

Section 9. Laboratory Considerations

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9. Introduction

9.1 Overview and General Guidance

This section contains information on the laboratory procedures performed in MTN-035.

As transmission of HIV and other infectious agents can occur through contact with contaminated needles, blood, blood products, rectal, 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 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). Table 9-1 lists each test, the testing location, specimen type, specimen container and kit/method (if specified). Table 9-2 specifies specimen collection for storage and shipment.

Regardless of whether tests are performed in clinic or laboratory settings, study staff that performs the tests must be trained in proper quality control (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.

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.

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

This section of the MTN-035 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). The MTN LC is available to assist in the creation of any SOPs upon request.

MTN-035 clinical research sites will complete the MTN-035 Laboratory Activation Checklist prior to study activation. This will document MTN LC approval of readiness for activation as described in the MTN Manual of Operations 14.9.

**Table 9-1
Overview of Laboratory Testing Locations, Specimens,
And Methods for MTN-035**

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.

Test	Testing Location	Specimen Type	Tube or Container and tube size (recommended)	Kit/Method
Pharyngeal NAAT for Gonorrhea and Chlamydia	Local Lab	Pharyngeal swab	Kit Specific Transport Tube	GenProbe Aptima or GeneXpert
Qualitative Urine hCG	Local Lab/Clinic	Urine	Plastic screw top cup	Not specified
Dipstick Urinalysis	Local Lab/Clinic	Urine	Plastic screw top cup	Not specified
Urine Culture	Local Lab	Urine	Plastic screw top cup	Not Specified
Urine NAAT for Trichomonas	Local Lab	Urine	Kit Specific Transport Tube	GenProbe Aptima or GeneXpert
Urine NAAT for Gonorrhea and Chlamydia	Local Lab	Urine	Kit Specific Transport Tube	GenProbe Aptima or GeneXpert
Syphilis Serology	Local Lab	Serum or Plasma	EDTA, plain or serum separator tube 4mL	Not specified
HIV-1/2 Testing	Local Lab/Clinic	Plasma, serum or whole blood	EDTA or plain tube 4mL	Not Specified
Plasma archive/storage	MTN LC	Plasma	EDTA tube 10mL	MTN LC Protocol
Vaginal NAAT for Gonorrhea and Chlamydia	Local Lab	Vaginal swab	Kit Specific Transport tube	GenProbe Aptima or GeneXpert
Vaginal NAAT for Trichomonas	Local Lab	Vaginal swab	Kit Specific Transport tube	GenProbe Aptima or GeneXpert
Anal HSV 1 and 2	Local Lab	Anal Swab	Consult local lab requirements	Not specified
Rectal NAAT for Gonorrhea and Chlamydia	Local Lab	Rectal Swab	Kit Specific Transport tube	GenProbe Aptima or GeneXpert

Volumes may vary depending upon each site's testing platforms. Please confirm with the testing lab to determine minimum volume requirements.

Notes: Additional blood may be collected for any clinically indicated testing.
Red top tubes contain no additive.
Purple top tubes contain EDTA.

**Table 9-2
Overview of Specimens for Storage and Shipment**

Specimen and Subsequent Testing	Additive	Tube type or size recommendation	Processing and Storage	Ship to:
Plasma Archive / Storage	EDTA	1x10mL	Spin 10 minutes at 1500xg (or double spin at 800xg). Aliquot and freeze.	Batch to MTN LC

9.2 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 of specimen collection should also be included on the label. If the date is handwritten, it should be in indelible ink (such as a black Sharpie pen).

When specimens are tested at the local lab, any additional labeling required for on-site specimen management and chain of custody will be performed in accordance with site SOPs. Specimens that are sent to the LC or are archived at the site will be entered into LDMS (Table 9-3) and labeled with LDMS-generated labels.

