Section 12. Laboratory Considerations

12.1 Overview and General Guidance

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

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 University or [*To be inserted*] **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.

Please refer all questions to the MTN Network Laboratory at:

mtnnetworklab@mtnstopshiv.org

Table 12-1
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)	MTN Network Lab, Regional or site lab	Urine	Plastic screw top Cup-Urine Preservative Tube for shipping	BD Probetec
Dipstick Urinalysis	Clinic/Local Lab	Urine	Plastic screw top cup	Bayer Multistix® 10 SG or Bayer Uristix 4
HIV antibody screen and Western Blot	Clinic/Local Lab	Plasma, whole blood, serum	EDTA or plain tube	FDA approved tests
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 and Antibody	Local Lab	Serum	Plain or serum separator	Not specified
Syphilis Serology	Local Lab	Serum or Plasma	EDTA tube, plain or serum separator	Not specified
Blood FTC and TDF level	Network Lab	Plasma	EDTA tube	JHU method
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
PBMC Isolation	Local Lab	Whole Blood	EDTA tube	HVTN method
Plasma Archive	Stored at Local Lab for shipment	Plasma	EDTA tube	N/A
Resistance Testing	MTN Network Lab	Plasma	EDTA tube	Standard resistance, allele specific and ultra sensitive
Pap Smear	Local Lab	Ecto- and Endocervical cells	Slides	Not specified
Vaginal pH	In clinic	N/A	N/A	S/P pH Indicator Strips
KOH wet preparation	Clinic/Local Lab	Vaginal fluid swab	N/A	Microscopy
Trichomonas Rapid Test	Clinic/Local Lab	Vaginal fluid swab	Plastic Tube	OSOM *Trichomonas* Rapid Test
BV Rapid Test	Clinic/Local Lab	Vaginal fluid swab	Plastic Tube	OSOM BV Blue
Vaginal Gram Stain	Stored at Local Lab for shipment	Vaginal fluid Swab	Slides	Network Lab procedure
Vaginal/Endocervical Swabs	Stored at Local Lab for shipment	Vaginal/Endocervical Swabs	Cryovial	Network Lab procedure

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 003 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.

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: Gram stain slides, vaginal and Endocervical swabs, plasma for storage (FTC and TDF levels, HIV testing at the NL, QA purposes, future research), PBMC.

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 and/or other documentation 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 003: plasma, serum, PMBC, vaginal biopsies, cervical cytobrushes 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 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 (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 12-2
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 12-3
LDMS Specimen Management Guide to Logging in MTN-003 Specimens
[*Draft-codes to be confirmed by FTSRF*]

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
Endocervical Swabs	CXS	PBS	2	Swabs	SWB	N/A	1	N/A
Vaginal Gram Stains	VAG	NON	2	Slides	SLD	N/A	1	N/A
PBMC	BLD	EDT	~50	ml	CEL	DMS	XX.X*	CEL
Plasma for storage	BLD	EDT	Variable	ml	PL 1/2	N/A	0.5-1 ml	N/A

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

The table above should be used as a guide when logging in MTN 003 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.

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 Multistix 9, Bayer Uristix 4 urine test strips or other Bayer 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 glucose, protein, leukocytes and nitrites. If leukocytes or nitrites are positive, perform a urine microscopy and a urine culture according to local SOP. 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.

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.

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 using the Becton Dickenson (BD) Probe Tec strand displacement assay (SDA). All current MTN-003 sites have been approved by the MTN NL to perform this assay. Sites will perform the testing per site SOPs and the package insert.

Specimen Stability:

- Neat
    - 2-30°C: 30 hours
    - 2-8°C: 7 days
    - ≤ -20°C: 2 months
- In Urine Preservation Tubes at 2-30°C: 30 days (Not generally used for this study)
- Lysed Specimens:
    - 18-30°C: 6 hours
    - 2-8°C: 5 days (must re-vortex and re-lyse)
    - ≤ -20°C: 98 days (must be thawed to room temperature, re-vortexed and re-lysed)

12.6 Blood Specimens for HIV testing, Syphilis, Hematology, Chemistries, Blood Tenofovir, Hepatitis B Surface Antigen and Antibody, Flow Cytometry for CD4+ T Cell Count, HIV-1 RNA Viral Load, Genotypic HIV Resistance Testing 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 (do not use serum separator for tenofovir).
- EDTA Tubes should be gently inverted at least eight times after specimen collection to prevent clotting. If whole blood for testing 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.

12.6.2 HIV Testing

Plasma, whole blood and/or serum will be tested for HIV using tests that have been validated at the study site.

