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10. Introduction

This section provides information and instructions for site clinical and laboratory staff related to the processing, storing, shipping and testing of MTN-042 laboratory specimens. Additional information for collection specimens from participant can be found in SSP Section 7 Clinical Considerations.

10.1. Overview and General Guidance

MTN-042 Clinical research sites will complete the MTN-042 Laboratory Activation Checklist prior to study activation. This will document MTN Laboratory Center (LC) approval of readiness for activation as described in the MTN Manual of Operational Procedures, Section 14.9.

As transmission of HIV and other infectious agents can occur through contact with contaminated needles, blood, and blood products, 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/hai/

Laboratory procedures may be performed in the study site clinics or laboratories, approved commercial laboratories and in the MTN Laboratory Center (LC), including the MTN Colorado Antiviral Pharmacology Laboratory (CAVP), Clinical Pharmacology Analytical Laboratory (CPAL), and MTN Virology Core (at the University of Pittsburgh). Appendix 10-1 and Appendix 10-2 highlight specimen, storage and shipment requirements.

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

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

Note: Additional blood may be collected for any clinically indicated testing.

All site laboratories will be monitored by the MTN LC 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 LC using the following email address: mtnnetworklab@mtnstopshiv.org

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 LC must be notified before the change and can provide further guidance on validation requirements. Similarly, the MTN LC must be notified 1 week before any reference (normal) range changes are made.

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

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 endpoint data derived from laboratory testing will be considered acceptable to all regulatory authorities across study sites.

This section of the 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 site Standard Operating Procedures (SOP) for specimen management, processing, and testing.

10.2. Specimen Labeling

All containers into which specimens are initially collected will be labeled with SCHARP-provided participant ID (PTID) labels. SCHARP will provide 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.

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 swabs
- Plasma
- Dried Blood spots
- Intravaginal rings

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

10.3. 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.

10.4. Use of LDMS (Laboratory Data Management System)

LDMS is a program used for the storage and shipping of laboratory specimens and supported by the Frontier Science Foundation (FSTRF). LDMS must be used at all sites to track the collection, storage, and shipment of the sample types described in Table 10-2.

Detailed instructions for use of LDMS are provided at: https://www.ldms.org/.

All sites will be required to maintain the current version 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. Samples must be separated by sample type when storing.

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). Backup and export are not required if sites are running Web LDMS.

Sites that have transitioned to Web LDMS can use the code LLT for for samples tracked in LDMS for Local Laboratory Testing that are not stored per protocol requirements. (Example HIV RNA).

LDMS records are used by the MTN Statistical Data and Management Center (SDMC) to generate monthly specimen discrepancy reports. These are used to resolve data entry errors when comparing information between LDMS and study case report forms (CRF). Sites are expected to resolve all discrepancies within two weeks of receipt of the report. MTN LC, while collaborating with the SDMC, will monitor this process and assist sites as needed to meet that timeline. All corrective action is documented in paper-based clinic and/or laboratory records as appropriate and entered in the details section of LDMS.

Questions related to use of LDMS in MTN-042 may be directed to MTN LC (or LDMS Technical User Support). Usual business hours for LDMS User Support are 7:00 am - 6:00 pm (US Eastern Time) from Monday through Friday. All other hours and weekends, an on-call user support -specialist will be available. Contact LDMS User Support at:

Email: ldmshelp@fstrf.org
Phone: +1-716-834-0900, ext. 7311 Fax: +1-716-898-7711

10.5. Documentation

Each lab test must have a defined source document that is the first place the result is recorded or generated; this must be described in an SOP. There must be quality control systems in place to ensure that results transcribed from source documents agree with reports going to clinics. Other laboratory records such as quality control results and calibrations should also be treated as source documents. Site labs will have a plan for the storage of these documents so that they are easily retrievable for auditors and network oversight visits.

All staff reporting results must:
- Be listed on site delegation logs
- Have documentation of training before reporting results
- Have documentation of competency assessment before reporting results, 6 months after training and annually thereafter

In most cases, lab results will be recorded from source document to CRF without any unit conversion. If unit conversion is required from source document to CRF, this should be automated rather than done manually. Contact the management team at mtn042mgmt@mtnstopshiv.org if you have questions.

10.6. 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.

Collect urine specimens before collecting any pelvic specimens. Heavy menses may interfere with pregnancy tests – sites should use discretion and contact the MTN LC if there are questions.

10.6.1 Specimen Collection

- As possible, the participant should not have urinated within one hour prior to urine collection.
- Provide the participant with a plastic, preservative-free screw-top urine collection cup labeled with a SCHARP-provided PTID label. Use a sterile container if culture is being performed.
- Collect and process urine samples per SOP (s) to accomdate all tests being performed.

10.6.2 Pregnancy Testing

Pregnancy testing is offered at the 6-week PPO visit. If the urine pregnancy test cannot adequately be interpreted because of interfering factors, for example excess blood or extreme cloudiness due to amorphous material, the sample can be spun down and the urine supernatant can be used. If the test continues to have interferences such as gross hemolysis making the test difficult to read, then 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, unless alternate kit approved by the LC. 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 LC or your Primary Network Lab (PNL) in case of questions regarding your EQA panels.

10.6.3 Urine dipstick

Sites will use a urine dipstick that contains at a minimum glucose, protein, leucocytes and nitrites.

10.6.4 Urine Culture

Perform urine culture per local standard of care if ordered by clinician for clinical indications.

10.7. 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.

10.7.1 Specimen Collection and Initial Processing

Sites must have processes in place to avoid specimen labeling errors. The MTN strongly recommends that specimens not be labeled in advance of collection. Specimen labeling must occur immediately at the time of collection. Participant Identification must be re-established each time a specimen is collected.

Sites should have local SOP's to describe procedures for infant blood collection (e.g. through heal sticks and/or venipuncture).