9.3 Procedures for Specimens that cannot be Evaluated

Specimen collection will be repeated (whenever possible) if samples 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. In cases where additional specimens need to be recollected either due to a laboratory error (lost, broken tube, clerical, etc.) or clinic error, a protocol deviation form may be required.

The site is responsible for notifying the LC in the following cases

- Any time a participant must return to the clinic for specimen collection
- Insufficient blood volume is collected for the plasma archive
- Any time specimens have been mishandled, possibly compromised specimen integrity
- Any situation that may indicate a protocol deviation

If site staff has any question regarding time windows or collection processes, contact LC staff as soon as possible for guidance.

9.4 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 to track the collection, storage, and shipment of specimens in Table 8-3

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).

Each site must export its LDMS data to 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 the site. Sites are expected to resolve all discrepancies 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 discrepancy reports for critical samples (e.g., blood 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.

Questions related to use of LDMS in MTN-035 may be directed to Edward Livant or LDMS Technical (User) Support. Usual business hours for LDMS User Support are 7:00 am - 6:00 pm (ET) 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

**Table 9-3
 LDMS Specimen Management Guide to Logging in MTN-035 Specimens**

The table below should be used as a guide when logging in MTN-035 specimens for each test listed. Tests that are listed as “local lab” and specimens are not stored, and are not required to be logged into the LDMS. The LDMS Tracking Sheet can be found on the MTN website (www.mtnstopshiv.org) under the MTN-035 study implementation materials.

Test	Primary	Additive	Primary Volume	No. of Aliquots	Aliquot Volume	Units	Derv	Sub Add/ Derv	Other Spec ID
Plasma Archive or Storage	BLD	EDT	10.0 ML	4-5	1.0	ML	PL1/ 2	N/A	

BLD: Whole Blood
 EDT: EDTA
 PL1: Single spun Plasma
 PL2: Double spun Plasma

9.5 Urine Testing

In general, at study visits when urine testing is required, a single specimen will be collected and aliquots will be made for each test when possible. When doing multiple tests from one specimen, an aliquot of urine should first be obtained for pregnancy testing (for participants who are able to get pregnant only) and the remaining specimen should be reserved for Trichomonas, Chlamydia and Gonorrhea testing. Collect urine specimens before collecting any pelvic specimens. Heavy menses may interfere with dipstick and pregnancy tests – sites should use discretion and contact the MTN LC if there are questions.

9.5.1 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 female participants not to clean the labia prior to specimen collection. Male

participants should withdraw foreskin if present.

- 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 dipstick urinalyses and/or pregnancy testing is indicated, aliquot 5 to 10 mL for this test and store the remaining urine at 2-8°C or introduce the urine immediately into the collection device for subsequent *Trichomonas*, Chlamydia and Gonorrhea testing.
- **Note:** only in situations where there is no NAAT testing and a clinician suspects a urinary tract infection, specimens may be collected per local specifications such as mid-stream clean catch.

9.5.2 Pregnancy Testing

At visits when pregnancy testing is required, aliquot approximately 5 to 10 mL of urine from the specimen collection cup and pipette from this aliquot for pregnancy testing. If the urine 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.

9.5.3 Urine Chlamydia, Gonorrhea and *Trichomonas* Testing

This testing will be done using the Gen-Probe Aptima, Cepheid GeneXpert NAAT or method approved by the MTN LC in the local or regional laboratory per local SOP.

9.5.4 Dipstick Urinalysis

Perform this test according to site SOPs and the package insert. Assess and record results for glucose, protein, leukocytes and nitrites. To avoid overgrowth of bacteria, refrigerate specimen before and during transport to laboratory.

9.5.5 Urine Culture

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

9.5.6 Pharyngeal Chlamydia and Gonorrhea Testing

This testing will be done using the Gen-Probe Aptima, Cepheid GeneXpert NAAT or method approved by the MTN LC in the local or regional laboratory per local SOP.

Note: Sites may conduct testing while validation is in progress with MTN LC approval.

9.6 Blood Testing

The blood tests performed depend 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.

