Laboratory Manual: Specimen Management and Laboratory Related Procedures

Protocol Laboratory Considerations for HPTN111

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Definitions

Ab	Antibody
Ag	Antigen
AIDS	Acquired Immunodeficiency Syndrome
Ambient/Room Temperature	*From CLSI PRE04-ED1:2023* [1] *:* Thermostatically maintained indoor air temperature that encompasses the normal working environment of 20 to 25°C with brief deviations. For the purposes of PRE04, room temperature can range from 15 to 25°C. *A temperature of 20℃ / 68°F also aligns with the National Institute of Standards and Technology, Common Temperature Reference Point, Room Temperature (https://www.nist.gov/p mL/owm/si-units-temperature)* The HPTN LC considers room temperature (ambient) to be 15 to 30°C for sample handling, processing, and testing unless otherwise stated in this manual or by a manufacturer of the process or assay being performed.
BLD	Blood (Whole - Venous); LDMS sample code
CDC	(United States) Centers for Disease Control and Prevention
Chain of Custody (CoC)	A written process detailing sample management for the life of a sample, starting with collection and ending with storage and destruction, specific to a clinic, protocol, laboratory, or storage facility. May be referred to by an alternate document, such as Specimen Management Document.
Confirmatory Visit	For site or local HIV testing; A follow-up visit to collect a new sample and perform HIV testing that occurs on a different day from the first initially reactive or positive (Index visit/result) HIV sample.
CT	*Chlamydia trachomatis*
DAIDS	NIH NIAID Division of AIDS
DPE	Spray Dried EDTA; LDMS sample code
EDTA	Ethylenediaminetetraacetic acid; anticoagulant, chelating agent
FDA	(United States) Food and Drug Administration
GC	*Neisseria gonorrhoeae*, also sometimes abbreviated “NG”
GCLP	Good Clinical Laboratory Practices
HIV	Human Immunodeficiency Virus
HPTN	HIV Prevention Trials Network
In-Clinic Laboratory	A laboratory located within a clinic that only performs Rapid Testing, Point of Care testing, or limited same-day testing; may perform specimen processing, handling, storage and shipping; not a full or complex clinical support laboratory.
Instrumented Laboratory-Based Assay	Testing performed using automation that is not in a clinic and is not a rapid testing platform; not a Point of Care assay
K2/K3 EDTA	Dipotassium (K2) / tripotassium (K3) EDTA;
LC (HPTN LC)	The Laboratory Center (The HPTN Laboratory Center)
LDMS	Laboratory Data Management System
LOC	The Leadership and Operations Center

[1] CLSI. *Handling, Transport, Processing, and Storage of Blood Specimens for Routine Laboratory Examinations.* 1st ed. CLSI guideline PRE04. Clinical and Laboratory Standards Institute; 2023.

Local Laboratory	A laboratory used by a CTU/CRS or protocol site indicated in the PAL or US laboratory spreadsheet for participant testing (e.g., safety testing) unrelated to the Laboratory Center
mL	Milliliter
MOP	Manual of Operations
NG	*Neisseria gonorrhoeae*
NIH/NIAID	(United States) National Institutes of Health/National Institute of Allergy and Infectious Diseases
NTF	Note-to-File
PAL	Protocol Analyte List; a listing of each protocol analyte and its specified assay method and laboratory, as well as backup method and laboratory; for international sites
PrEP	Preexposure Prophylaxis
Primary container	The initial receptacle for a sample at the time of collection, such as a vacutainer blood collection tube or a urine cup
Processing Laboratory	A laboratory for the receiving and handling of specimens, but does not perform testing. e.g., creates plasma from collected whole blood and makes LDMS entries and stores temporarily.
PTID	Participant Identifier; Unique number assigned by Medidata RAVE or SCHARP for protocol participants within a study and LDMS
QA	Quality Assurance
QC	Quality Control
SCHARP	The Statistical Center for HIV/AIDS Research and Prevention
SDMC	The Statistical and Data Management Center
SOE	Schedule of Events
SOP	Standard Operating Procedure
Specimen Management Document	A written process detailing the life span of each sample, starting with collection and ending with storage and destruction, specific to a clinic, protocol, laboratory, or storage facility. May be referred to by an alternate name, such as Chain of Custody.

Overview

This protocol laboratory manual, as part of the Study Specific Procedures Manual, contains laboratory considerations and information for sample collection, processing, testing, and other sample handling required for HPTN 111. For any inconsistency between this manual, the HPTN MOP, and the protocol, follow the protocol and notify the LC and CRM of the inconsistency.

