11. Laboratory and Specimen Management Procedures

11.1 Overview of Section 11

This section contains information on the laboratory procedures performed in HPTN 094.

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

1. Clinics or mobile units
2. Local laboratories
3. The HPTN Laboratory Center ("LC", Baltimore, MD and Aurora, CO, USA)
4. Other laboratories designated by the HPTN LC

Tables in this document list the time points, testing location(s), and specimen requirements for each test. In all settings, laboratory procedures will be performed according to the guidelines included in this section of the SSP and in addition study site Standard Operating Procedures (SOPs) that have been reviewed and approved by the HPTN LC. In addition, package insert instructions must be followed.

Ideally, one method, test kit, and/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 is required.**

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 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.html and
https://www.cdc.gov/niosh/topics/bbp/

Additional reference information can be requested from the HPTN LC. The information provided below is intended to standardize laboratory procedures for HPTN 094 across the study sites. Adherence to the specifications detailed in this section is essential to ensure that primary, secondary and exploratory endpoint data derived from laboratory testing will be considered acceptable to regulatory authorities.

11.2 Specimen Labeling

All containers into which specimens are initially collected (e.g., blood collection tubes) will be appropriately labeled according to local practices. Participant Identification (PTID) labels will be provided by the HPTN Statistical Data and Management Center (SDMC, SCHARP) if required for this function. Lab Data Management System (LDMS) Tracking Forms will also be provided for use if required, although sites may use their own specimen transport documentation. The staff member who collects the samples will ensure the visit code (as found in section 13 of this SSP), specimen collection date and time, as well as their initials or code is fully documented.

More detailed information about the labeling procedures must be provided in the site's Chain of Custody SOP.

When specimens are tested at the laboratories, any additional labeling required for in-country specimen management or chain of custody will be performed in accordance with site-specific SOPs. Stored specimens will be entered into the LDMS and labeled with LDMS-generated labels.

11.2.1 Local Specimen Processing and Storage

For samples that are processed and stored locally, each sample will be entered into the LDMS and labeled with the LDMS generated labels. If needed, any temporary labels (e.g. during plasma processing) for samples will include at least the full PTID, in addition to any other information required by lab SOPs.

11.2.2 Local Specimen Testing

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

11.2.3 Remote Specimen Testing

Samples that will be sent to the HPTN LC will be entered into the LDMS and labeled with the LDMS generated labels.

11.2.4 Use of the LDMS

LDMS must be used at all sites to track specimens that will be tested, stored, or shipped off-site for testing. All of the sites participating in HPTN 094 will be using Web (Cloud-Based) LDMS. Detailed instructions for use of LDMS are available in the LDMS User Manual:

https://www.ldms.org/resources/ldms/web/

All sites are responsible for ensuring they are using the most recent version of LDMS and that they have validated the LDMS. All sites must use the *HPTN barcode* label format in order to ensure that both the specimen ID and the global specimen ID assigned to each specimen are printed on LDMS-generated labels.

An examples of a two-dimensional Web LDMS-generated barcode label is shown below:

Row 1: Global Specimen ID

Row 2: Patient Identifier (ID1) and Study/Protocol Identifier (ID2)

Row 3: Specimen Date or Harvest Date and Specimen Collection Time

Row 4: Primary Type, Additive Type, Derivative Type, and Sub Additive/Derivative Type

Row 5: Volume/Volume Unit and Visit/Visit Unit (VID)

Row 6: Other Specimen ID (if applicable)

Questions related to use of LDMS for HPTN 094 should be directed to Paul Richardson (pricha18@jhmi.edu).

Technical support for the general use of LDMS is available from Frontier Science. (LDMS.org)

LDMS User Support at Frontier Science

LDMS user support is available 24 hours per day by telephone or email to answer your questions about using LDMS, diagnosing problems, and helping your laboratory get the most out of the software. When contacting LDMS User Support, be sure to include your LDMS laboratory number.

Please note LDMS User Support cannot be contacted during the following U.S. Holidays – Thanksgiving Day, Christmas Day, New Year's Day, Memorial Day, Independence Day.

https://www.ldms.org/contact/

Phone: +1 (716) 834-0900, extension 7311

Email: ldmshelp@fstrf.org

When you contact LDMS user support, there are certain pieces of information that you can provide to help them better respond to your question. Please provide the following information in your email support:

1. **Your name**

2. **Your laboratory's LDMS ID number**
This is a 3-digit number assigned by Frontier Science to uniquely identify your laboratory. It appears when you start LDMS, and can also be found in the bottom-right corner of the screen.

3. **A full explanation of the issue**
Your explanation should include any error messages or error numbers that appeared, what you were doing in LDMS at the time the issue occurred, and steps needed to reproduce the issue. The more details that you can provide, the faster LDMS User Support can help you.

4. **How you want to be contacted**
If you want LDMS user support to call a specific telephone number, please provide that number and extension.

5. **(If applicable) The license code or challenge code being generated by LDMS**

Note: If you are contacting user support about a license or challenge code, do not close the window with the code. Doing so will cause LDMS to generate a new code.

Below are a few other details that can also be helpful to include in your email:

1. Have there been any recent changes to the computer with LDMS, such as new hardware installed, a firewall upgrade, a network name change, or another change?

2. Are you or another user able to repeat the issue?

3. If you have LDMS installed on multiple computers, does the issue occur on all of them or does it only occur on a specific computer?

11.2.5 LDMS Reconciliation

All sites must follow the HPTN LC approved site-specific SOP for regular reconciliation and verification of specimens that are stored; these independent SOPs or detailed Chain of Custody procedures must be followed throughout the study. All sites must also create an HPTN 094 Primary Specimen report upon LC request. In the event that the required volume or number of sample aliquots is not obtained at any time point, designated site clinic and lab staff must immediately inform the HPTN LOC, HPTN SDMC, and the LC. The HPTN LOC, SDMC, and LC will provide guidance on how to respond to the problem. 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 and stored in the LDMS. Emailed reconciliation reports require a documented response within one week of the original email date.

11.3 Protocol Related Testing and Sample Collection

Samples will be collected and processed at the screening, enrollment, and follow-up visits as indicated in Tables 11.1 – 11.3.

Collect specimens and label tubes according to local regulations and the Blood, Swab and Urine Collection SOPs. Blood collection tubes must be filled to the appropriate fill level as indicated by the tube manufacturer. After collection:

- Ethylenediaminetetraacetic acid (EDTA) tubes should be gently inverted at least 8 times (or as specified by manufacturer) after specimen collection to prevent clotting.
- For plasma storage, approximately 20 mL of whole blood should be collected into spray dried EDTA tubes, e.g. BD 366643 or other, to yield 5 x 1.8 mL plasma aliquots.
- Sites will follow the tube manufacturer's processing instructions for collection tubes.

For example:

BD Plus serum tubes and Plus Silica tubes should be gently inverted 180^{o} and back 5-6 times following collection.

Note: Biological samples must be transported in a sturdy, closeable container with a Bio-Hazard sticker/label per local safety regulations.

Table 11-1: Schedule of Study Visits and Specimen Collection for Participants who are HIV Positive at Enrollment.

	Screening	Enrollment	Care Visit(s)	26 Weeks	52 Weeks
Rapid Testing in the Mobile Unit					
HIV testing[1]	X	X			
Urine MOUD testing (to include buprenorphine and methadone)	X	X		X	X
Urine substance use testing (to include opioid, cocaine, amphetamines, benzodiazepines, methamphetamine, oxycodone)	X	X	(X)	X	X
Urine pregnancy testing[2]		X	(X)	(X)	(X)
Testing at Local CLIA Certified Laboratory					
Laboratory-based HIV testing	X[3]				
HIV viral load		X	(X)	X	X
CD4 cell count		X	(X)		
Syphilis testing		X	(X)	X	X
Urine testing for fentanyl	X	(X)	(X)		
Urine/Swabs for GC/CT NAAT[4]		X	(X)	X	X
HCV Ab testing[5]		X			X
HCV viral load (RNA)[6]		X		X	X
HBV testing (HBsAg, HBsAb, HBcAb)		X			
Other HBV related testing[7]		(X)			
HAV testing (HAV IgG)		X			
Hematology and chemistry testing (to include hemoglobin, creatinine, ALT, AST, and total bilirubin)		X			
Specimen Processing and Storage					
Plasma storage[8]	X	X		X	X
Urine storage[9]		X		X	X
Serum storage for SARS-CoV-2 testing[11]		X		X	X

Footnotes for Table 11-1.