Before starting MTN 003, sites will be required to complete a review of HIV testing: methods/kits to be used, validations performed, testing locations, staff competency, specimen types, QA and QC. This review will be approved by the MTN NL as part of study activation.

All tests, and associated QC procedures, must be documented on local laboratory log sheets or other laboratory source documents. At all sites, HIV infection status will be assessed per the MTN 003 HIV testing algorithm (see appendix 12-1 in this section of the MTN 003 SSP or appendix III in the current version of the MTN 003 protocol).

<u>SCREENING</u>

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 rapids are non-reactive, the participant will be considered HIV-uninfected.

If the non rapid ELISA or the two rapid tests are 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 one FDA approved rapid test. If the ELISA or rapid is non-reactive, the participant will be considered HIV-uninfected. Sites may add an additional rapid test at their discretion.

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, an HIV RNA Viral Load will be performed (always contact the MTN NL).

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 003. 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 MTN 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 003 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 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 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 ≤ -70°C and batched onsite until the MTN 003 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.

12.6.7 Genotypic HIV Resistance testing

HIV genotypic resistance testing is performed when sample 2 is drawn for the HIV Testing algorithm. Plasma will then be stored for shipment to the MTN NL, where resistance testing will be performed. The plasma used for resistance testing must be double spun (approximately 3000 RPM) and 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 NL if fewer than four 1 mL aliquots are stored for any participant requiring resistance testing.

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.

12.6.8 Hepatitis B Surface Antigen and Surface Antibody

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

12.6.9 Flow Cytometry CD4+ T Cells

<u>Flow Cytometry for CD4+ T Cells is only performed for participants who have seroconverted.</u>

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.

12.6.10 HIV RNA Viral Load

HIV RNA Viral Load Testing is only performed to confirm seroconversion or for participants who have seroconverted.

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.

The sites may first perform the standard assay which has a lower limit of 400 copies. For samples with result <400 copies, 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.

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.

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:

- During pelvic examination, touch a pH indicator strip to the vaginal wall just until the paper is moistened. Avoid contact with cervical mucus, which has a high pH. Alternatively, vaginal fluids may be collected via swab and then swabbed onto the pH strip (instead of inserting the pH strip into the vagina).
- 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 Wet Mount (KOH) for Candidiasis

Wet mount (KOH) for Candidiasis testing for MTN 003 is only done when clinically indicated.

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.

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 one microscope slide. Affix a SCHARP-provided PTID label to the other side of the slide (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.
- If testing will not be done immediately, 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 a slide upon receipt from the collecting clinician; KOH would then be applied to the swab.
- Examine the KOH slide at both 10× and 40× magnification for yeast and pseudohyphae.
- Positive if pseudohyphae and/or budding yeast are observed.

12.7.3 Rapid BV Test
[Draft-details to follow]

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

12.7.4 Rapid Trich Test
[Draft-details to follow]

This testing will be done using the Genzyme Rapid Trichomonas test with vaginal swabs per site SOP's approved by the MTN NL.

12.7.5 Vaginal Gram Stain

In addition to the wet mounts described above, 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 SDMC that the slide may be discarded. Instructions for slide preparation and shipping are provided below.