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

- Allow plain tubes (red, tiger top or gold top SST non-additive tubes or serum separator tubes) to clot, then centrifuge per site SOPs to yield serum. Serum may be used for tests such as chemistry or syphilis serology as defined in local testing SOP.
- Gently invert EDTA 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 plasma aliquots are made. If whole blood is to be used for multiple tests, ensure that the tube is well mixed before removing any specimen.

When collecting infant blood samples, if enough blood is not obtained for all required samples, sites should prioritize samples for diagnosis and treatment over research-only samples. This may not always be possible depending on the situation and while mainating proper tube collection order. Prioritziation of samples would be:

- HIV rapid and/or confirmatory samples
- Creatinine
- AST/ALT if indicated
- CBC if indicated
- Plasma storage (could be for resistance or DPV PK)
- DBS

10.7.2 HIV Testing-ADULT

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 SSP Appendix 10-3.

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 rapid 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 rapid test results within the specified timeframes and prior to disclosure of results to participants; this documentation must include the read time for the second checker.

Site laboratory management is responsible to ensure that all HIV testing is done per manufacturer's directions.

Send all HIV testing queries and algorithm related notifications to mtnvirology@mtnstopshiv.org using the MTN Laboratory Core HIV Query Form (Appendix 13-5).

At any time point where HIV rapid testing is performed, two different rapid tests will be used:

- The first rapid test will be the (Fourth Generation) CE-marked Alere HIV Combo rapid test, Alere product number 7D2846 (20 tests) or 7D2847 (100 tests). The Alere Company was purchased by Abbott and the HIV Combo kit was renamed Abbott Determine™ HIV Ultra. The Abbott 24SEP20 letter of equivalence should be kept on site for auditing purposes. *Note that there are several different Abbott/Alere products with similar names and formulations. It is imperative to obtain this specific kit.*
- Sites may use the WHO-prequalified version of the kit with catalog numbers 7D2842, 7D2843 if the CE-marked version is not available. The Abbott 13MAR20 letter of equivalence should be kept on site for auditing purposes.
- The second rapid test will be an FDA approved (Third Generation) test, either OraQuick, Unigold or other kit approved by the MTN LC. *Note that there are FDA and non-FDA approved versions of these kits – please ensure that only the FDA-approved version is obtained.*
- In cases of potential kit shortages, sites must contact the MTN LC for guidance on backup kit selection. A backup third generation test may be used if the fourth generation kit is unavailable; the LC must be notified via email if this occurs and may send additional guidance.

Study participants may report potential exposures to HIV that would increase the likelihood they are acutely infected during screening, enrollment or post enrollment study visits. Participants may also present with signs and symptoms that are consistent with acute infection. In these cases, even if the participant has 2 negative rapid test results, site clinicians may request an HIV RNA be performed which may detect infection before other tests. For RNA tests done at the clinician's discretion for suspected acute infection, sites must notify the MTN Virology core with an HIV Query form. The Virology core will track these cases and send guidance as needed.

SCREENING/ENROLLMENT

Sites will use two rapid HIV tests at screening and enrollment, the fourth generation CE Marked Alere Combo and a third generation FDA-approved test.
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:

- Inform the MTN LC by submitting a query form (Appendix 13-5) to mtnvirology@mtnstopshiv.org. The MTN LC will send guidance within 1 business day.
- In common circumstances, guidance from the MTN LC will be to collect blood and perform a Geenius confirmatory test and plasma viral load (HIV RNA PCR). The site may immediately proceed with these tests as part of HIV status determination as long as a query form is also submitted on the same day. Please note, the participant has not completed enrollment procedures, so blood or plasma may not be stored for future testing at this time.

FOLLOW UP

Sites will use two rapid HIV tests at each follow up visit the fourth generation CE Marked Alere Combo and a third generation FDA-approved test.

If the rapid tests are negative, the participant will be considered HIV-uninfected.

If both rapid tests are positive, the CE marked Geenius confirmatory assay will be performed from a separate blood draw which is collected on the same day. With this additional sample, the site will also collect blood for CD4, viral load (HIV RNA PCR), plasma storage, CBC with platelets, AST/ALT, Blood creatinine and calculation of creatinine clearance, and Collection of drug level and biomarker specimens. If the site is unable to collect the sample because the participant is unwilling or other reason, they should try to recall the participant as soon as possible.

If one rapid test is positive and one rapid test is negative (discordant results), follow procedures identical to those after two positive rapids. Collect blood from a separate blood draw on the same day and proceed immediately with Geenius testing, CD4, and HIV RNA viral load at the local lab. You may also notify the MTN LC using the query form (Appendix 13-5) if technical guidance is needed. However, do not wait for MTN approval or MTN LC response to the query form to proceed with Geenius, CD4,HIV RNA viral load testing, CBC with platelets, AST/ALT, Blood creatinine and calculation of creatinine clearance, and Collection of drug level and biomarker specimens. SCHARP will send the LC monthly reports of discordant rapids encountered during follow up. The LC will monitor these reports and may request kit lot information from sites.

If the Geenius is positive, HIV infection is considered confirmed for study purposes per the algorithm.

If the Geenius is negative or indeterminate, notify the MTN LC using the query form (Appendix 13-5) and use the results of the HIV RNA viral load to determine the need for further testing. A viral load result above the limit of detection will be considered positive and the Geenius will be repeated on a new sample taken approximately 1 month later for confirmation. A viral load result below the limit of detection will be considered negative; based on this result, the participant will be considered HIV-uninfected. A viral load result of “detected, below the limit of detection” may require further guidance before HIV status is finalized.

When collecting blood to repeat Geenius, even though seroconversion is not yet confirmed at this point, collect additional blood for post seroconversion sample testing (CD4, RNA and plasma storage) along with the repeat Geenius. Testing for the RNA and CD4 should proceed immediately.