The information provided in this manual is intended to standardize laboratory procedures for HPTN 111. Adherence to the specifications detailed in this manual is essential to ensure that primary and secondary as well as endpoint data derived from laboratory testing will be considered acceptable to the study team, study sponsor, and regulatory authorities, as applicable.

Laboratory procedures will be performed in a variety of settings, including:

- In-Clinic Laboratories
- Local Laboratories

- Non-traditional settings, such as a barber shops, mobile van, in homes, event spaces, tents, as allowable by regulatory approval and participant consent.

Figures in this document describe various aspects of the related protocol laboratory activities, and may include the testing location(s) and specimen requirements for each assay/test. In all settings, laboratory procedures will be performed according to the guidelines included in this manual, study site Standard Operating Procedures (SOPs) that have been reviewed and approved by the HPTN LC, manufacturer guidelines, the HPTN protocol, HPTN MOP, Cross Laboratory SOPs, and DAIDS policies such as GCLP.

Ideally, one method, test kit, or combination of test kits will be used for each test throughout the duration of the study. If for any reason a new or alternative method, kit, or test must be used after study initiation, site laboratory staff must inform the HPTN LC to determine if any test kit validation or other action is required before protocol use. Any such changes need to also be documented through the International PAL, and may require a NTF.

Regardless of whether tests are performed in clinic or laboratory settings, study staff that perform the tests must be trained in proper testing and associated quality control (QC) procedures before performing the tests for study purposes; documentation of training/competency should be available for inspection at any time.

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. References on healthcare worker safety and prevention are available from the US Centers for Disease Control and Prevention at:

- https://www.cdc.gov/niosh/topics/healthcare/default.ht mL
- https://www.cdc.gov/niosh/topics/bbp/

Additional reference information for laboratory safety may be requested from the HPTN LC and site institutional policies.

Specimen Labeling

All containers into which specimens are initially collected (e.g., blood collection tubes, collection cups, transport tubes) will be appropriately labeled at a minimum according to local practices and requirements. Participant Identification (PTID) labels may be provided by the HPTN Statistical Data and Management Center (SDMC, SCHARP) if required for this function. Specimen requisitions will also be provided if required, although sites may use their own specimen transport or requisition documentation with LC approval. The staff member who collects the samples will ensure the participant identification, visit code, specimen collection date and time, as well as the collector initials or code is fully documented.

More detailed information about the labeling procedures must be provided in the site's Specimen Management Document (Chain of Custody SOP).

When specimens are tested at the laboratories, any additional labeling required for local specimen management or chain of custody will be performed in accordance with site-specific SOPs.

1. Local Specimen Processing and Storage

For samples that are processed and stored locally, any labels for samples will include at least the full PTID, in addition to any other information required by lab SOPs or information needed to uniquely identify one sample from others.

2. Local Specimen Testing

Sites must follow local testing arrangements for the collection and testing of samples, this will be described in the site-specific SOPs. All lab results must be recorded and reported following local guidelines.

3. LDMS Usage

The local laboratory will not be utilizing LDMS for this study.

Protocol Related Testing and Sample Collection

Samples will be collected and processed at the screening, enrollment, and follow-up visits as indicated in the protocol SOE.

Collect specimens and label collection containers/tubes according to local regulations and the laboratory specific Sample Collection SOPs. Sometimes K2 and K3 EDTA anticoagulant may be specified for a sample type, as these are interchangeable for some assays, but not for all testing. If not explicitly indicated in this manual, verify with the LC what is needed for protocol testing and with your local lab for local sample testing. Other sample preservatives may have similar multi-choice options (urine containers and you should also verify the specific type required. Blood collection tubes must be filled to the appropriate fill level as indicated by the tube manufacturer. After collection:

- EDTA tubes should be gently inverted at least 8 times (or as specified by manufacturer) after specimen collection to prevent clotting.

Note: Biological samples must be transported in a sturdy, non-breakable, closeable container labeled with the international symbol for Bio-Hazard (sticker/label) and meet local safety regulations.

1. HIV Testing

HIV testing will be performed using blood collected by phlebotomy at participant visits in accordance with the testing algorithms described in Figures 11-1 through 11-2. For further help on implementing the HIV testing algorithm, seek guidance from the HPTN LC.

Whole blood will be collected according to site-specific procedures.

Participants with one or more reactive HIV test results at either the screening or enrollment visit will not be eligible for enrollment, regardless of subsequent test results.

