonstrate proficiency in isolation and storage of viable cells before they can perform this procedure for MTN-015. Isolated PBMCs should be stored frozen on site per HVTN guidelines (-80°C then Liquid Nitrogen) and logged into LDMS.

Unless an alternate specimen collection volume has been approved by the MTN LC, 50 mL of whole blood will be drawn for PBMC isolation. During specimen processing for PBMC isolation, all available plasma will be used for protocol-specified testing and archive purposes.

10.5.4 HIV Genotypic Resistance

HIV genotypic resistance testing will take place at the MTN Virology Core Laboratory in Pittsburgh, PA. This testing may be done using plasma collected at the Screening and Enrollment visit and as clinically indicated during follow-up. The test requires spun EDTA plasma. The preferred sample is five 1 mL aliquots; the minimum sample is four 1 mL aliquots. The specimens are stored at ≤ -70°C.

Follow plasma archive specimen requirements in Section 10.5.9.

10.5.5 CD4+ T Cell Count

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.

10.5.6 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). 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.

Clinical management of syphilis infections should include repeat RPR testing at six-month intervals following diagnosis of a new infection to confirm treatment effectiveness. As per Section 8.7 of this manual, if the RPR titer does not decrease four-fold or revert to seronegative within six months of treatment, treatment should be repeated. Any questions related to RPR testing to confirm treatment effectiveness, interpretation of unusual syphilis test results, and/or appropriate clinical management of test results should be emailed to the MTN-015 Clinical Management Group (mtn015ClinMgt@mtnstopshiv.org).

10.5.7 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
- Red blood cells
- Mean corpuscular volume
- Platelets
- White blood cell count with differential (% and absolute count)
    - Neutrophils
    - Lymphocytes
    - Monocytes

- Eosinophils
- Basophils

These tests will be performed on EDTA whole blood.

10.5.8 Liver and Renal Function Testing

The following tests will be performed to evaluate liver and renal function:

Liver Function
- Aspartate aminotransferase (AST)
- Alanine transaminase (ALT)
- Total bilirubin
- Alkaline Phosphatase

Renal Function
- Creatinine

These chemistry tests will be performed on serum.

10.5.9 Plasma Storage

Plasma will be processed and stored at all visits.

Spin blood at room temperature in a centrifuge according to either one of these techniques:
- Single spun: Spin blood at 1200-1500 RCF (g-force) for 10 minutes, remove plasma.
- Double spun: Spin blood at 800 g for 10 minutes, place plasma in a tube to spin again at 800 g for 10 minutes, remove plasma.

As noted in Section 10.5.3, unless an alternate specimen collection volume has been approved by the MTN LC, 50 mL of whole blood will be collected in EDTA tubes at each visit for PBMC isolation. During specimen processing for PBMC isolation, all available plasma will be used for protocol-specified testing and archive purposes. Plasma will be used as needed at the Local Lab to perform the HIV RNA viral load.

At Screening and Enrollment visits, and at any follow-up visits when HIV genotypic resistance testing is clinically indicated, plasma will then be stored for shipment to the MTN LC, where resistance testing will be performed. The plasma used for resistance testing must be frozen at ≤ -70°C in 1 mL aliquots. The preferred sample is five 1 mL aliquots; the minimum sample is four 1 mL aliquots. Site staff must notify the MTN LC if fewer than four 1 mL aliquots are stored for any participant requiring resistance testing.

At all visits, after the above-listed processing has been performed (as applicable), store at least three 1.0 mL aliquots of plasma and store at ≤ -70°C. Any additional remaining plasma should be stored in aliquot sizes of site discretion.

- At Screening and Enrollment visits, and at any follow-up visits when resistance testing is required, at least three 1.0 mL aliquots of plasma should be stored *in addition to* the aliquots stored for resistance testing, for a total of at least 7 mL (8 mL preferred). Site staff must notify the MTN LC if this minimum storage requirement is not met for any participant.
- At visits when resistance testing is not required, at least three 1.0 mL aliquots of plasma should be stored; site staff must notify the MTN LC if this minimum storage requirement is not met for any participant.
- In any situation, store all available plasma and notify the MTN LC when minimum volumes are not available.

If held at room temperature, plasma must be frozen within 4 hours of collection. If refrigerated or on ice, plasma must be frozen within 24 hours of collection.

Table 10-3
Quick Reference Plasma Storage

Archive Type	Tube type	Minimum Volume Required	Aliquot Sizes	Comments
Plasma for Resistance Testing	EDTA	4 mL	1mL	5 mL preferred. Please store all plasma available in 1 mL aliquots.
Routine Plasma	EDTA	3 mL	1mL	Please store all plasma available in 1 mL aliquots.

10.5.10 HIV Serology

HIV serology will be done at enrollment and may be done at a follow-up visit to ensure that the participant's HIV status was correctly characterized in the parent protocol.

Perform two validated HIV rapid tests. At least one of the HIV rapid tests must be FDA approved. Use the same two rapid tests as the parent protocol unless otherwise approved by the LC.