HIV DNA testing will only be used in rare circumstances where HIV infection status cannot be determined from Geenius and HIV RNA viral load results (for example, if Geenius is indeterminate with one major band such as p24, and HIV RNA viral load is detected but below the limit of detection). Samples for DNA testing can only be collected with approval from the MTN LC.

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

Additional Guidance

- Fourth Generation rapids
    - Fourth Generation rapids contain two test bands: one for antigen and one for antibody
    - For purposes of the testing algorithms, if either test line is present, the result is positive.
    - Testing logs need to differentiate Antigen positive, Antibody positive, Dual positive, negative.
    - The Quality Control must include antigen and antibody positive samples. Sites will use the Abbott/Alere Combo control (Cat# 7D2252). Alternate controls require LC approval.
- Geenius
    - Manual reading and reporting of test cassettes is not allowed in MTN-042. In the event that the Reader Instrument is down, sites must revert to backup.
    - Sites must maintain printouts of Geenius reports that include the PTID, Visit code, sample date and testing date.

- QC must be run each week study participants are tested, in addition to requirements in the package insert.
- The LC suggests labelling cassettes so that the label can be seen in the image displayed on the Geenius reports.
- HIV-1 Indeterminate results
    - Staff members should be observing the cassette when they place it in the instrument.
    - If no bands are visible on the cassette but the reader gives and indeterminate result, the technician can cancel the run and re-read the cassette. This must be done within the allowable read time for the cassette.
        - If the second read is negative, this result can be accepted.
        - If the second read is still indeterminate:
            - Accept the result
            - Proceed with the algorithm and notify the LC as required for enrolled participants.
- HIV-2 positive or indeterminate results
    - HIV-2 Positive results: if the Geenius reports an HIV-2 positive result, contact the LC with an HIV query form. The LC will send guidance on a case by case basis. Wait for guidance from LC before proceeding with the algorithm.
    - HIV-2 Indeterminate results:
        - If no bands are visible on the cassette but the reader gives an indeterminate result, the technician can cancel the run and re-read the cassette. This must be done within the allowable read time for the cassette.
        - If the second read is negative, this result can be accepted.
        - If the second read is still indeterminate:
            - Accept the result
            - Notify the LC as required for enrolled participants. The LC will send guidance on a case by case basis. Wait for guidance from LC before proceeding with the algorithm.

NOTE: HIV-2 is rare in the countries where MTN-042 is conducted, but all HIV-2 positive or indeterminate results must be evaluated. In cases of HIV-2 positive or indeterminate results, product should continue to be held and the MTN-042 PSRT consulted on further product use management, including progression to permanent discontinuation if HIV-2 infection is confirmed, and clinical care (see also SSP section 8.26).

10.7.3 HIV Testing-INFANT

HIV testing will occur on infants of HIV positive mothers. MTN-042 Infant HIV testing and diagnosis will be performed per local standard of care. This typically involves HIV DNA but may include other tests.

10.7.4 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.

10.7.5 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
    - 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.

10.7.6 Serum Chemistries

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

- Aspartate aminotransferase (AST)
- Alanine transaminase (ALT)
- Creatinine : Note SCHARP has an online tool that can be used for creatinine clearance calculation: https://atlas.scharp.org/cpas/project/Collaborators/Lab%20Unit%20Conversion%20Tool/begin.view?

10.7.7 Plasma Storage

Note: in MTN-042, the term “plasma archive” will only be used for enrollment storage. Plasma will also be collected for dapivirine levels and in response to positive HIV tests or confirmed infection.

For plasma storage, 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 placed 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 LC.

Table 10-1 notes the required plasma volumes to be drawn, where to record plasma storage on CRF and required codes to be entered into the “Other SPEC ID”. The Specimen Storage CRF has multiple fields for plasma storage.

Table 10-1: Plasma Storage Volume, CRF linkage and LMDS Other SPEC ID Code Guide

Plasma Specimen	Draw volume	Minimum Plasma Required	Record Storage on this CRF	LDMS “Other Spec ID”
Enrollment archive	~10 mL	4.0 mL	NA	Specimen Storage
Follow-up HIV testing algorithm Storage	~15 mL	6.0 mL	CON	HIV Confirmatory Results
Post-seroconversion	~15 mL	6.0 mL	SER	Seroconverter Results
Dapivirine Levels-adults	~ 4 mL	2.0 mL	PK	Specimen Storage
Dapivirine Levels-infants	~ 2 mL	0.75 mL	PK	Infant Specimen Storage

For all types of plasma listed in Table 10-1:

- If the minimum volume specified in Table 10-1 is not obtained, notify the MTN LC.
- Use LDMS to label and track all aliquots.
- Store all aliquots frozen on site ≤ -70°C.
- The MTN LC will send instructions when shipping and/or testing is required.

For routine plasma storage, standard processing per site SOPs should be performed.

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.

Plasma must be stored in 1 ml aliquots. Prepare as many 1 mL aliquots as possible.

Plasma storage is allowable in the protocol at any visit after enrollment "as indicated". Any positive HIV test results after enrollment is to be considered an indication for plasma storage. Store plasma for all post enrollment positive HIV results.

Short draws / missed collections:

If adult plasma collection is less than the required amount by ≥ 1mL, contact the LC immediately for guidance.

Contact the LC for any infant draws that do not meet minimum requirements.

Leftover Specimens: Leftover specimens may be temporarily stored for site QA purposes and problem resolution for all participants. This process must be described in an SOP or on-site policy that indicates how long the samples will be stored. Local guidelines and regulations must be followed in these situations. Only specimens from participants who have consented to long-term storage may be stored longer for future research. Sites that save these specimens for long-term storage must have a plan to identify which participants have consented to this. Contact the management team for assistance as needed.

10.7.8 Dry Blood Spots (DBS)

DBS will be used for determining the concentrations of tenofovir, emtricitabine and/or tenofovir diphosphate.