Additional HIV testing may be performed at any time at the discretion of the site investigator.

All tests and associated QC procedures must be documented on local laboratory log sheets or other laboratory source documents. Kit lot numbers and expiration dates must also be documented and tracked by the laboratory.

All staff involved in HIV testing and verification of HIV test results should be aware of the testing time frame for the HIV test, so that all tests are performed, read, and confirmed within the manufacturer specified time frame of testing. Place appropriate calibrated or verified timekeeping devices in all test settings to ensure that each test is read and verified at appropriate time points. Documentation is required for the testing start and stop times, as well as, result confirmation and verification times (second trained staff member confirms initial reading) if applicable/available. These must be recorded on testing log sheets.

If a participant has a reactive or positive HIV test at any time after enrollment, additional blood draw and testing is required as detailed in the protocol and or SOE.

Irrespective of local testing standards, HIV infection must be confirmed using two independent samples collected on different days.

Figure 1 Screening and Enrollment

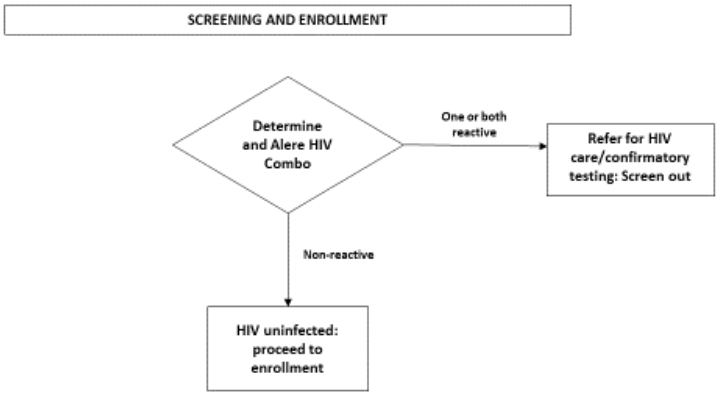
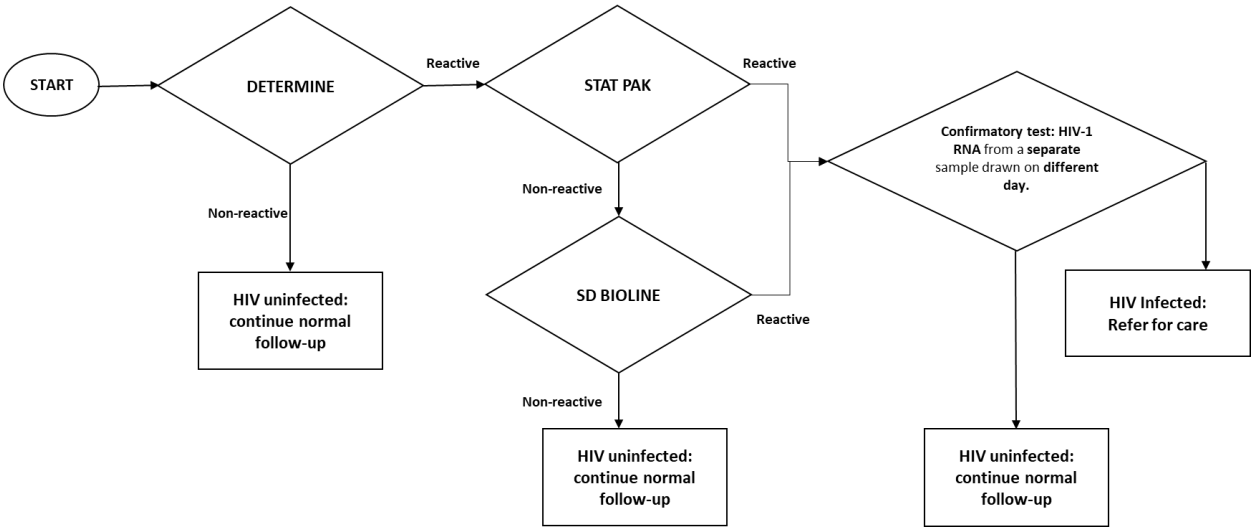


Figure 2 Follow up



2. GC/CT Testing

Participants should not urinate for about 1 hour prior to collection. The participant should be instructed to collect first void urine with a collection between 10 and 50 mL. Urine should be transferred by the laboratory staff to the appropriate transport tube containing the required preservative following the instructions provided by the local laboratory.