9.6.1 Specimen Collection and Initial Processing

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

After collection:

- Allow plain tubes (no additive or serum separator) to clot, then centrifuge per site SOPs.
- Lavender top tubes (additive = EDTA) should be gently inverted at least eight times after specimen collection to prevent clotting. 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 aliquoted. If whole blood is to be used for multiple tests, ensure that the tube is well mixed before removing any specimen.

Note: If locally available tube top colors do not correspond with the tube additives specified above, use appropriate tubes based on the additives, not the listed tube top colors.

9.6.2 HIV Testing

Although the HIV algorithm (Appendix II of the MTN-035 protocol) allows for EIA testing, rapid testing is recommended to obtain immediate results confirming participant eligibility throughout the study.

Non-CLIA sites performing HIV rapid testing must perform two HIV rapids, one of which is FDA approved.

HIV testing must be performed at the study site per the CLIA standards (US sites) and DAIDS laboratory policy (see MTN-035 protocol section 7.11). All tests, and associated QC procedures, must be documented on local laboratory log sheets or other laboratory source documents.

HIV infection status at screening will be assessed per the HIV testing algorithm (see Appendix II of the MTN-035 protocol).

- If the initial test(s) are negative, the participant will be considered HIV-seronegative.

If the initial test(s) are positive, discordant or indeterminate, a confirmatory test will be performed on the original sample; this is referred to as "Sample 1". If there is insufficient sample to perform confirmatory testing, then additional blood must be collected. If the confirmatory test is negative or indeterminate, contact the MTN LC for guidance.

- If Sample 1 confirmatory testing is negative or indeterminate, contact the MTN Virology Core (mtnvirology@mtnstopshiv.org) for guidance by submitting an MTN LC HIV Query Form, which can be found on the MTN website.
- If Sample 1 is positive:
 - Screening Participants: the participant is ineligible for enrollment.
 - Enrolled Participants: If the Sample 1 Confirmatory test is positive, the participant is recalled for a new blood draw (Sample 2) for local confirmatory testing. Collect plasma for storage per SSP Section 9.6.4 with Sample 2.
- If Sample 2 is negative or indeterminate, contact the MTN Virology Core (mtnvirology@mtnstopshiv.org) for guidance by submitting an MTN LC HIV Query Form, which can be found on the MTN website.