Procedure for Dried Blood Spot processing, Storage, and Shipping

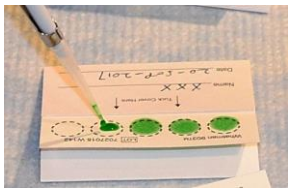
Supplies:

- 4 mL (smaller volume tubes acceptable) EDTA tube for adults
- 0.5-1.0 mL EDTA tubes or microtainers for infants (250 µL required for the procedure)
- *Whatman Protein Saver Card #903 (Whatman 10534612 or Fisher Scientific #05-715-121)
- Gas-impermeable plastic sealable storage bag (LasecSA) or Whatman Plastic Zipper Seal Sample Bags (Whatman 10548232 or Fisher Scientific#50-853-570)
- Desiccant pack (Gel Silica Sachets – 1 gm) (LasecSA or Whatman WB100003 or Fisher Scientific#09-923-360)
- Humidity indicator cards (Multisorb Des Manufacture # MS200032, DESCO Industries #13870 or Fisher Scientific # NC0281067 or NC9511648)
- Whatman card drying rack (VWR catalogue # 89015-592)
- Power free (preferable) Latex or nitrile gloves
- Water proof marker
- 10-100 µL or 20-200 µL micropipette and appropriate tips with filters. Sites should check with local suppliers for appropriate tips for their micropipette

*Note: This procedure REQUIRES the use of this exact type of filter paper for sampling.

DBS Processing:

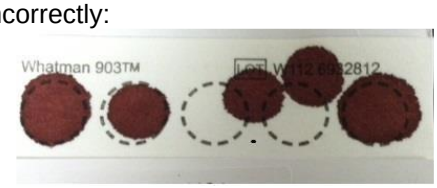
1. Gently invert the EDTA tube (8 to 10 times) to mix the blood thoroughly.
2. Within 4 hours (and preferably keeping the blood collection tube on ice), blood must be pipetted onto the filter paper spots in the card.
3. Pipette exactly 50 µL of the whole blood into each single spot on a PTID labeled Whatman Protein Saver 903 Card.
    - Performed with a calibrated 50 µL pipette and a disposable pipette tip using the wet tip technique (pre-wet the tip by aspirating liquid into the tip once and then dispensing all liquid out first, do this two to three times before dispensing into the circles).
    - Do not touch the filter paper with your hands.
    - Do not touch the card with the pipette tip.
    - Leaving the card slightly tilted may be beneficial for blood absorption.
    - Slowly expel blood from the tip and touch the drop to the paper, allowing the blood to absorb. Care must be taken when applying larger volumes of blood to ensure the spots do not run outside of the circle.
    - A single tip may be used to load the card.
    - Do not touch the DBS circle once blood is applied



4. All five spots on the card should be filled (each spot 50 uL).
    - If a difficult (short volume) blood draw was encountered, a minimum number of 50 uL spots is 3.
    - In the event you cannot fill 3 spots, fill as many as possible, store the sample and contact the MTN LC or MTN-042 management team for guidance.

Examples of DBS done correctly:

Examples of DBS done incorrectly:



5. Sample Drying:
    - Allow the blood spot to air dry without the card flap covering the spots in a clean, dry place (i.e. biosafety cabinet, drying racks) that is protected from rodents, insects and direct sunlight for at least 2 hours (drying overnight may be necessary in areas with higher humidity).
        - Do not heat, stack or allow DBS to touch other surfaces during the drying process.
    - Once confirmed to be completely dry, tuck in the flap of the Whatman Protein Saver 903 Card as indicated on the card to protect the samples from contamination.
    - Store the card in a PTID labelled sealed plastic bag with a desiccant pack (sachet of desiccant).
    - Do not store more than one card per bag.
    - Store with a humidity indicator card with each sampled card.
6. Specimen Storage**:**
    - If processing does not occur immediately, EDTA tubes are required to be refrigerated directly after being drawn.
    - DBS spots are stable for up to 7 days at ambient temperature, 2 months at 4.0°C, and 6 months at -20°C.
    - DBS processing sites are to store plastic bags (containing DBS, desiccant, and humidity cards) frozen at ≤-70°C.
    - Make sure the bags are sealed tightly to prevent deterioration due to moisture.

10.7.9 CD4+ T Cell Count

CD4+ T cell counts are only performed for participants in conjunction with 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.

10.7.10 HIV RNA PCR

HIV RNA PCR (viral load) testing is only performed for participants in the follow-up HIV testing algorithm, and during post-seroconversion follow up, if applicable, per protocol Section 7.6.1. On a case-by-case basis, sites may perform HIV RNA PCR on screening participants with discordant rapid test results, per guidance by the MTN LC.

All sites will participate in the Viral Quality Assurance (VQA) program. HIV RNA viral loads will be performed on EDTA plasma using methods approved by the MTN LC. All testing will be performed according to site SOPs and package inserts.

10.7.11 Hepatitis B Surface Antigen

Perform Hepatitis B Surface Antigen per local SOP.

10.8. Testing of Vaginal Specimens

Refer to the current Pelvic Exam checklist on the MTN-025 website under *study implementation tools* for further information on the required sequence of specimen collection and diagnostic procedures to be performed during scheduled pelvic exams.

When collecting multiple swabs, sites do not need to differentiate a different collection time for each swab. The collection time can be recorded as 1 time point for all swabs collected at one time. This could be either the end of the collection process or the beginning. If swabs are collected at different times during a visit because of specific circumstances, these different times should be noted.

Information on participant self-collection of multiple vaginal swabs can be found in SSP Section 7.12.5.

10.8.1 NAAT for GC/CT/Trich

Perform vaginal swab NAAT GC/CT/Trich per local SOP on platform approved by the MTN LC. In the event of laboratory supply chain disruptions, the MTN Laboratory Center can approve alternate methodologies to be used as backup. Contact the LC in advance of any possible supply chain disruptions.

10.8.2 Wet Mount for Candidiasis and BV

Wet mount testing for candidiasis and BV is only done when clinically indicated.