The expected result is dual positive. If both rapid tests are not positive, contact the MTN-015 management team immediately. Investigate the situation and continue with all study procedures until other guidance is received. The LC will give guidance on further testing and investigation.

10.6 Testing of Vaginal and Cervical Specimens

Refer to the visit checklists on the MTN-015 Study Implementation Materials webpage (http://www.mtnstopshiv.org/node/468) for further information of the required sequence of specimen collection and diagnostic procedures to be performed during study gynecologic exams.

The MTN 015 protocol has been amended to allow for two different types of BV/Trichomonas testing:

1. Standard Saline (for Clue cells and motile trichomonads) and KOH (for yeast) wet mount testing.

2. A combination of OSOM Rapid Trichomonas testing, and Standard Wet Mount (if indicated).

This change was to allow for consistency of testing for sites with on-going parent protocols.

Please refer to the relevant sections below for further guidance.

10.6.1 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.

Assess vaginal pH as follows:

- During gynecologic examination, vaginal fluids are collected via swabbing the vaginal walls and then swabbing onto the pH strip (instead of inserting the pH strip into the vagina). Avoid collecting the swab from the cervix and the pooled secretions in the fornix which have 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.

10.6.2 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 10-5. If the OSOM rapid Trichomonas test is used the samples for clue cells and yeast it can be read immediately or placed in saline and read up to 4 hours later. If Trichomonas is being detected by wet mount the samples must be read within 30 minutes of collection.

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.

The MTN LC maintains a semi-annual proficiency testing program for clinic and laboratory staff designated to perform wet mounts. If your site is only performing the KOH wet mount, you will only be graded on presences or absence of yeast. The MTN LC posts wet mount slides on the MTN web site every 6 months; all site staff who perform wet mounts must evaluate these slides and enter their results directly on the website (contact: Lorna Rabe rabelk@upmc.edu). The MTN LC reports results back to the site Laboratory Manager and will specify any corrective action that may be needed based on the results. Contact the MTN LC for additional information and guidance on performing and documenting the proficiency testing. Also contact the MTN LC when new laboratory staff is hired, so that appropriate training can take place prior to such staff performing wet mounts for study purposes.

Table 10-4
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 (*Gardnerella vaginalis* 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 and/or anaerobic GNR*). Clue cells must comprise at least 20% of the observed epithelial cells in order 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.

10.6.3 Rapid Test for *Trichomonas vaginalis*

This test can be used instead of detection on a wet mount.
The test will be done using the OSOM Rapid Trichomonas test with vaginal swabs per site SOPs approved by the MTN LC. 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.

10.6.4 Vaginal Fluid Swabs for Storage

- During gynecologic exam, swab the posterior fornix of the vagina with each of two plastic shaft Dacron swabs; the vaginal tissue should be gently swabbed until the tip of the swab is saturated with fluid.

- Immediately place the swab in a 2 mL cryovial containing 0.4 mL phosphate buffered saline (calcium and magnesium free; 1X concentration) and labeled with a SCHARP-provided PTID label.

- Break off the end of the swab to allow closure of the cryovial and securely attach the cap.

- Place the cryovial in a plastic ziplock biohazard bag and immediately place the bag in a refrigerator or in a cooler with an ice pack. All cryovials should be frozen on the day of collection, such that specimens are ideally stored refrigerated for no more than eight hours prior to freezing.

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

- Log the cryovial into LDMS and generate an LDMS cryovial label. Affix the LDMS label to the cryovial (over the SCHARP-provided PTID label).

- Within 8 hours of collection, store the cryovial in the freezer location assigned in LDMS at –70^{o} C.

10.6.5 Cervicovaginal Lavage (CVL) for Storage

CVL specimens are collected and processed according to the following procedures.

<u>Collection</u>

1. Arrange materials before participant visit.
2. Do not collect CVL if there is menstrual blood present.
3. Explain procedure to study participant.
4. Position patient for pelvic examination.
5. Wash hands thoroughly prior to procedure and put on gloves.
6. Examine external genitalia.
7. Carefully insert the speculum about halfway into the vagina.
8. Open speculum gently to visualize anatomy/positioning. Close speculum and gently advance it. Repeat opening the speculum to guide insertion until part of cervix is visible.
9. Carefully open the speculum, without hitting the cervix, to position cervix into view.
10. Visually inspect cervix and vagina. If required by the SSP or protocol, document and report findings.
11. Draw 10mL of sterile saline warmed in the water bath into 20-50 mLsyringe.
12. Carefully insert tip of syringe into the vagina using care not to touch vaginal walls with syringe. With tip of syringe aimed at the cervix, dispense all 10 mL of saline onto the cervix. Gently tilt speculum if necessary to avoid leakage of saline.
13. Place tip of a 2mL pipette onto posterior blade of the speculum and draw fluid into pipette, using care not to touch the vagina or cervix.
14. Use the 10mL of saline to lavage the cervix, fornices and vaginal walls. Be sure to lavage each side wall at least twice. Only use the original 10mL of saline. DO NOT use any additional saline to perform lavage.