Parentheses around an X indicate that this will be done as needed.

[1] See HIV testing notes Section 11.3.1

[2] Urine human chorionic gonadotropin (HCG) testing for any participant who could potentially be pregnant at that visit (unless already known to be pregnant).

[3] Other HIV testing may be performed for clinical care. This may include drug resistance testing and/or HLA-B5701 testing. If indicated, this testing should be performed at a local laboratory: these results will not be reported to the SDMC.

Separate samples should be collected for this testing.

[4] Oropharyngeal swab, rectal swab, and vaginal swab for female participants. Oral swab, rectal swab and urine for male participants. Swabs may be self-collected or clinician-collected; participants have the option to opt out of oropharyngeal or rectal swabs. Urine may be collected for women as a less preferable alternative to vaginal swab.

[5] Perform HCV Ab testing at enrollment for all participants; perform HCV Ab testing at week 52 for participants who tested HCV Ab negative at enrollment.

[6] Perform HCV viral load testing at enrollment, 26 weeks, and 52 weeks for participants who have a positive HCV Ab test at enrollment. Perform HCV viral load at 52 weeks for participants who have a positive HCV Ab test at the 52-week visit. HCV RNA viral load testing may be performed on a date after HCV Ab results are available.

[7] Perform HBV viral load testing for participants who have chronic HBV infection (HBsAg+) or have an isolated HBcAb positive result (HBsAg negative and HBsAb negative); this testing is performed for clinical care management (intervention arm only).

[8] Stored plasma will be used for testing at the HPTN LC, as described in Section 10 of the Protocol.

[9] Stored urine will be used for testing at the HPTN LC, as described in Section 10 of the Protocol.

[10] Stored serum will be used for retrospective testing at the HPTN LC to determine the prevalence of SARS-CoV-2 seropositivity at baseline, 26 and 52 weeks; stored samples may also be used for specialized testing related to COVID-19 (see Section 10 of the Protocol and Section 11.3.8).

Table 11-2: Schedule of Study Visits and Specimen Collection for Participants who are HIV Negative at Enrollment.

	Screening	Enrollment	Care Visit(s)	26 Weeks	52 Weeks
Rapid Testing in the Mobile Unit					
HIV testing[1]	X	X	(X)	X	X
Urine MOUD testing (to include buprenorphine and methadone)	X	X		X	X
Urine substance use testing (to include opioid, cocaine, amphetamines, benzodiazepines, methamphetamine, oxycodone)	X	X	(X)	X	X
Urine pregnancy testing[2]		X	(X)	(X)	(X)
Testing at Local CLIA Certified Laboratory					
Laboratory-based HIV testing	X	X	(X)	X	X
Syphilis testing		X	(X)	X	X
Urine testing for fentanyl	X	(X)	(X)		
Urine/Swabs for GC/CT NAAT[3]		X	(X)	X	X
HCV Ab testing[4]		X			X
HCV viral load (RNA)[5]		X		X	X
HBV testing (HBsAg, HBsAb, HBcAb)		X			
Other HBV related testing[6]		(X)			
HAV testing (HAV IgG)		X			
Hematology and chemistry testing (to include hemoglobin, creatinine, ALT, AST, and total bilirubin)		X			
Specimen Processing and Storage					
Plasma storage[7]	X	X	(X)	X	X
Urine storage[8]		X		X	X
Dried blood spot (DBS) storage[9]		X		X	X
Serum storage for SARS-CoV-2 testing[10]		X		X	X

Footnotes for Table 11-2.

Parentheses around an X indicate that this will be done as needed.

[1] Not required if participant has been confirmed as infected at a prior visit. See HIV testing notes Section 11.3.1

[2] Urine HCG testing for any participant who could potentially be pregnant at that visit (unless already known to be pregnant).

[3] Oropharyngeal swab, rectal swab, and vaginal swab for female participants. Oral swab, rectal swab and urine for

male participants. Swabs may be self-collected or clinician-collected; participants have the option to opt out of oropharyngeal or rectal swabs. Urine may be collected for women as a less preferable alternative to vaginal swab.

[4] Perform HCV Ab testing at enrollment for all participants; perform HCV Ab testing at week 52 for participants who tested HCV Ab negative at enrollment.

[5] Perform HCV viral load testing at enrollment, 26 weeks, and 52 weeks for participants who have a positive HCV Ab test at enrollment. Perform HCV viral load at 52 weeks for participants who have a positive HCV Ab test at the 52-week visit. HCV RNA viral load testing may be performed on a date after HCV Ab results are available.

[6] Perform HBV viral load testing for participants who have chronic HBV infection (HBsAg+) or have an isolated HBcAb positive result (HBsAg negative and HBsAb negative); this testing is performed for clinical care management (intervention arm only).

[7] Plasma will also be stored at any visit where laboratory-based HIV testing is performed. Stored plasma will be used for testing at the HPTN LC, as described in Section 10 of the Protocol.

[8] Stored urine will be used for testing at the HPTN LC, as described in Section 10 of the Protocol.

[9] Stored DBS will be used for testing at the HPTN LC, as described in Section 10 of the Protocol.

[10] Stored serum will be used for retrospective testing at the HPTN LC to determine the prevalence of SARS-CoV-2 seropositivity at baseline, 26 and 52 weeks; stored samples may also be used for specialized testing related to COVID-19 (see Section 10 of the Protocol and Section 11.3.8).

Table 11-3: Additional Procedures for Participants who have a Reactive or Positive HIV test after Enrollment.

The Confirmation visit for HIV testing and plasma storage should be performed on a different date than the blood draw that gave the initial reactive or positive HIV test.

	HIV Confirmation visit
HIV testing[1]	X
HIV viral load	X
Other HIV testing per local standard	X
CD4 cell count	X
Plasma storage[2]	X
DBS storage[3]	X

Footnotes for Table 11-3.

[1] The site should ensure that local guidelines for HIV confirmatory testing are followed.

[2] Stored plasma will be used for testing at the HPTN LC or a laboratory designated by the HPTN LC, as described in Section 10 of the Protocol.

[3] Stored DBS samples will be used for testing at the HPTN LC or a laboratory designated by the HPTN LC, as described in Section 10 of the Protocol.

11.3.1 HIV Testing

HIV rapid testing will be performed using blood collected by phlebotomy or finger-stick at participant visits in accordance with the testing algorithms described in Figures 11.1-11.3.

Additional HIV testing for this study will be performed in accordance with local standards for diagnostic testing as determined by the site clinical staff.

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 expiry dates must also be documented.

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 specified time frame of testing. Place appropriate 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 when possible (second trained staff member confirms initial reading). 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 Table 11.3.

HIV infection must be confirmed according to local guidelines. Plasma storage is required at every visit where laboratory-based HIV testing is performed. Plasma storage is required at Enrollment for all participants. Every time a blood specimen is drawn for phlebotomy for HIV testing, additional blood must be drawn for plasma storage if it does not exceed the visit blood draw limits stated in your local consent forms. This includes split visits, interim visits, and all visits for repeat HIV testing and confirmatory testing. The amount of blood drawn if not limited by consent forms should be sufficient to yield 5 x 1.8 mL (approximately) plasma aliquots. Note that plasma storage is also required for other assessments.

Individuals who have a reactive instrumented HIV Ag/Ab immunoassay at Screening may be eligible for enrollment as a HIV-positive participant if HIV infection is confirmed. For these cases, HIV infection status will be confirmed using local HIV testing guidelines. In addition to having reactive HIV test results at the Screening visit, participants must have a reactive HIV rapid test result at the Enrollment visit before they are allowed to enroll as a HIV-positive participant.

Individuals who have all non-reactive/negative HIV test results at Screening may be eligible for enrollment as a HIV-negative participant. In addition to having all non-reactive/negative HIV test results at the Screening visit, the participant must have a non-reactive HIV rapid test at the Enrollment visit before they are allowed to enroll as a HIV-negative participant. Results of the instrumented HIV Ag/Ab immunoassay from the Enrollment visit are NOT required prior to enrollment. If a reactive result is obtained for the instrumented HIV Ag/Ab immunoassay after

enrollment for participants enrolled as HIV negative, contact the Site PI and HPTN Laboratory Center for further guidance.