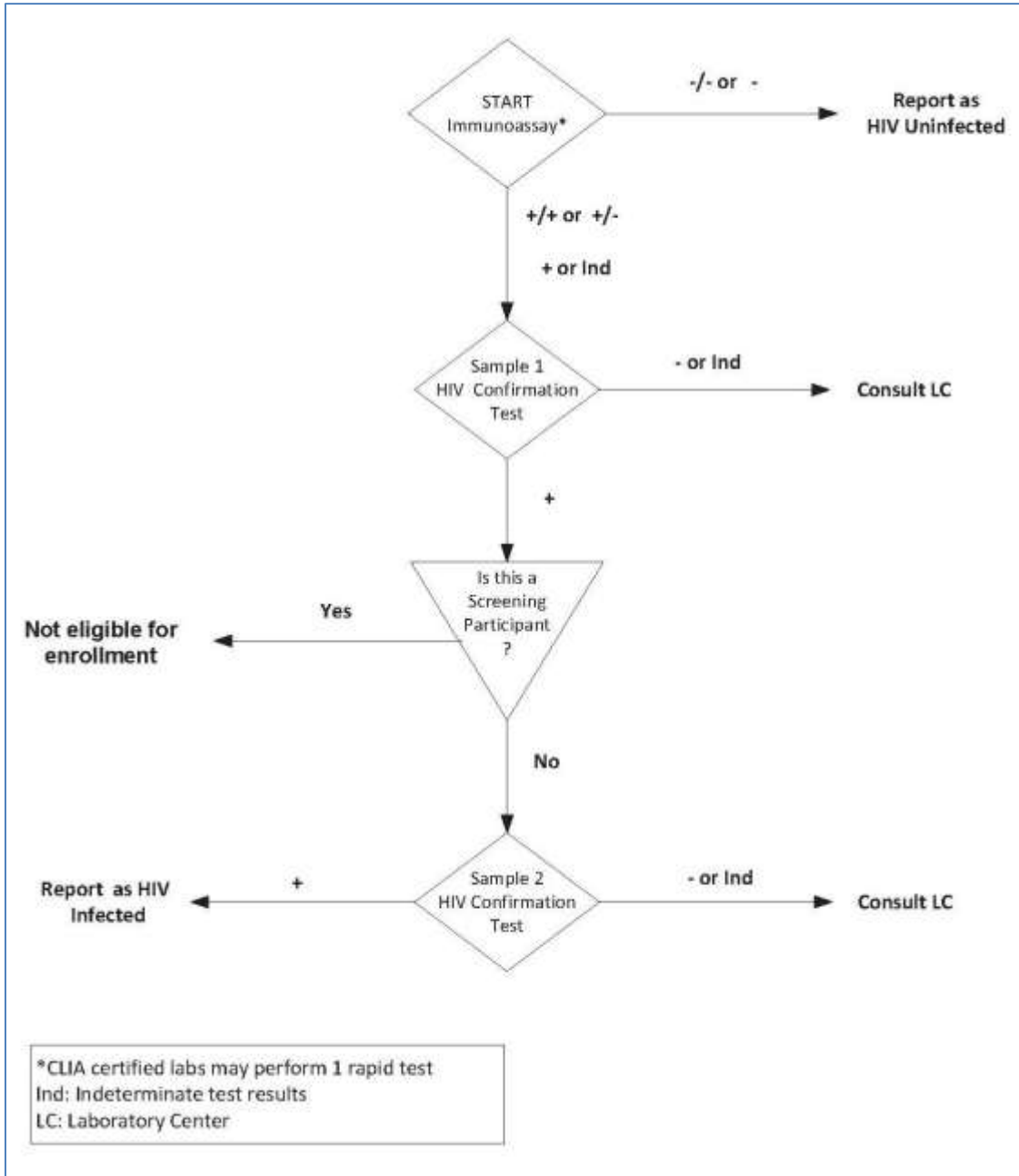
If Sample 2 is positive, the participant is considered HIV infected. Notify the MTN Virology Core (mtnvirology@mtnstopshiv.org) via e-mail of all possible seroconverters identified during a follow up visit by submitting an MTN LC HIV Query Form, which can be found on the MTN website.

Once the MTN Virology Core has reviewed the form, a request for plasma storage to be shipped on dry ice to the MTN Virology Core may be issued; the LC will send shipping instructions at that time. Be sure to provide the lab with the tracking number and details of each specimen prior to shipping.

All test results must be documented on local laboratory log sheets or other laboratory source documents. For non-CLIA sites, 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.

Figure 9-1

ALGORITHM FOR HIV TESTING FOR SCREENING AND FOLLOW-UP



9.6.3 Syphilis Testing

Syphilis testing can be performed using in one of two ways:

- Rapid Plasma Reagin (RPR) or Venereal Disease Research Laboratory (VDRL) screening test followed by a confirmatory test for *Treponema pallidum* such as the Enzyme Immunoassay (EIA), microhemagglutinin assay for *Treponema pallidum* (MHA-TP), *Treponema pallidum* hemagglutination assay (TPHA), *Treponema pallidum* particle

agglutination (TPPA), or fluorescent treponemal antibody (FTA-ABS). All positive RPR or VDRL results must have a titer reported. For reactive RPR or VDRL tests observed during screening, a confirmatory test is performed and appropriate clinical management action must be taken prior to enrollment in the study. MTN LC recommends for enrolled participants considered positive, repeat non-treponemal assay tests at quarterly intervals following syphilis diagnosis to evaluate treatment effectiveness. If the RPR or VDRL titer does not decrease four-fold or revert to seronegative within three months after treatment, further investigation and/or treatment may be warranted.