Wet mount procedures for this study consist of two different preparations —saline prep and potassium hydroxide (KOH) prep —for diagnosis of bacterial vaginosis, and candidiasis. Trichomoniasis may also be observed on saline wet mounts.

Prior to site activation and throughout the study, MTN LC requires semi-annual wet mount proficiency testing and administers a web-based proficiency test approximately every six months. The MTN LC will post wet mount slides on the MTN website for this purpose every 6 months; results will be entered directly on the website (contact: Michele Austin: maustin@mwri.magee.edu). The MTN LC will report results back to the Laboratory Manager and also specify any corrective action that may be needed based on the results. Contact the MTN 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.

Wet mount results are recorded directly onto appropriate laboratory log sheets or other laboratory source documents as specified in site SOPs.

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

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 for immediate examination.
- Use a pencil to write the PTID and specimen collection date on one side of the frosted end of two microscope slides (one for KOH and one for clue cells). 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.
- Remove the swab from the saline and smear vaginal fluid specimens onto each slide.
- Apply one drop of 10% KOH to one slide and immediately perform whiff test for a "fishy" amine odor. Then apply cover slip and allow a couple minutes for the bacteria and epithelial cells to lyse before reading.
- 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, budding yeast, and pseudohyphae. Examine at 40X magnification to determine whether observed epithelial cells are clue cells and quantitate the cells. Clue cells are irregularly bordered squamous epithelial cells that are completely covered with bacteria (*Gardnerella vaginalis*). Clue cells must comprise at least 20% of the observed epithelial cells for the saline prep to be considered positive for clue cells.
- Examine the KOH slide at both 10X and 40X magnification for yeast and pseudohyphae.
- Note: if motile trichomonads are noted on the saline wet prep, these can be reported to the clinician. If Trichomonas vaginalis is seen on the wet mount but the OSOM Rapid Trichomonas test is negative, report as positive by wet mount only

If wet prep slides are read in-clinic by clinical staff, results may be recorded directly on to STI Test Results CRF. If slides are read by lab staff (either in the local laboratory or a designated in-clinic lab area), results must be recorded on laboratory log sheets or other laboratory source documents and then transcribed onto the CRF.

10.8.3 Vaginal Swabs for Microbioata

- Refer to the current version of Section 7 of the SSP for specimen collection procedures.
- At each required timepoint, **2** dacron swabs will be collected for Microbioata.
- Place the swabs in an empty labeled cryovial with no preservative and cap the vial.
- Deliver the tube and an LDMS Specimen Tracking Sheet to the local LDMS laboratory.
- Using the LDMS Tracking Sheet, log the cryovial into LDMS (See Section Appendix 13-4 for LDMS for additive codes) and label the vial with a LDMS label.
- Freeze at ≤ -70°C within 8 hours of collection.
- Assign the code "**MIC**" in the "Other SPEC ID" in LDMS

10.8.4 Vaginal Swabs for biomarkers

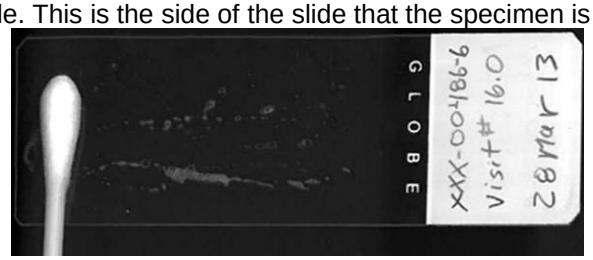
- Refer to the current version of Section 7 of the SSP for specimen collection procedures.
- At each required timepoint, **1** dacron swabs will be collected for Biomarkers.
- lace the swab in an empty labeled cryovial with no preservative and cap the vial.
- Deliver the tube and an LDMS Specimen Tracking Sheet to the local LDMS laboratory.
- Using the LDMS Tracking Sheet, log the cryovial into LDMS (See Section Appendix 13-4 for LDMS for additive codes) and label the vial with a LDMS label.
- Freeze at ≤ -70°C within 8 hours of collection.
- Assign the code "**BIO**" in the "Other SPEC ID" in LDMS

10.8.5 Vaginal Gram Stains

Dried vaginal fluid smears will be prepared for Gram staining and assessment for bacterial vaginosis at the MTN LC. Two slides (one designated as primary and the other as secondary) will be prepared using 1 swab. Both slides will be entered into LDMS. The primary slide will be shipped to the MTN LC and the secondary will be archived on site until written notification is received from the that the slide may be discarded.

Instructions for slide preparation and shipping are provided below:

1. Use a pencil to write the PTID and specimen collection date on one side of the frosted end of the slide. This is the side of the slide that the specimen is to be applied.

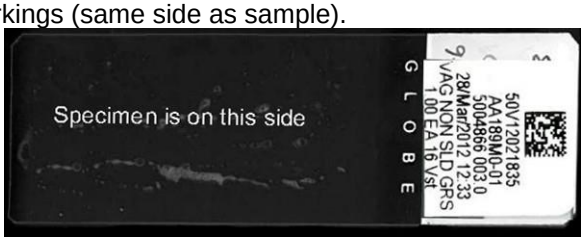


2. Immediately following specimen collection from the lateral vaginal wall via 3 turns of a swab (polyester or cotton), roll the swab across each of the slides. (Be sure to collect the specimen from opposite the vaginal wall used for the wet mount specimen collection.) Do not place the swab in saline, transport medium, or any transport container prior to slide preparation.

3. A SCHARP-provided PTID label is to be placed on the underside of the slides (on the frosted end, under the pencil markings); write the specimen collection date in indelible ink (e.g. pen) on each label.

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

5. Vaginal smears for gram stain are to be logged into LDMS (specimen type = VAG) and label the slides with LDMS labels. Place the LDMS label on the frosted end of the slide on top of the pencil markings (same side as sample).