Figure 11.1 HIV Testing Algorithm at the Screening Visit

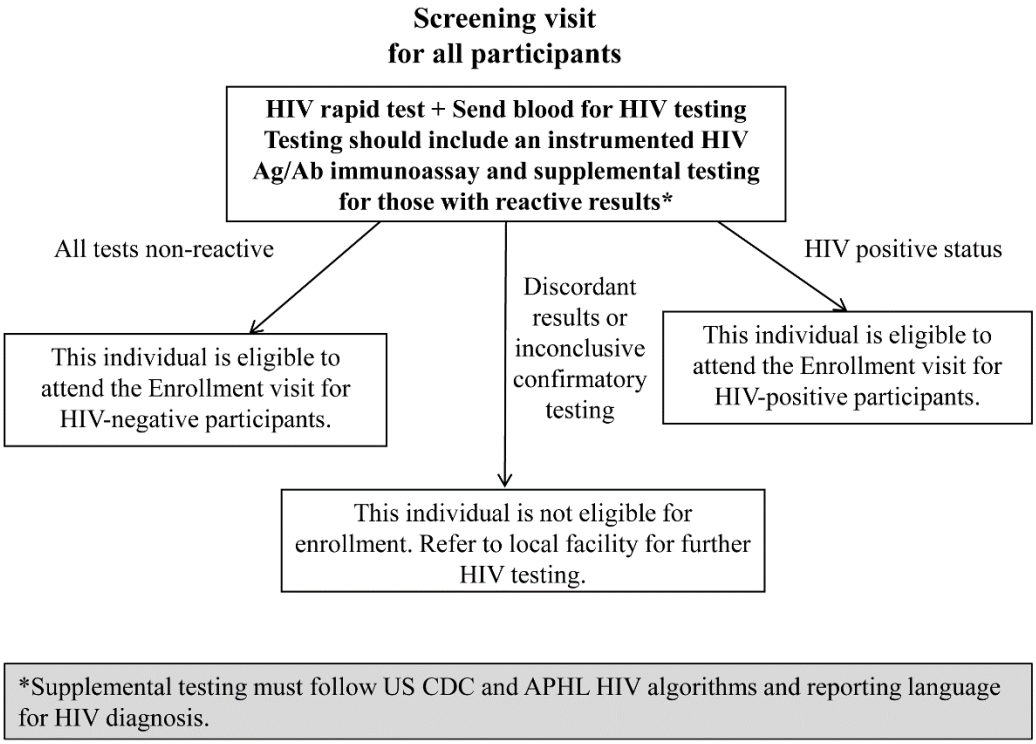
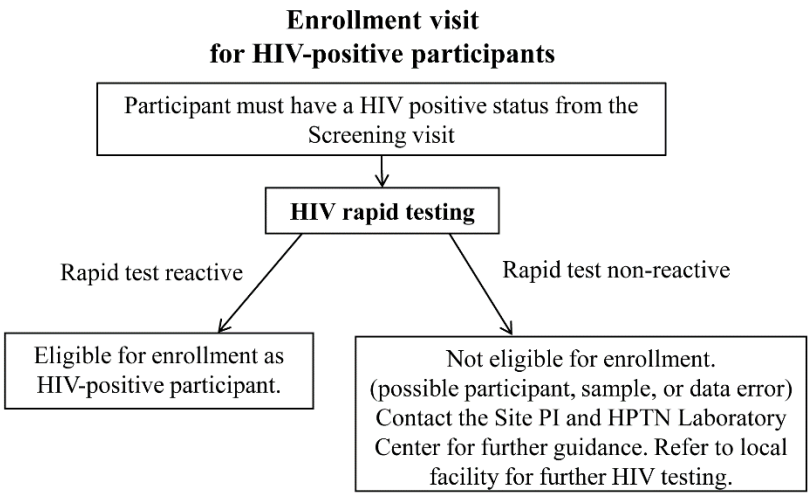
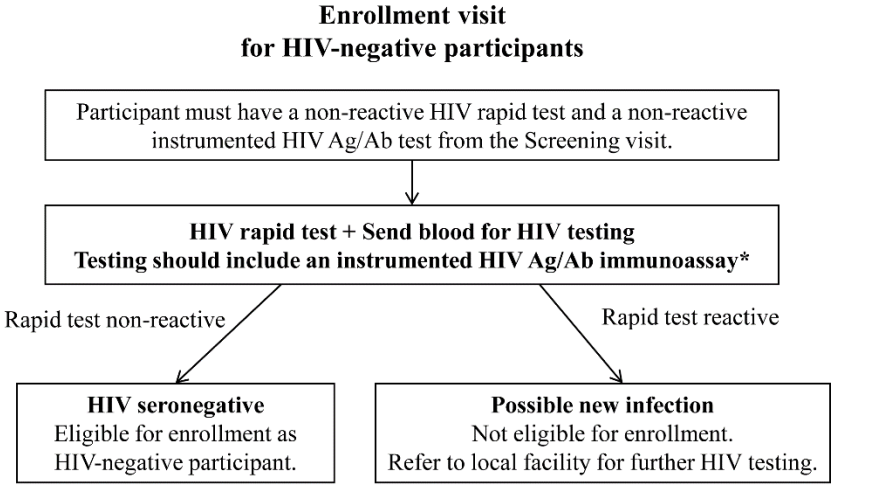


Figure 11.2 HIV Testing Algorithm at the Enrollment Visit

A

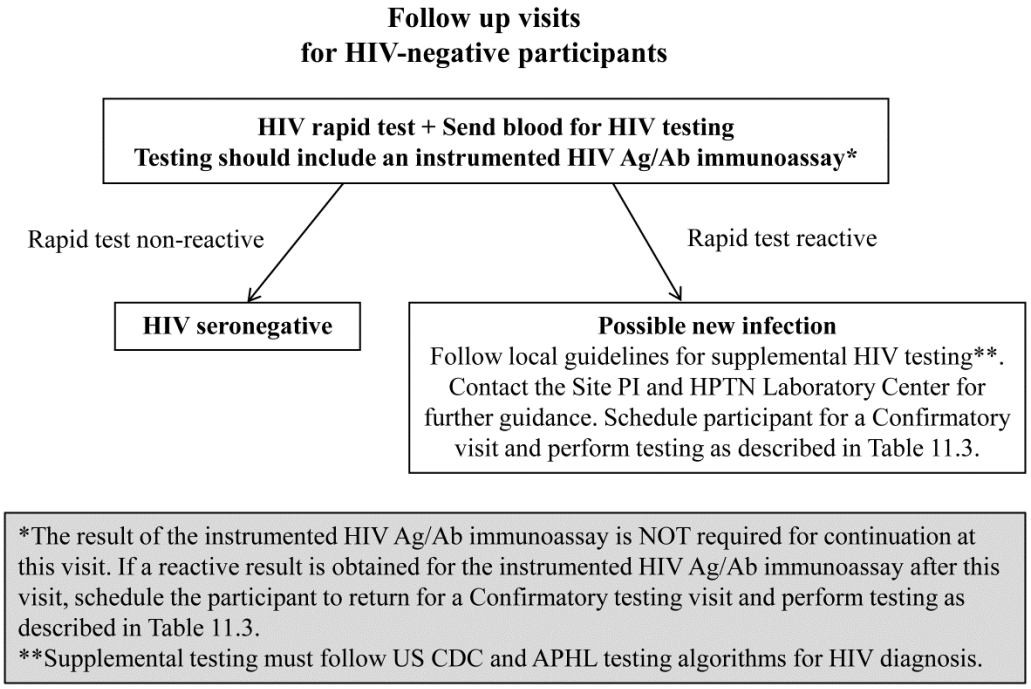


B



*Results of the instrumented HIV Ag/Ab immunoassay from the Enrollment visit are NOT required prior to enrollment. If a reactive result is obtained for the instrumented HIV Ag/Ab immunoassay after enrollment, contact the Site PI and HPTN Laboratory Center for further guidance. Schedule the participant for a Confirmatory testing visit and perform testing as described in Table 11.3.