3. Other STI testing

A. STI testing for Syphilis (RPR and reflex confirmation, if appropriate)

Syphilis testing will be performed using blood collected by phlebotomy at participant visits in accordance with the protocol SOE. The blood sample collected for HIV Diagnostics testing can be utilized for this testing.

4. Timing and Temperature Considerations for Sample Collection Through Storage or Testing

The following chart details important aspects of protocol samples, such as amount of time allowed during a process or the acceptable temperature range for handling of specimens, starting with collections and going through storage, testing, or sample shipping.

Sample	Collection Notes	Transport/ Handling Notes	Processing Notes	Storage Notes	Testing Notes	Shipping Notes
Serum for HIV Diagnostics and Syphilis	Evacuated blood collection tube sample should fill tube in under 2 minutes	Blood samples for serum should be transported at room temp	Leave the blood sample undisturbed for 15-30 minutes and centrifuge at 1000 xg for 10 minutes to collect serum	Store serum at +4°C- +8°C until for one week	Follow the SOP for syphilis testing	Ship samples to the testing lab (Entebbe) at cold chain (+2°C to 8°C)
plasma for RNA	Evacuated blood collection tube sample should fill tube in under 2 minutes	keep at room temperature must arrive at the processing laboratory for processing within 1 hour from collection	centrifuge at controlled temperature in RT range process and aliquot within 1-2 hours from collection must use amber/opaque cryovial	store at at +4°C- to+8°C pending transportation to Entebbe	do not hold for batching	Ship samples to the testing lab (Entebbe) at cold chain (+2°C to 8°C)
Urine for CTNG	Collect first catch urine, 10 to 50mL	Transfer approx. 8mls of urine to the to the urine specimen collection tube	No processing is required	Store the collection tubes at +4°C- +8°C pending transportation to Entebbe	Follow SOP for CT/NG testing	Ship samples to the testing lab (Entebbe) at cold chain (+2°C to 8°C)

Sample Processing

1. Serum and Plasma Processing for Testing

Approximately 4mL of EDTA whole blood should be drawn into spray dried EDTA (DPE) tubes for HIV RNA testing at each time point as indicated by the HIV algorithm.

Approximately 5mL of whole blood should be drawn into a yellow topped (SST) tube for HIV Diagnostics and Syphilis testing.

Sites will follow the instructions below or may follow site specific SOPs for processing which will include the following:

- Collect blood into appropriate tube type labeled with a SDMC-provided PTID label. An alternate, site-specific labeling process may be used if an SOP is in place, and HPTN LC approved, but still must use the PTID with other identifiers. Size and number of collection tubes may vary depending on local lab requirements.
- Deliver the samples to the local laboratory along with the site-specific requisition that contains the required information. Samples without tracking forms or requisitions may be rejected by the laboratory, follow local SOPs.
- Blood processing for HIV RNA testing should be performed within 1-2 hours of sample collection.
- Centrifuge tubes at 1000 g for 10 minutes to separate cells and plasma.

Local laboratory should plan to store specimens until all the requested testing has been completed and following any local requirements.

Sample Shipping

1. Shipping of Samples to the Local Testing Laboratory

Site will ship plasma, serum, or urine samples to the UVRI-IAVI laboratory following a shipping schedule on a weekly basis.

Contact the UVRI-IAVI Lab manager (Annemarie) to coordinate the timing and logistics of each shipment.

Site will ship samples to the local lab using the site-specific Specimen Management SOP. Site will utilize any additional tracking documentation as needed

Personnel involved in the shipping process must be IATA trained and certified for the shipping of Biological Substance, Category B UN 3373 (Diagnostic) Packing Instructions 650.

Local Lab Testing

1. HIV QA Testing

Selected (10%) of participant samples will be tested at the Local Laboratory (UVRI-IAVI) with a Laboratory based instrumented assay as a Quality Assurance check. Results will be reported back to the site for comparison.

2. STI Testing

HPTN111 includes testing for STIs including Syphilis and CT/NG.

Laboratory Monitoring

1. Reconciliation

The site must follow the HPTN LC approved site-specific SOP for regular reconciliation and verification of specimens that are sent to the Local Laboratory for testing. The detailed

Specimen Management (Chain of Custody) procedures must be followed throughout the study. In addition to following this guidance, designated site and lab staff will work together to document the problem, take appropriate corrective and preventive action, and document all action taken. Reconciliation must be performed for all specimen types that are received by the laboratory.

2. Laboratory Visits

LC staff will conduct periodic site visits to review in-clinic documentation, specimen storage, and other laboratory documentation relevant to this protocol.

####### End of draft document #########