6. The primary slides will be stored in a slide storage box and sent to MTN LC upon request. If possible, gram stain slides will be shipped with other samples that are to be sent to the Magee-Womens Research Institute. (See shipping instructions below next section).

7. Store the secondary slide in the slide box location assigned in LDMS at room temperature. (This is a backup slide in case the first is lost, broken, or unreadable).

10.8.6 Vaginal Fluid pH

Vaginal fluid pH will be assessed as part of on-site evaluations for bacterial vaginosis. pH Indicator Strips (pH range 3.6 to 6.1) with brand names S/P Cardinal Health, Baker-pHIX, Whatman, or Machery-Nagel must be used at all sites.

Vaginal fluid pH swab (polyester or cotton) may be collected in any of 3 ways depending on that particular visit:

1. Obtained by the clinician during the pelvic examination
2. Collected by the clinician in a non-speculum exam
3. Self collected by participant

Note: a speculum is not required for pH sample collection.

Vaginal Fluid pH Procedure (Clinician Collected):

1. Swab onto the pH strip (Do not insert the pH strip into the vagina).
2. Match the resulting color of the indicator strip to the color scale provided with the strips to determine the pH value.
3. Record the pH value directly onto the STI Test Results CRF. It is not necessary to record pH values onto laboratory log sheets or other source documents prior to recording values onto the CRF.

Note: If wet mount testing is required (if indicated), then the pH swab can be used for that testing.

10.8.7 Intra-Vaginal Ring Storage

All returned used rings will be stored in the laboratory. If returned rings are noted to have been partially eaten by rodents, these should not be sent to the lab for transport to Paraxel. The rings should be placed in Biohazard bin for destruction and documented per your site product accountability SOP. The ring should be noted as “not stored” in Rave with a comment.

If the damage is not noted in the clinic and the ring is transported to your local lab, the lab should notify the clinic and destroy the ring. If the ring has already been entered in LDMS, change the sample status to DSR and add a comment.

Please notify the LC when rings are noted to have been damaged by rodents.

The key outcome of this process is storing a dry ring to prevent microbial growth on the ring.

Returned Ring Processing:

1. All used returned rings are transported to the laboratory labeled with PTID, Date, Visit code.
2. Rinse the ring in water.
    a. If not processing in a biological safety hood the person should wear protective eye wear, lab coat or gown, and gloves when rinsing. Do not rinse in a sink because the ring is covered with potentially infectious material.
    b. To prevent aerosols, place the ring in a disposable container with tap water, swirl the ring gently, remove and blot dry with disposable paper towels.
    c. Discard the towels with other biohazardous material. Decontaminate the water used for rinsing before discarding per local guidelines for biohazard waste disposal. Decontaminate the area used to process the ring.
    d. Do not use any soaps, cleaners or chemicals to rinse the ring. Use only tap water.
3. Place the ring in a new unused bag.
4. Affix a label to the new bag with PTID, visit code and date.
    a.
5. Store the ring at room temperature.
6. Shipping guidance will be provided by the LC.

10.9. General Specimen Shipping Specifications

General Specifications

- All samples will be stored on site until the MTN Laboratory Center or MTN-042 Management Team requests a sample shipment.
- Ensure temperature sensitive shipments arrive on business days when receiving lab staff is available. When in doubt (such as holiday schedules), contact the receiving lab before scheduling the shipment.
- Copy the MTN LC on all MTN sample shipment notification emails, regardless of destination.
- Shipping labs are primarily responsible to confirm receipt of shipments. The LC may also track this.
- Sites must QC shipments before sending, including but not limited to:
    - If there is a shipping request, make sure that all requested samples have been included. If you do not have samples available for any of the requests, contact the LC for guidance. Maintain the LC response for documentation.
    - Make sure to include the number of aliquots indicated on the sample shipping list (Excel sheet attached).
    - Make sure that all physical aliquot global ID's match exactly what is on the shipping manifest, including their positioning in the box.
    - Shipping Box structure and labelling must meet all IATA and local regulations for the type of shipment being sent.
    - Make sure that all relevant import/export permits are included.
    - Cryobox Box labeling
        - The top of the box should have:
            - Site name
            - LDMS batch number
            - Box X of X (example: Box 1 of 2) for all boxes in the LDMS batch (at least the box number is required).
            - Studies (Protocol number)
            - Sample type
        - The side of the box should have:
            - Site name
            - LDMS batch number
            - Box number
    - You must notify the receiving lab when sending shipments. Make sure to include at a minimum:
        - Shipment date
        - Tracking information
        - Electronic version of shipping manifest: format may vary
        - Include a paper copy of the manifest or box map in the shipment in addition to other shipping documentation.
    - Shipment destination addresses are not included in this SSP because they may change. The LC will relay these to the sites via email.
    - Sites may use the Courier accounts only with written authorization. Sites will be invoiced for unauthorized charges.

Appendix 10-1:Overview of Laboratory Testing Locations, Specimens, and Methods for MTN-042

Assay	Testing Location	Specimen Type	Tube/Container	Kit/Method
Urine Pregnancy Test	Clinic/Local Lab	Urine	Plastic screw top cup	Quidel Quick Vue
Urine Dipstick	Clinic/Local Lab	Urine	Plastic screw top cup	Not specified
Urine Culture	Local Lab	Urine	Plastic screw top cup	Not specified
HIV Rapid Tests	Clinic/Local Lab	Plasma, Whole Blood, Or Serum	EDTA or plain tube	At least one FDA approved test
HIV Geenius	Local Lab	Plasma, Whole Blood, Or Serum	EDTA or plain tube	CE approved Geenius
Complete Blood Count	Local Lab	Whole Blood	EDTA tube	Not specified
Chemistries (AST, ALT, Creatinine)	Local Lab	Serum	Plain or serum separator tube	Not specified
HBsAG	Local 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	Approved method
HIV-1 DNA PCR*	Local Lab	Plasma	EDTA tube	Approved method
Plasma	Stored at Local Lab	Plasma	EDTA tube	N/A
Vaginal Gram stain	MTN Network Lab	Vaginal	Slide	MTN LC procedure
Vaginal wet preparation	Clinic/Local Lab	Vaginal Swab	sterile tube	Microscopy
Vaginal Swabs	Stored at Local Lab	Vaginal Swab	Cryovial	MTN LC procedure
Vaginal NAAT for Gonorrhea, Chlamydia and Trichomonas	Local, Regional, or MTN Network Lab	Urine	Plastic screw top cup	Cepheid Gene Expert or LC approved alternative method
Residual Drug Analysis	Stored at Local Lab	Vaginal ring	Biohazard Bags	Contract Laboratory procedure

***** These tests are only done for participants who have positive HIV rapid tests in the follow-up HIV testing algorithm and for post seroconversion follow up when applicable.