Figure 11.3 HIV Testing Algorithm at Follow-up Visit:



11.3.2 Hepatitis Testing

Testing for HBV (HBsAg, HBsAb, HBcAb), HCV Ab, HAV IgG, HCV RNA (if HCV positive), and HBV DNA (if needed for clinical management) will be performed at enrollment, and other time points as dictated by Tables 11-1 and 11-2. Sites will follow local testing arrangements for the collection and testing of samples, this will be described in the site SOPs.

11.3.3 Safety Testing

Hemoglobin, creatinine, ALT, AST, and total bilirubin testing are required at the enrollment visit. Sites will follow local testing arrangements for the collection and testing of samples which may include a Complete Blood Count (CBC) and Complete Metabolic Panel (CMP), this will be described in the site SOPs.

This testing is not required at other visits.

11.3.4 Urine Testing for MOUD and Substance Use

Sites will follow local testing arrangements for the collection and testing of urine for MOUD and substance use as indicated in Tables 11-1 and 11-2.

This will include:

Urine MOUD testing - buprenorphine, methadone.
Urine substance use testing - opioid, cocaine, amphetamines, benzodiazepines, methamphetamine,oxycodone, fentanyl
.

Note: Fentanyl test strips are not CLIA waived. Testing for fentanyl must be performed in a CLIA certified facility

This will be described in the site SOPs.

11.3.5 Urine Pregnancy Testing

Sites will follow local arrangements guidelines for the collection and rapid testing of urine for pregnancy.

Testing for pregnancy will be performed for any participant who could potentially be pregnant at that visit (unless already known to be pregnant).

Urine pregnancy testing will be performed at visits indicated in Tables 11-1 and 11-2.

11.3.6 Syphilis Testing

Sites will follow local testing arrangements for the collection and testing of serum or plasma for syphilis testing. This will be described in the site SOPs.
Syphilis testing will be performed at visits as shown in Tables 11-1 and 11-2.

11.3.7 Samples for GC/CT Testing

Sites will follow local testing arrangements for the collection and testing of samples for GC/CT nucleic acid testing. This will be described in the site SOPs.
GC/CT testing will be performed at visits shown in Tables 11-1 and 11-2.

Samples collected will be as follows:

Men – Oropharyngeal swab, rectal swab, urine.

Women – Oropharyngeal swab, rectal swab, vaginal swab (or urine as a less prefered sample type).

Participants may opt out of oropharyngeal and/or rectal swab collection/testing.

11.3.8 Serum for SARS-CoV-2 Testing

Serum samples will be collected for SARS-CoV-2 serology testing at the HPTN LC, as show in Tables 11-1 and 11-2. Testing may also be performed to characterize the host response to infection and factors associated with SARS-CoV-2 seropositivity. Results from SARS-CoV-2 testing performed at the HPTN LC will not be returned to study sites or participants.

11.4 Plasma Processing for Storage

Approximately 20 mL of EDTA whole blood should be drawn into spray dried EDTA tubes for plasma storage at each time point at which HIV testing is performed as indicated in Tables 11-1 and 11-2. Sites are requested to store 5 x 1.8 mL aliquots of plasma if possible. The HPTN LC should be informed any time that three or fewer aliquots with 1.8 mL or less are stored.

Note. The 1.8 mL plasma volume stated in this SSP is an approximate volume. This can be estimated using for example the volume markings on the cryovials. The use of a precision pipette is not required for this purpose. See section 11.7.

An additional approximate 20 mL of EDTA whole blood will be drawn for plasma storage for participants with a reactive or positive HIV test at any time after enrollment, as indicated in Table 11-3. This additional plasma will be stored in the same way.

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

- Collect blood into lavender top blood collection tubes (EDTA) labeled with a SCHARP-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 LDMS laboratory along with the LDMS Specimen Tracking Sheet or site specific requisition that contains the required information.
- Using the LDMS Specimen Tracking Sheet or site specific requisition, log the sample into LDMS (specimen type = BLD) and generate the appropriate number of LDMS cryovial labels. The lab should store plasma in labeled cryovials.
- Blood processing and plasma storage should be performed within 6 hours of sample collection.
- Log samples into LDMS and generate LDMS labels (PL1). Each aliquot will have its own individual identification number (Global Specimen ID).
- Centrifuge tube at 800 - 1000 x g for 10 minutes to separate cells and plasma.
- Carefully remove plasma and avoid disturbing the cell layer. Transfer the plasma to an appropriately labelled sterile centrifuge tube.
- Store plasma in aliquot number order. For example, if there is only 3 mL of plasma for storage: store 1.8 mL in aliquot 1, then store the remaining 1.2 mL of plasma in aliquot 2 and adjust the aliquot volume in LDMS to indicate 1.2 mL. The remaining aliquots (3, 4, and 5) should be entered as QNS.
- Additional sample condition codes besides QNS, to be used as directed by the HPTN LC, include “SNP” and “SNR”.
- Store the aliquots in the freezer locations assigned in LDMS in a minus 70° to minus 90° freezer.

Plasma for storage will be stored on site until all protocol-related testing is complete. Note that some testing will be performed after study visits have been completed.

LDMS Entry:

PL1 aliquots from the 20 mL EDTA draw as follows:

- Several possible tube combinations equaling at least 20 mL (per individual site chain of custody)
- A single primary container of EDTA whole blood is created
- 5 PL1 aliquots of 1.8 mL are created (adjusted to approximate aliquot volume as needed during storage)

- Primary container and aliquot entries should have the "SHV" (short volume) condition code used as needed when incomplete volumes are obtained
- Please remember to contact the HPTN LC when using condition codes not listed in the SSP

- No other aliquots are created from this primary container
- See Figures 11.10 and 11.11 for LDMS examples

LDMS Specimen Code for Plasma Storage

Test	Primary LDMS Code	Additive	Derivative	Sub Add/Deriv
Plasma Storage	**BLD**	**DPE**	**PL1**	**N/A**

Codes used in table:

BLD	**Blood**
DPE	**Spray Dried EDTA**
PL1	**Plasma, Single Spun**
N/A	**Not Applicable**

- All plasma vials are stored electronically in the LDMS and physically in a minus 70°C to minus 90°C freezer. Selected aliquots will be shipped to HPTN LC when requested.

11.5 Serum Processing for Storage

A suggested 10 mL of whole blood should be drawn into serum or serum separator tubes for serum storage at visits indicated in Tables 11-1 and 11-2. Sites are requested to store 2 x 1.8 mL (approximate) aliquots of serum if possible.

See section 11.7. for cryovials filling instructions.

Sites will follow the tube manufacturer's processing instructions for collection tubes. Gravity separation is not sufficient for specimen preparation:

The clotting of a sample must be fully completed before centrifugation of serum tubes. Specimens must be left a minimum of 30 minutes prior to centrifugation. Samples should ideally be processed and stored within 2 hours of collection. Because this may not always be possible, please ensure collection date and time, and frozen date and time are entered into LDMS so that this time range is captured.
Centrifugation Instructions: Centrifuge tube at room temperature (20°C to 25°C) at 1100 to 1300 x g for 10 minutes in swinging bucket rotor units or centrifuge at 1100 to 1300 x g for 15 minutes in fixed angle units.

- Collect blood into serum collection tubes labeled with a SCHARP-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 LDMS laboratory along with the LDMS Specimen Tracking Sheet or site specific requisition that contains the required information.
- Using the LDMS Specimen Tracking Sheet or site specific requisition, log the sample into LDMS (specimen type = BLD) and generate the appropriate number of LDMS cryovial labels. The lab should store plasma in labeled cryovials. See section 11.7.
- Log samples into LDMS and generate LDMS labels (SER). Each aliquot will have its own individual identification number (Global Specimen ID).
- Transfer labeled cryovials to the freezer locations assigned in the LDMS, store at minus 70° to minus 90°C.
- Store serum in aliquot number order. For example, if there is only 3 mL of serum for storage: store 1.8 mL in aliquot 1, then store the remaining 1.2 mL of plasma in aliquot 2 and adjust the aliquot volume in LDMS to indicate 1.2 mL. Empty aliquots should be entered as QNS.
- Additional sample condition codes besides QNS, to be used as directed by the HPTN LC, include “SNP” and “SNR”.
- Store the aliquots in the freezer locations assigned in LDMS in a minus 70° to minus 90° freezer.

Serum for storage will be stored on site until all protocol-related testing is complete. Note that some testing will be performed after study visits have been completed.