- Perform syphilis assessment using a specific treponemal test (such as EIA, MHA-TP, TPHA, TPPA, or FTA-ABS) and confirming positive test results with a non-treponemal assay (RPR or VDRL). If the confirmatory non-treponemal assay is reactive at screening visit, appropriate clinical management action must be taken. If the RPR or VDRL is negative, this may indicate prior treatment, late latent disease, or a false positive. MTN LC recommends additional testing using an alternative treponemal test other than the original treponemal test used for the original assessment so the participant can be correctly evaluated. (Of note, the FTA-ABS should not be used as the alternative confirmatory test due to performance issues). If the second confirmation test is negative, the participant is not considered infected with syphilis. If the second confirmation test is positive, the participant has had prior exposure to syphilis and depending on clinical scenario may or may not require treatment.

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

Questions related to result interpretation concerning eligibility and enrollment in the study should be directed to the MTN-035 Protocol Safety Physicians (mtn035safetymd@mtnstopshiv.org).

9.6.4 Plasma Archive (baseline) and storage

Plasma archive is required at Enrollment. Additionally, plasma for storage is required for further MTN LC HIV testing of enrolled participants with “Sample 2” of the HIV testing algorithm (see Appendix II of the MTN-035 protocol). Plasma may be stored on enrolled participants at additional time points only for specific indications; contact the MTN LC for guidance.

For plasma archive and plasma storage, use collection tubes with EDTA anticoagulant. Aliquot plasma into 2 mL cryovials, store at $\leq -70^{\circ}\text{C}$, and batch onsite until the MTN LC requests shipping and/or testing.

1. If sample is collected and held at room temperature, freeze plasma within 4 hours. If refrigerated or on ice after collection, freeze plasma within 24 hours.
2. If total whole blood volume is less than 2.0 mL, redraw as soon as possible.
3. Spin blood at room temperature in a centrifuge according to one of these techniques:
 - Single spun: Spin blood at $1500\times g$ for 10 minutes and remove plasma.
 - Double spun: Spin blood at $800\times g$ for 10 minutes, recover plasma and place in a tube to spin again at $800\times g$ for 10 minutes, remove plasma.
4. Prepare as many 1.0 mL aliquots as possible with a total volume of aliquots greater than or equal (\geq) to 4ml
5. If less than 4 mL of plasma is available, store that plasma and inform the MTN LC for instruction.
6. If samples are hemolyzed, store the aliquots as per normal and enter comments in LDMS.
7. The MTN LC will send instructions to the site when shipping and/or testing is required.

9.7 Testing of Vaginal and Cervical Specimens

Cervicovaginal specimens will be collected in the order and manner stated in Section 7 (Clinical Considerations) of this SSP.

9.7.1 Cervicovaginal Testing for GC/CT (Neisseria gonorrhoea and Chlamydia trachomatis) and Trichomonas by NAAT

Testing for chlamydia, gonorrhoea and trichomonas is performed using the Gen-Probe Aptima, Cepheid GeneXpert NAAT or method approved by the MTN LC in the local or regional laboratory per local SOP. When performing all three tests, more than one swab may need to be collected.

9.8 Testing of Anorectal Specimens

Rectal samples should be collected in the following order:

1. Anal swab for HSV 1/2
2. Rectal swab for GC/CT

9.8.1 Anal HSV-1/2

Testing will be performed from an anal swab collected per local SOP at sites with capacity only.

9.8.2 Rectal NAAT for Gonorrhoea and Chlamydia

This testing will be done using the Gen-Probe Aptima, Cepheid GeneXpert NAAT or method approved by the MTN LC in the local or regional laboratory per local SOP.

Note: Sites may conduct testing while validation is in progress with MTN LC approval.