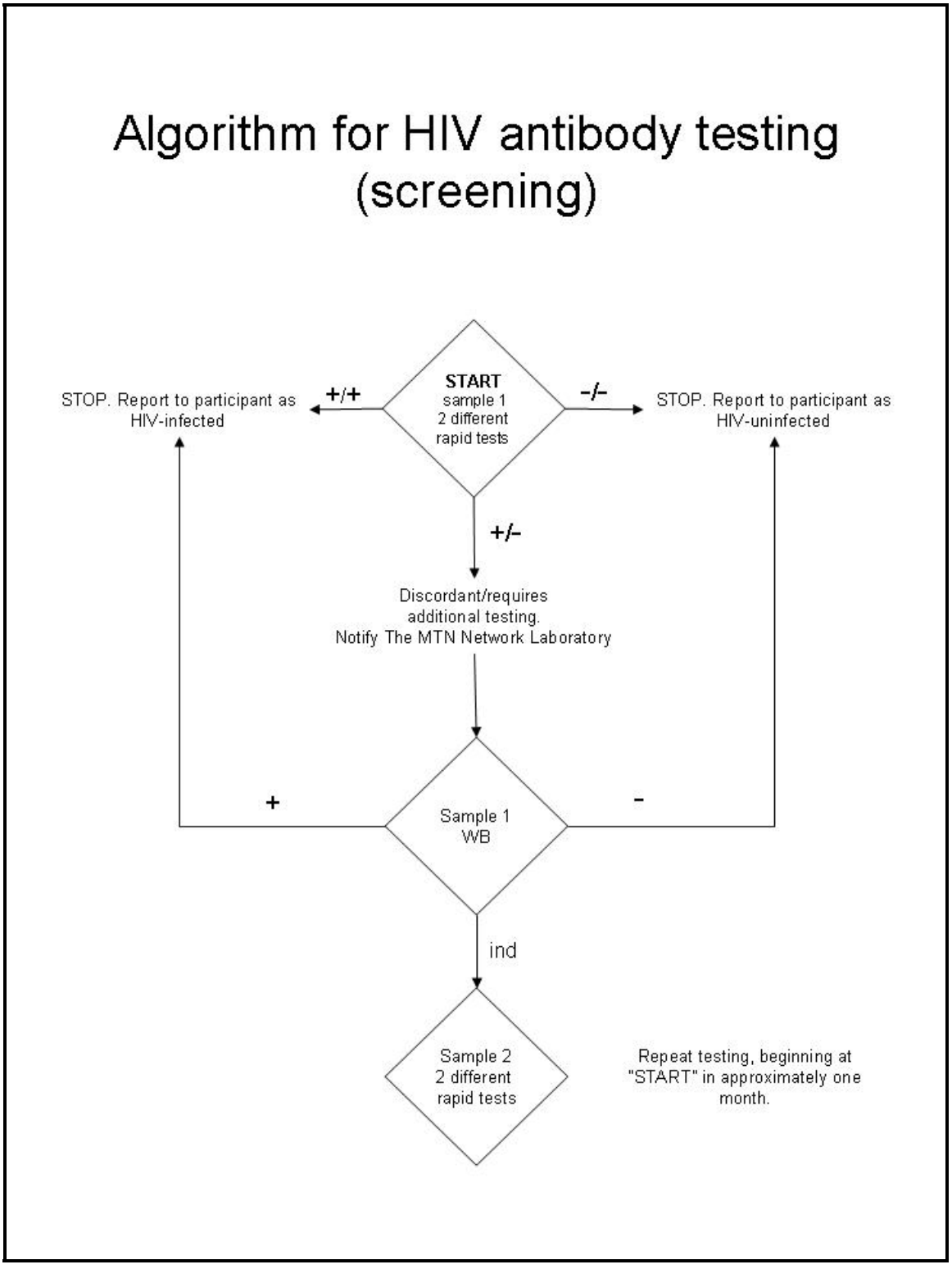
- Use a pencil to write the PTID and specimen collection date on one side of the frosted end of one microscope slide. Affix a SCHARP-provided PTID label to the other side of the slides (on the frosted end, under the pencil markings) and write the specimen collection date in indelible ink (e.g. Sharpie pen) on each label. Also write "V" for vaginal on each label.
- Immediately following specimen collection from the lateral vaginal wall via swab, roll the swab across each of the slide. (Be sure to collect the specimen from opposite the vaginal wall used for the wet mount specimen collection.) 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 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.
- Place one slide in a plastic slide holder and send to the MTN NL at Magee-Womens Research Institute with the vaginal swab for culture. (See shipping instructions below).
- The slide from the screening visit should be sent with the enrollment visit specimens.
- Store the second slide in the slide box location assigned in LDMS at room temperature. (This is a backup slide incase the first is lost or unreadable).