Study sites should plan to store specimens until all of the protocol-specified testing (including assessments at the HPTN LC) has been completed and at least for one year after the primary research paper has been published. The sites will be notified by the HPTN LC when they can destroy samples from participants that did not consent to long term storage and when the remaining samples can be destroyed. The HPTN LC will seek permission from protocol leadership and network leadership prior to this destruction process. Any samples that are collected in error etc. should not be destroyed without the permission of the HPTN LC.

LDMS Entry:

SER aliquots as follows:

- Several possible tube combinations equaling a suggested 10 mL (per individual site chain of custody)
- A single primary container of whole blood is created
- 2 SER aliquots of 1.8 mL are created (adjusted to approximate aliquot volume as needed during storage)

- Primary container and aliquot entries should have the "SHV" (short volume) condition code used as needed when incomplete volumes are obtained
- Please remember to contact the HPTN LC when using condition codes not listed in the SSP

- No other aliquots are created from this primary container
- See Figures 11.10 and 11.11 for LDMS examples

LDMS Specimen Code for Serum Storage

Test	Primary LDMS Code	Additive	Derivative	Sub Add/Deriv
Serum Storage	**BLD**	**NON**	**SER**	**N/A**
Serum Storage	**BLD**	**SST**	**SER**	**N/A**

Codes used in table:

BLD	**Blood**
NON	**None**
SST	**Serum Separator Tube**
SER	**Serum**
N/A	**Not Applicable**

Specimen collection date and time, and frozen date and time should be documented in LDMS.

- All serum vials are stored electronically in the LDMS and physically in a minus 70°C to minus 90°C freezer. Selected aliquots will be shipped to HPTN LC when requested.

All enrolled study participants must consent to collection and storage of their serum for the duration of their study participation and until all protocol-specified testing has been completed. Participants are asked to consent separately to indefinite storage and possible future research testing of their serum after the study is completed. Participants may refuse to consent to indefinite storage and possible future research testing and still enroll in the study. After all protocol-specified testing has been completed; the stored serum of participants who do not consent to indefinite storage and possible future research testing must be destroyed. After all protocol-specified testing has been completed, the HPTN SDMC will provide each site with a list of participants who did not consent to indefinite storage and possible future research testing and the HPTN LC will provide detailed instructions for specimen destruction and documentation thereof.

11.6 Frozen Urine Storage

Urine will be received at the laboratory or clinic for entry into the LDMS, processing, and frozen storage.

The date and time of collection will be noted on the appropriate documentation or LDMS tracking sheet or alternate tracking documentation.

Using the LDMS Specimen Tracking Sheet, log the sample into LDMS (primary specimen type = URN) and generate the appropriate number of LDMS aliquot labels:

- 2 aliquots labels are required for frozen urine storage.

LDMS Specimen Code for Frozen Urine Storage

Test	Primary LDMS Code	Additive	Derivative	Sub Add/Deriv
Urine Storage	**URN**	**NON**	**URN**	**N/A**

Codes used in table:

URN	**Urine**
NON	**NONE**
N/A	**Not Applicable**

- Transfer approximately 1.8 mL into each of two 2 mL cryovials, labeled with a LDMS label (Global Spec ID -001 and 002). See Figures 11.11 and 11.12 for LDMS examples.
- See Section 11.7. for cryovials storage instructions.
- Transfer labeled cryovials to the freezer locations assigned in the LDMS, store at minus 70° to minus 90°C.

Cryovial size may vary, but 2.0 mL is recommended. Reminder these vials hold 1.8 mL of liquid. Do not add more than 1.8 mL due to expansion issues when freezing.

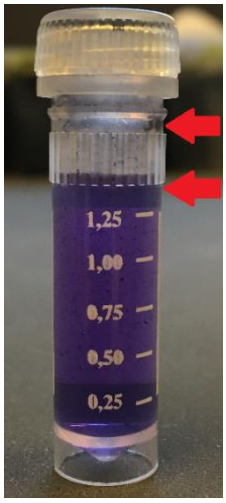
Note. The 1.8 mL urine volume stated in this SSP is an approximate volume. This can be estimated using for example the volume markings on the cryovials. The use of a precision pipette is not required for this purpose.

11.7 Cryovial Storage for Plasma, Serum and Urine

The HPTN LC request the use of Sarstedt (cat# 72.694.006) 2 mL Cryovials, or cryovials of the same dimensions. Reminder: Do not add more than 1.8 mL due to expansion of plasma during freezing.

Other cryovials types may only be used if specifically approved by the HPTN LC.

Figure 11-4



The manufacturer of this example tube stops gradations at 1.25 mL for the 2 mL cryovial. The 1.8 mL needed is an approximate volume. For these cryovials, the top of the vertical striped area is an estimated maximum fill 'line' for a limit fill volume to prevent cracking of the container during freezing, and will provide an acceptable 1.8 mL estimate. See photo to the right for reference (Figure 11.4). The plasma/serum/urine level needs to be between the two arrows for 1.8 mL to be delivered and stored. The optimal level is at the indicated top arrow, near the cryovial 'ring' below the cap. Various methods for achieving the desired volume are possible. Example list, other methods are not prohibited or excluded:

- Use of a pipette with a precise measurement (not required)
- Use of a graduated disposable transfer pipette
- A marked-up a cryovial, or filled cryovial with a liquid for a comparison level (properly labeled as a blank for lab safety requirements)

Study sites should plan to store specimens until all of the protocol-specified testing (including assessments at the HPTN LC) has been completed and at least for one year after the primary research paper has been published. The sites will be notified by the HPTN LC when they can destroy samples from participants that did not consent to long term storage and when the remaining samples can be destroyed. The HPTN LC will seek permission from protocol leadership and network leadership prior to this destruction process. Any samples that are collected in error etc. should not be destroyed without the permission of the HPTN LC.

See section 11.9 for suggested storage procedure.

11.8 Dried Blood Spots (DBS)

11.8.1 DBS Supplies

Possible vendors for DBS supplies: Thermo Fisher Scientific, VWR, Sigma Aldrich, and Market Lab. Some Whatman items may be listed as GE Healthcare Life Sciences. The following supplies may be used. Contact HPTN LC if alternate supplies are to be used.

- EDTA spray dried Blood Collection Tubes
- Whatman Protein Saver Card #903 (Whatman 10534612 or Fisher Scientific # 05-715-121). Please handle with gloves and do not touch spot areas
- Whatman Plastic Sample Bags (Whatman 10548232 or Fisher Scientific # 09-800-16) or Whatman Foil-Barrier Sample Bags (Whatman 10534321 or Sigma Aldrich # WHA10534321)
- Desiccant pack (GE Healthcare Life Sciences (Whatman) 10548234 or Fisher Scientific # 09-

800-17)

- Humidity indicator Cards (Manufacturer # MS200032 or MS200033; ADCOA # MS20003-2 or MS20003-3; Fisher Scientific # NC9511648). Or similar products with similar indicator levels, suitable for storage bag size
- Whatman card drying rack (VWR # 89015-592 or Sigma Aldrich # WHA10539521) or other suitable drying rack
- Gloves, preferably powder free
- Water proof marker (Fisher Scientific# 50853571 or VWR # 95042-566)
- LDMS labels
- A fixed 25 µl, variable 10-100 µl, or 20-200 µl micropipette with appropriate filtered pipette tips. Sites should check with local suppliers for appropriate tips for their micropipettes

11.8.2 DBS Preparation and Storage

The use of a negative airflow biosafety cabinet is not required for this specimen processing and storage. Sites will follow the instructions below or may follow site specific SOPs for DBS processing and storage which will include the following:
DBS will be prepared and stored at visits indicated in Tables 11-2 and 11-3.

DBS should be prepared from an EDTA blood tube received in the laboratory. For HPTN 094, it is acceptable to use one of the tubes received for plasma storage before it is processed for plasma storage or a sample received for HIV rapid testing after testing has been performed.

The EDTA tube should be well mixed before preparing the DBS. Pipette 25 µl of whole blood directly onto the center of each spot on the filter paper so that it is contained within the circle (Figure 11.6).