15. **The saline must be in contact with the vaginal vault for at least 1 minute.**
16. After at least one minute of contact, remove lavage fluid with 20-50 mL syringe and sterile tubing or 2ml pipette.
17. **SAVE LAVAGE FLUID FOR ANALYSIS**. Transfer fluid to 20-50mL conical centrifuge tube.
18. Once lavage procedure is complete, visually inspect cervix and vagina. If required by the protocol, document and report findings per the study protocol and SSP Manual.
19. Gently remove speculum.
20. Verify labeling of all specimens with study identifiers, visit code, date of collection.
21. Place specimen in refrigerator or on ice or cold packs immediately after collection.
22. Transport specimen to the laboratory on ice or cold packs.
23. Discard syringe, pipette and tubing in biohazard bag.
24. Remove gloves and wash hands thoroughly.

Processing and Storage

1. CVL specimens are kept on ice or refrigerated after collection until they processed.
2. All the CVL liquid will be spun at 800×*g* for 10 minutes in a conical collection tube.
3. Remove supernatant from the cell pellet and save fluid in 1 mL cryovials.
4. Re-spin the 15 mL conical tube containing cells for 10 minutes at 800×*g*.
5. Pull off and save any additional supernatant making sure not to remove any cells or debris.
6. Store all supernatant in as many 1 mL aliquots as possible in 1 mL cryovials.
7. Freeze all aliquots at ≤-70˚C within 8 hours of collection and track in LDMS.
8. Cell pellets will be suspended in 0.5 mL normal saline in a plastic cryovial and frozen at ≤-70˚C within 8 hours of collection.
9. The MTN LC will send instructions to the site when shipping is required.

10.6.6 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 sexually transmitted infections (e.g., trichomoniasis). Please see Section 8.7 of this manual for guidance on how to interpret and respond to such results.

Section Appendix 10-1 Specimen Requirements Summary
MTN-015 LAB SPECIMEN PROCESSING GUIDELINES-PELVIC AND URINE SPECIMENS

Assay	Primary Specimen	Additive/Container	Minimum Volume	Testing Specifications	Handling Requirements
NAAT for GC/CT	First Void Urine	Instrument Specific Transport container	15-60 mL	Locally in real time or Shipped and performed weekly or as often as needed	According to the manufacturer's guidelines
Vaginal Swabs for Storage	Dacron Swab	1X PBS	2 swabs	Stored and shipped for analysis in batches.	Place each swab in a separate cryovial container containing 400 µL of PBS, break off the stick and close. Freeze within 8 hours.
hCG	Urine	Urine Container- No additive	3 drops	Locally in real time	Room temp-test within 8 hours Refrigerate-test within 72 hours
CVL	Saline	Conical Vial	10 mL's of saline used-save all fluid recovered-at least 3 aliquots of 1-2 mLs. If less than 6 mLs recovered, contact LC	Stored and shipped for analysis in batches.	Keep on ice or refrigerate until specimen is frozen long term. Centrifuge (~800 g) and Freeze supernatant within 8 hours of collection.
Pap Smear	Cervical Cells	Slide	N/A	Locally in real time	Locally Defined
Wet Mount	Vaginal Fluid Swab	Variable	N/A	Locally in real time	Place swab in tube with 5 drops of saline and transport to lab. Read within 30 minutes of collection if not using OSOM rapid Trich kit and 4 hours if using OSOM kit.
Vaginal pH	Vaginal Fluid	None-performed at bedside	N/A	Locally in real time	Done immediately at bedside
OSOM Rapid Trichomonas Test	Vaginal Fluid Swab	Plastic Tube	N/A	Locally in real time	Test within 24 hours at room temp or 36 hours if refrigerated.

Appendix 10-1 Specimen Requirements Summary
MTN-015 LAB SPECIMEN PROCESSING GUIDELINES-BLOOD SPECIMENS

Assay	Primary Specimen	Additive/Container	Minimum Volume	Testing Specifications	Handling Requirements
LFT and Serum Chemistry	Blood	Plain Tube-No additive	Locally defined	Locally in real time	Locally Defined
HIV RNA Viral Load	Blood	EDTA Tube	Locally defined	Locally in real time-specimens may be shipped for ultra sensitive method if indicated	Locally Defined
Syphilis Serology	Blood	Plain Tube-No additive	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	Blood	EDTA Tube	Locally defined	Locally in real time	Locally Defined
Plasma Storage – Routine	Blood	EDTA Tube	3 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.
Plasma Storage – for Resistance Testing	Blood	EDTA Tube	4 mLs plasma (Prefer 5 mLs)	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	Blood	EDTA Tube	Variable-approximately 50 mLs of blood will be collected and processed	Stored and shipped for analysis in batches.	Snap freeze should commence within eight hours of collection.