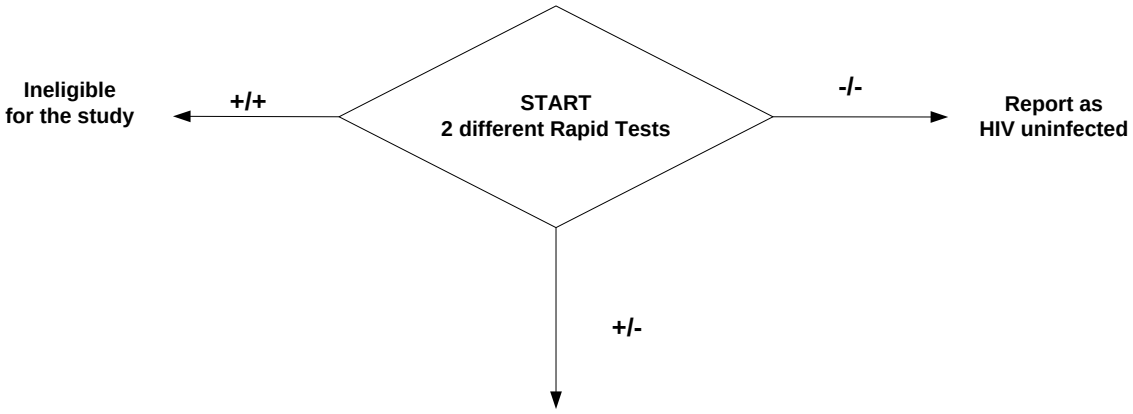
Appendix 10-2: LDMS Specimen Management Guide to Logging in MTN-042 Specimens

Sample	Primary	Primary Additive	Primary Volume	Primary Units	Other SPEC ID	Aliquot Derivative	Aliquot Sub Add/Derv	Aliquot Volume	Aliquot Units
Vaginal Swab (Microbiota)	VAG	NON	2	EA	MIC	SWB	N/A	2	EA
Vaginal Swab (Biomarkers)	VAG	NON	1	EA	BIO	SWB	N/A	1	EA
Enrollment Plasma	BLD	EDT	Variable	mL	NA	PL 1/2	N/A	1	mL
Plasma for HIV Algorithm*	BLD	EDT	Variable	mL	CON	PL 1/2	N/A	1	mL
Plasma for HIV Seroconverters*	BLD	EDT	Variable	mL	SER	PL 1/2	N/A	1	mL
Plasma for DPV Mothers	BLD	EDT	Variable	mL	PK	PL 1/2	N/A	1	mL
Plasma for DPV Infants	BLD	EDT	Variable	mL	PK	PL 1/2	N/A	0.75	mL
Dry Blood Spots	BLD	EDT	4	mL	NA	DBS	NA	250	µL
Ring for storage*	IVR	NON	1	EA	NA	IVR	NA	1	EA
Vaginal Gram Stain Slides	VAG	NON	2	EA	NA	SLD	GRS	1	EA

**Samples collected as required in protocol Section 7.7.1*

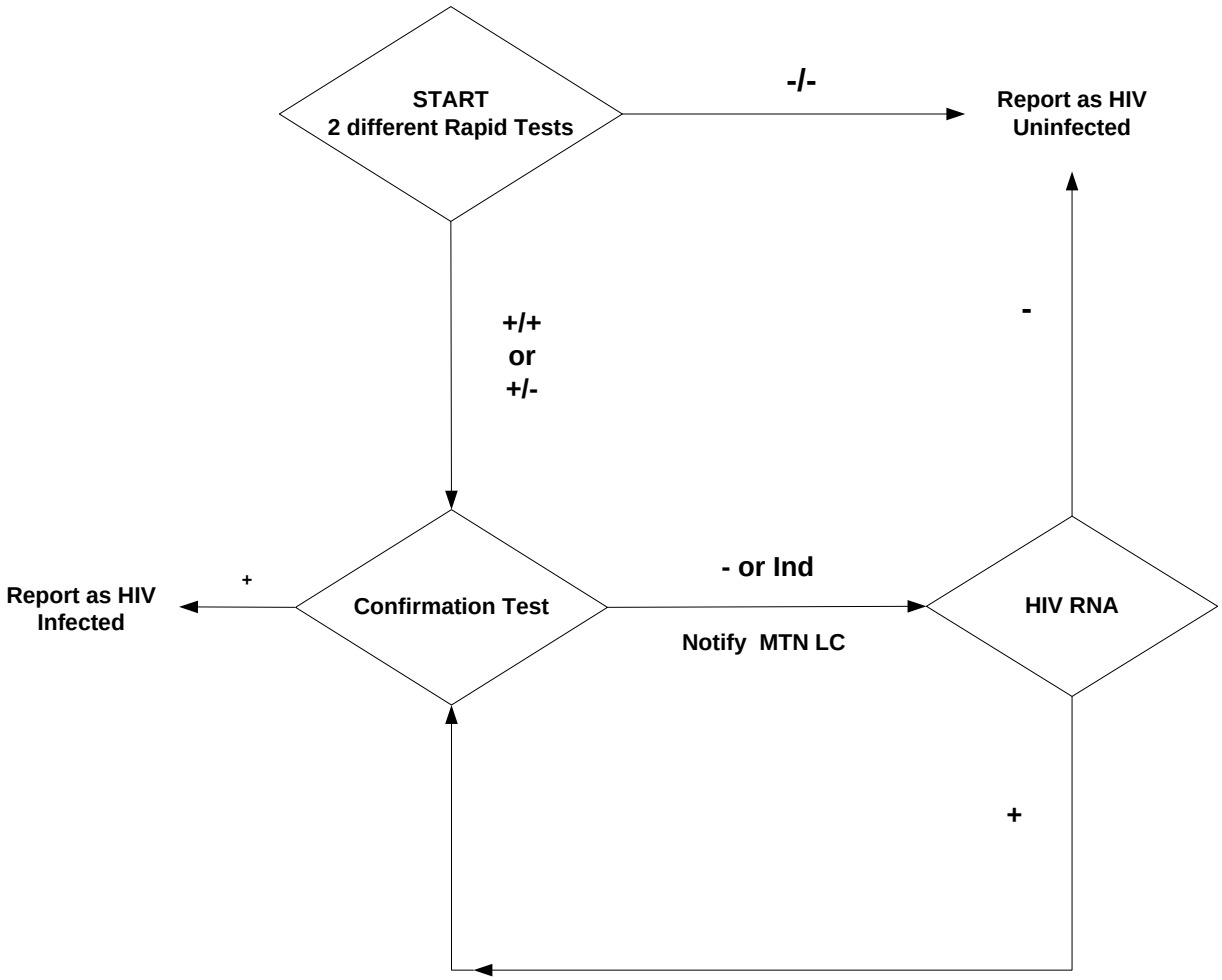
Appendix 10-3: MTN-042 HIV Testing Algorithms