- There will be a total of 5 blood spots created.
- Whole blood for DBS should be stored at room temperature (15°C to 25°C) until spots have been created.
- Samples should be processed (spotted) within 6 hours of the time of collection; the actual time of collection should be recorded on the Case Report Form, as well as DBS creation time.
- Ensure that both hands are gloved before handling the Protein Saver (DBS) card; do not touch the areas where the blood spots will be placed (the filter paper portion).
- Label each Protein Saver Card with the study protocol number, PTID#, Specimen collection date and time of sample collection. Use a waterproof pen or a non-removable label.
- Create an LDMS label and enter specimen information into LDMS. See Figures 11.5 and 11.13.
    - Additional sample condition codes besides QNS, to be used as directed by the HPTN

LC, include “SNP” and “SNR”.

- Primary container and aliquot entries should have the “SHV” (short volume) condition code used as needed when incomplete volumes are obtained.
- Please remember to contact the HPTN LC when using condition codes not listed in the SSP.

- Assure the blood tube has been inverted 8 times and is well mixed. Remove the cap from the EDTA tube and spot 25 µl of blood, using a pipette, onto the center of the designated circles on the Protein Saver Cards (see Figures 11.6 to 11.8 below). Return the cap to the tube and process for other lab tests (i.e. plasma processing) as needed.
    a. The pipette tip should be held approximately 3 mm above the spot location and the blood dispensed onto the card with one single dispensing motion from the micropipette. Do not touch, press, or smear the spots.
- Air dry the cards in a card holder or other drying rack (Figure 11.9). Ideally, drying time should be between 2 and 16 hours. If storage cannot take place within 16 hours for example over a weekend, an appropriate comment must be made in LDMS to indicate the drying time.
- Keep the DBS cards away from direct sunlight. DBS cards should be dried at the designated lab room temperature which should be between 15 and 40^{o}C. DBS cards should not be dried in excess of 40^{o}C. Do not dry the DBS cards with a fan or any heat source in an attempt to decrease drying time. Air dry only. The use of a biosafety cabinet is not required for the drying of DBS for HPTN 094. If a cabinet is used there is no requirement for the airflow to be operational or documented for DBS purposes.
- After DBS cards have dried, place DBS card in low gas-permeability plastic bags with humidity indicator and desiccant pack to reduce humidity. See Figure 11.10. Indicator cards and desiccant packs should be kept in their manufacturer stock containers (airtight) until the DBS card is dried and ready for freezer storage.
- Store bag in an appropriately labeled box at minus 70 to minus 90°C.
    a. If the indicator indicates too much humidity exposure (color change from blue to pink 40% to 50% level or higher), replace the old desiccant pack and indicator card with a new one and comment the change in LDMS.
    b. There is no need to check the humidity indicators unless DBS are handled for another purpose (i.e. shipping), and action is needed if a problem is noticed.

LDMS Entry:

DBS from EDTA whole blood (example 4 mL draw) as follows:

- A single primary container of 4 mL EDTA whole blood is created
- 5 aliquots of 25 µl each are created (1 for each spot on the DBS card)
- See Figures 11.11 - 11.13 for additional LDMS entry and DBS label information

LDMS Specimen Code for DBS Storage

Test	Primary LDMS Code	Additive	Derivative	Sub Add/Deriv
Dried Blood Spots	**BLD**	**DPE**	**DBS**	**N/A**

Codes used in table:

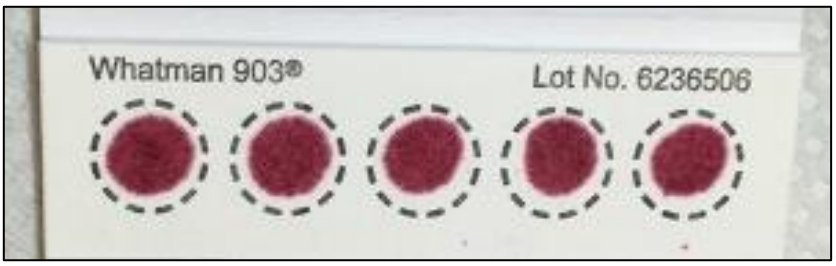
BLD	**Blood**
DPE	**Spray Dried EDTA**
DBS	**Died Blood Spot**
N/A	**Not Applicable**

- All DBS are stored electronically in the LDMS and physically in a minus 70°C to minus 90°C freezer. Selected cards will be shipped to the HPTN LC when requested.

- In addition to the illustrations, include the date and time of specimen receipt, date and time of DBS processing (spot time), and date and time of DBS completion and storage for each aliquot. Note the primary aliquot is BLD with 5 aliquots created from the primary specimen. Each aliquot will be 25uL having its own Global Specimen ID. DBS need to be entered into LDMS and stored in appropriate location so they can be easily retrieved when necessary.

Figure 11.5 Suggested labeling of DBS cards

Figure 11.6 Example of correctly spotted DBS card (25 µl spot volume)



Note: 25 µl spot volume may not completely fill target circle on DBS card.

Figure 11.7 Example of *incorrectly* spotted DBS card

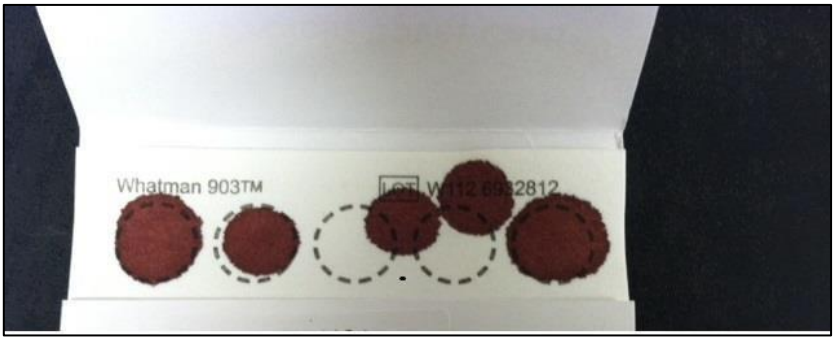


Figure 11.8 Example of *incorrectly* spotted DBS card (continued)

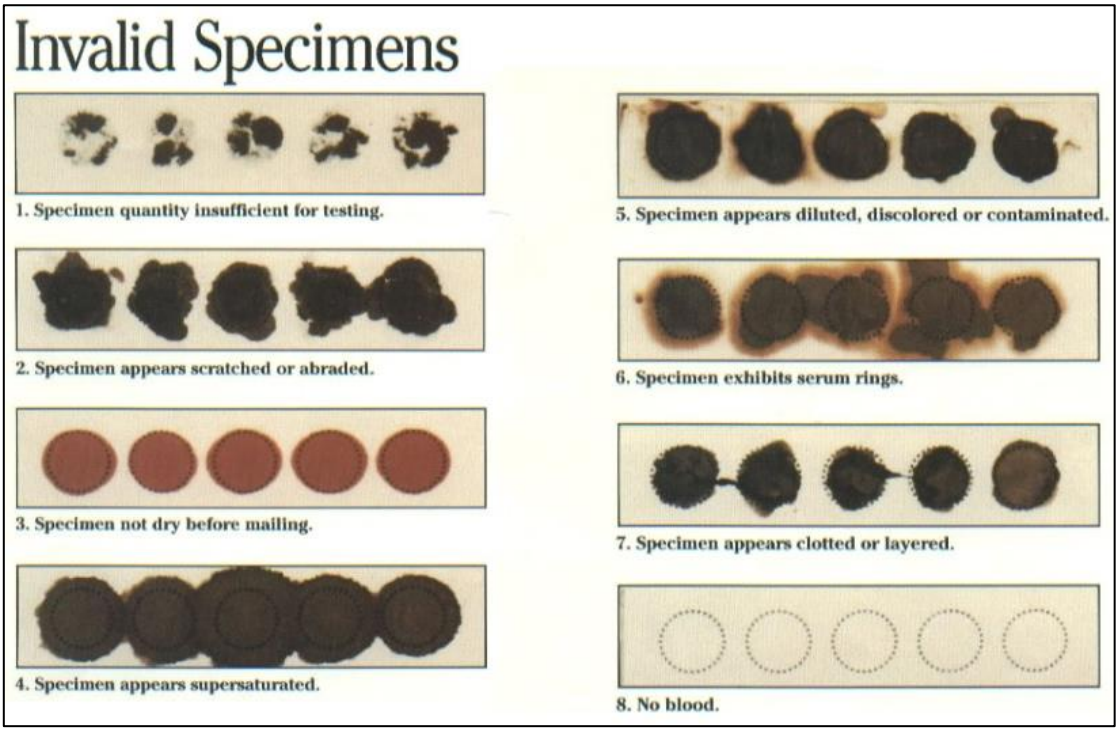


Figure 11.9 Whatman card drying rack (VWR catalogue # 89015-592)