12.7.6 Papanicolaou (Pap) Test

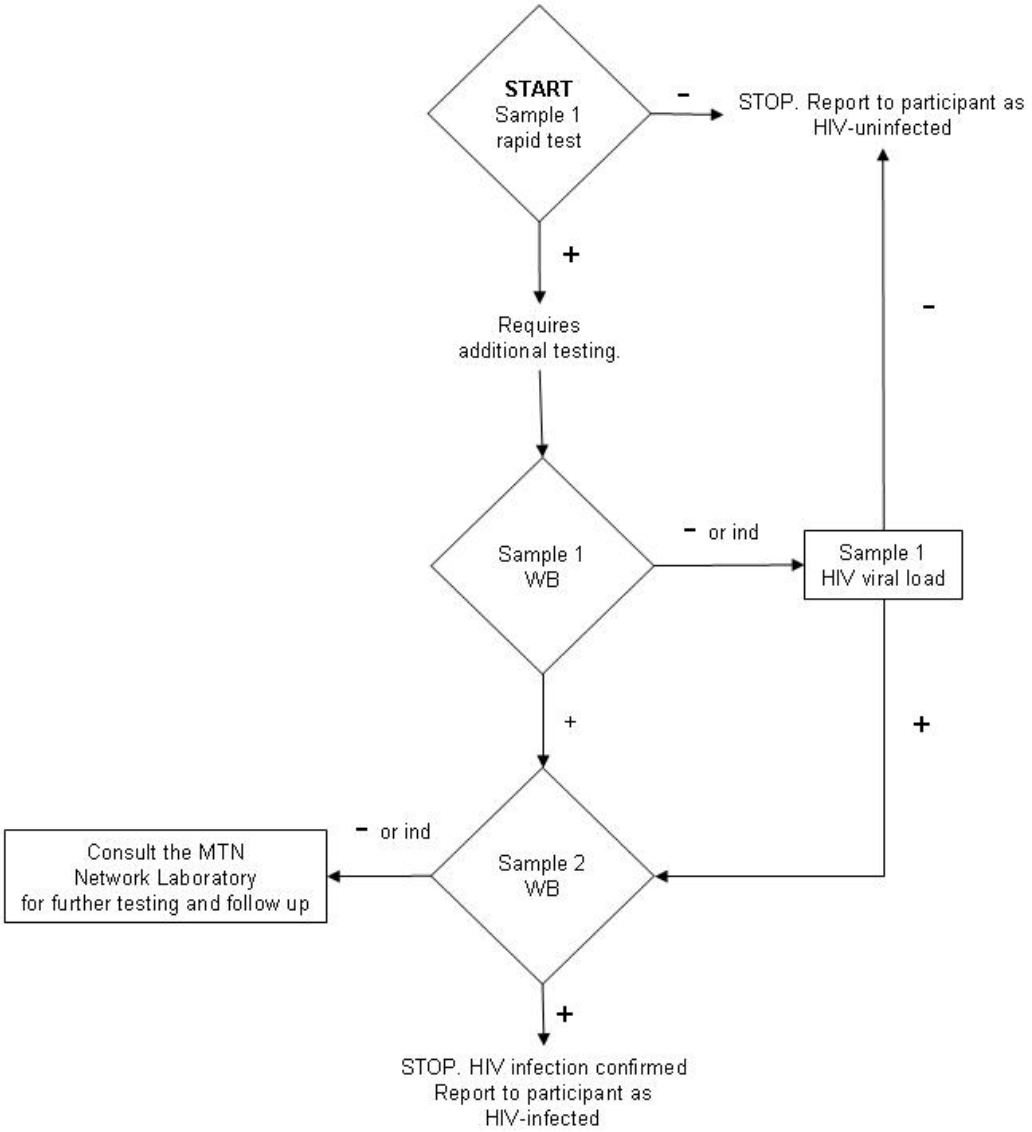
Pap smears will be performed at selected sites. At visits when Pap smears are required, ecto- and endocervical cells will be collected after all tissues have been visually inspected and all other required specimens have been collected. Specimen collection, slide preparation, slide interpretation, and 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 of any STi's during follow up.

Appendix 12-1 HIV Testing Algorithms



Algorithm for HIV antibody testing (follow-up and primary endpoint determination)



Appendix 12-2 Specimen Requirements Overview

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)
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	Variable	N/A	Locally in real time	TBD
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	N/A
Vaginal Swabs	Vaginal Fluid Swab	Cryovial	N/A	Stored Locally for Shipment	Freeze Within 8 hours
Endocervical swabs	Endocervical swabs	Cryovial	N/A	Stored Locally for Shipment	Freeze Within 8 hours

MTN 003 LAB SPECIMEN PROCESSING GUIDELINES-BLOOD SPECIMENS

Assay	Additive/Container	Minimum Volume	Testing Specifications	Handling Requirements
AST, ALT, Phosphorus and Creatinine	Plain Tube-No additive	Locally defined	Locally in real time	Locally Defined
Syphilis Serology	Plain Tube-No additive	Locally defined	Locally in real time	Locally Defined
HBsAG and HBsAB	Plain Tube-No additive	Locally defined	Locally in real time	Locally Defined
Full Blood Count	EDTA Tube	Locally defined	Locally in real time	Locally Defined
HIV-1 Screening and Confirmation	EDTA Tube	Locally defined	Locally in real time	Locally Defined
Flow Cytometry (CD4)	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	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	EDTA Tube	50 mls whole blood	Stored and shipped for analysis in batches.	Snap freeze should commence within eight hours of collection.