ALGORITHM FOR HIV ANTIBODY TESTING- SCREENING/ENROLLMENT



Notify the MTN Laboratory Center for follow-up.

ALGORITHM FOR HIV ANTIBODY TESTING FOR FOLLOW-UP



Ind: Indeterminate test results
LC: Laboratory Center

Appendix 10-4: MTN Network Lab HIV Testing Query Form

MTN Laboratory Center HIV Testing Notification & Query Form

Study	MTN-042
PTID	
Site/Contact Person	
Last Update	Click here to enter a date.

Form Closed ☐

Please check one:
☐ Notification (LC response not required)
☐ Query (Waiting for LC Response)

VISIT CODE:

VISIT DATE: Click here to enter a date.

SITE COMMENTS/QUERY:

	Rapid Test 1	Rapid Test 2	Confirmatory Assay	HIV RNA	Other Test
Testing Date					
Kit Name			Geenius		
Result					

LC RESPONSE:

Participant Final Outcome
☐ HIV NEGATIVE
☐ HIV POSITIVE
☐ OTHER (Please describe further)

MTN-042 Visit LDMS Specimen Tracking Sheet

For login of stored specimens into LDMS

Participant ID ☐☐☐-☐☐☐☐-☐ (Site Number – Participant Number – Chk)

Visit Code ☐☐.☐☐

Specimen Collection Date ☐☐ ☐☐☐ ☐☐ (DD MMM YY)

# of TUBES or SPECIMENS	PRIMARY SPECIMEN	PRIMARY ADDITIVE	ALIQUOT DERIVATIVE	OTHER SPEC ID	COLLECTION TIME	INSTRUCTIONS FOR CLINIC and PROCESSING LAB
	Blood **(BLD)** *Enrollment plasma*	**EDT**	**PL1/2**	**N/A**	__ __:__ __ **hour : min**	Blood Collected at Enrollment: Leave "other SPEC ID" blank
	Blood **(BLD)** *Resulting from positive HIV test result*	**EDT**	**PL1/2**	**CON**	__ __:__ __ **hour : min**	When an enrolled participant has a positive HIV rapid test: use LDMS "other Spec ID" **CON**
	Blood **(BLD)** *For Dapivirine PK*	**EDT**	**PL1/2**	**PK**	__ __:__ __ **hour : min**	Adult and infant blood collected for Dapivirine PK: Use LDMS "other Spec ID" **PK**
	Blood **(BLD)** *Seroconverter Plasma Storage*	**EDT**	**PL1/2**	**SER**	__ __:__ __ **hour : min**	Blood collected after an enrolled participant been confirmed HIV positive: use LDMS "other Spec ID" **SER**
☐	Vaginal swab for Biomarkers **(VAG)**	**NON**	**SWB**	**BIO**	__ __:__ __ **hour : min**	Place Dacron swab in a labeled cryovial containing no additive. Store sample tubes at ≤-70°C.
	Vaginal Gram Stain **(VAG)**	**NON**	**SLD**	**NA**	__ __:__ __ **hour : min**	**Aliquot Sub Add/Derv: GRS** Make 2 slides (one Primary and the other Secondary).
☐	Vaginal swab for Microbiome **(VAG)**	**NON**	**SWB**	**MIC**	__ __:__ __ **hour : min**	Place 2 Dacron swabs in 2 labeled cryovials containing no additive. Store sample tubes at ≤-70°C.
	Dried Blood Spot **(BLD)**	**EDT**	**DBS**	**NA**	__ __:__ __ **hour : min**	After creating Dried Blood Spot Cards, store at ≤-70°C.
☐	Used vaginal ring **(IVR)**	**NON**	**IVR**	**NA**	__ __:__ __ **hour : min**	Store at room temperature.

Comments: _____

Initials: _____ (Sending Staff) _____ (Receiving Staff) **LDMS Data Entry Date:** ☐☐ ☐☐☐ ☐☐ (dd MMM yy) / _____ (LDMS Staff)

Version 1.4, 18JAN21