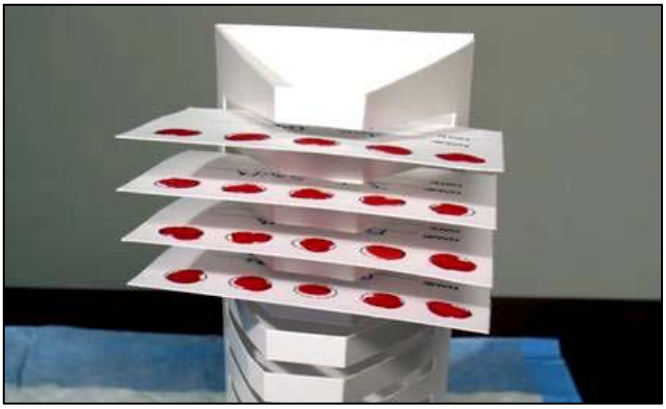


Figure 11.10 Properly labeled and packaged DBS cards for storage

Figure 11.11 Example LDMS (Web) Record Entry Visit 2.0

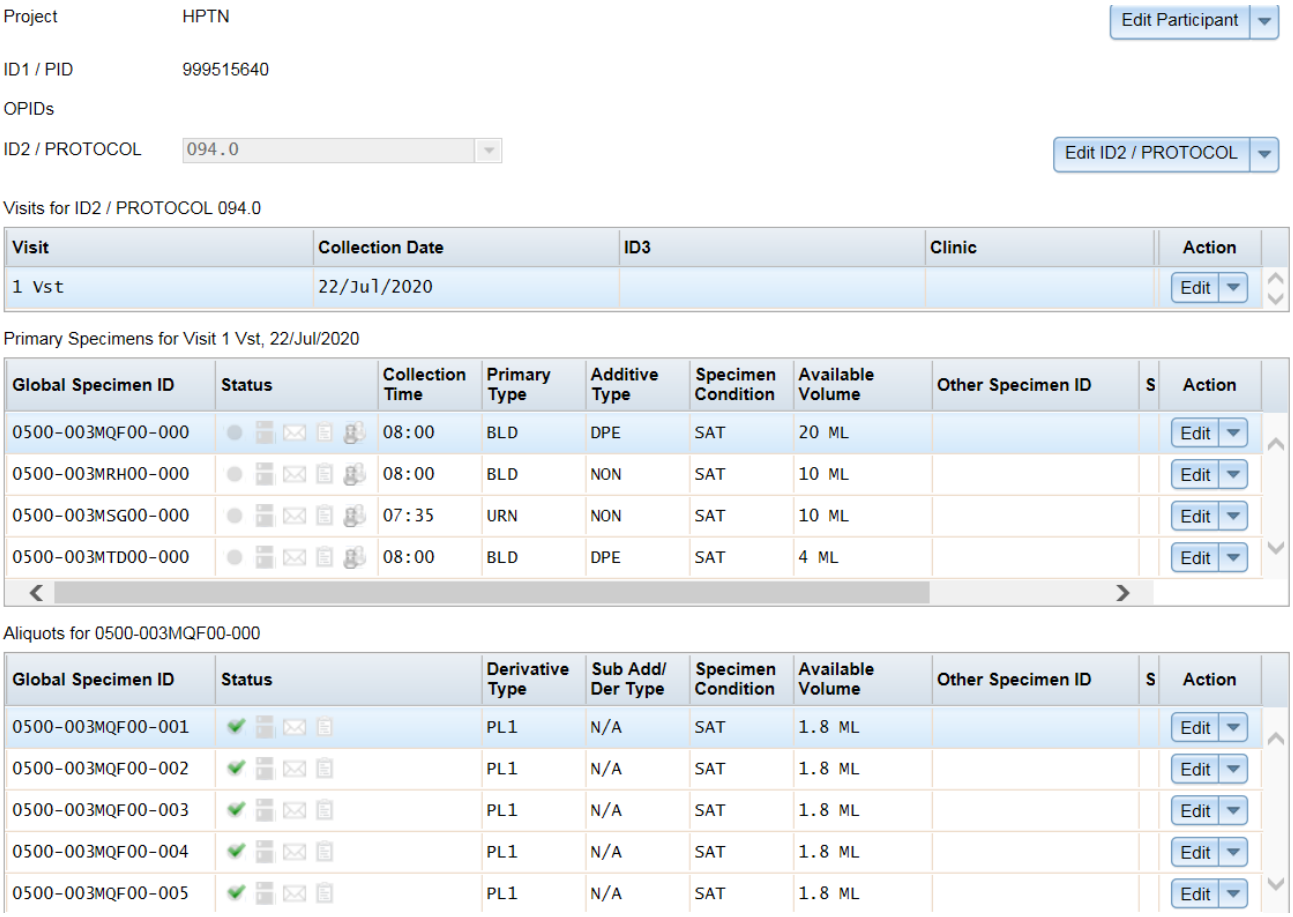
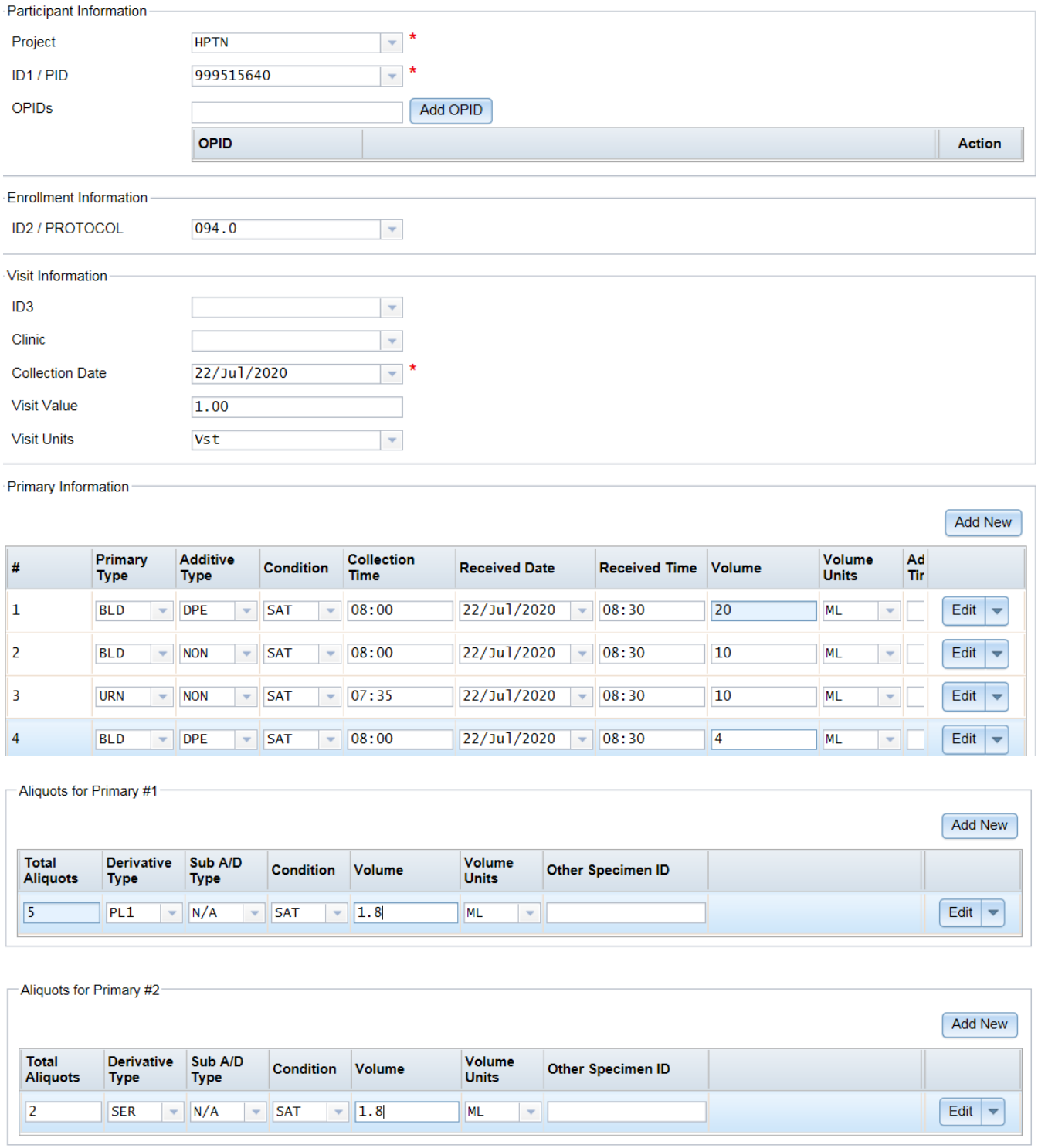


Figure 11.12 Example LDMS (web) Entry Visit 2.0 (Quick Add)



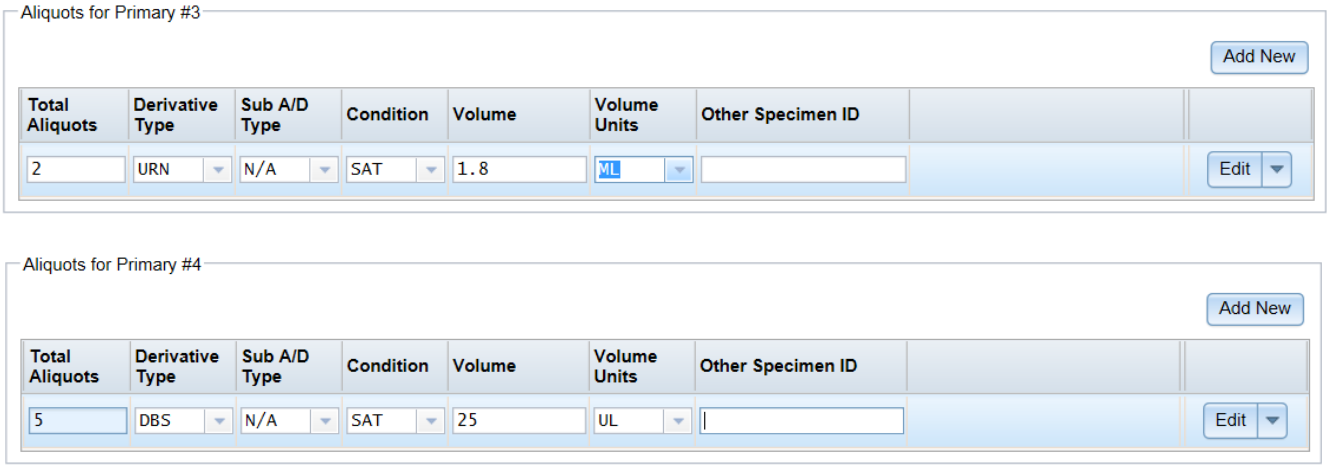
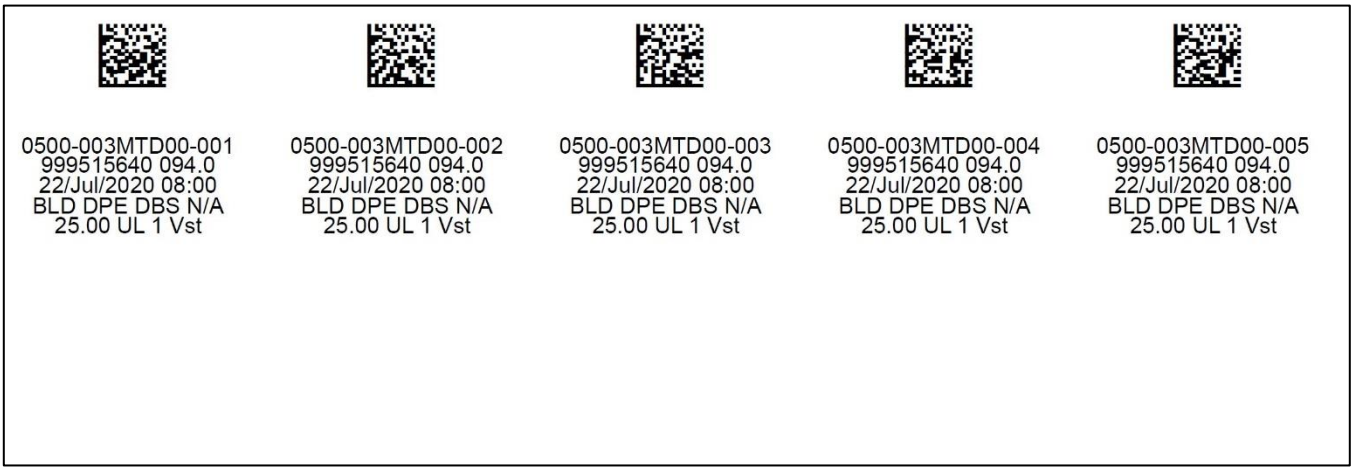


Figure 11.13 Example DBS LDMS Labels for each aliquot (Web)



11.9 Storage and Shipping of Samples to the HPTN Laboratory Center

The HPTN LC recommends (not mandated) the following for ease of storage and future shipping of samples (enrollment visit onwards):

Plasma 5 aliquots

- Store plasma in aliquot number order. Any of the protocol related testing to be performed at the LC may be performed on any aliquot but for ease of shipping the Global Spec IDs should be stored as follows:
    - Global Spec ID -001 - Store all global ID-001s from all participants in a box

identified for example - **LC Plasma Designated to be Shipped**.

- Global Spec ID -002 to -005- Store all global ID-002 thru -005 from all participants in a box identified for example – **LC Plasma Designated for On Site Storage**.

Serum 2 aliquots

- Store serum in aliquot number order. Any of the protocol related testing to be performed at the LC may be performed on any aliquot but for ease of shipping the Global Spec IDs should be stored as follows:

    - Global Spec ID -001 - Store all global ID-001s from all participants in a box identified for example - **LC Serum Designated to be Shipped**.

    - Global Spec ID -002 - Store all global ID-002s from all participants in a box identified for example – **LC Serum Designated for On Site Storage**.

Urine 2 aliquots

- Store urine in aliquot number order. Any of the protocol related testing to be performed at the LC may be performed on any aliquot but for ease of shipping the Global Spec IDs should be stored as follows:

    - Global Spec ID -001 - Store all global ID-001s from all participants in a box identified for example - **LC Urine Designated to be Shipped**.

    - Global Spec ID -002 - Store all global ID-002s from all participants in a box identified for example – **LC Urine Designated for On Site Storage**.

Samples in the boxes labelled **"Designated to be Shipped"** will be sent to the LC testing laboratory upon request.

Samples in boxes labelled **"Designated for On Site Storage"** will be stored on site until a request for shipment to the LC, or permission to destroy has been given.

DBS can be stored per local lab standards.

All enrolled study participants must consent to collection and storage of their plasma, serum, urine and DBS for the duration of their study participation and until all protocol-specified testing has been

completed. Participants are asked to consent separately to indefinite storage and possible future research testing of their plasma after the study is completed. Participants may refuse to consent to indefinite storage and possible future research testing and still enroll in the study. After all protocol-specified testing has been completed; the stored plasma of participants who do not consent to indefinite storage and possible future research testing must be destroyed. After all protocol-specified testing has been completed, the HPTN SDMC will provide each site with a list of participants who did not consent to indefinite storage and possible future research testing and the HPTN LC will provide detailed instructions for specimen destruction and documentation thereof.

Each site will ship urine, plasma, and DBS samples to the LC or designated laboratory upon request or following a shipping schedule as determined by the LC. The site will batch the shipment, export the LDMS data, and notify the SDMC and LC. Additional samples may be specifically requested by the HPTN LC (e.g., archive/back-up samples); in this case, the SDMC will provide the site(s) with specific shipping lists.

Contact the HPTN LC at Johns Hopkins University (Estelle Piwowar-Manning: epiwowa@jhmi.edu, +410-614-6736 and Paul Richardson pricha18@jhmi.edu, +410-614-6737) to coordinate the timing and logistics of each shipment.

Sites will ship samples to the LC using the LDMS following the LC approved Shipping SOP indicating the LDMS Lab number as the ship to lab ID number.

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

Include a copy of the shipping manifest and box map with the shipment. For dry ice shipments, use diagnostics packing code 650, UN 3373, and address the shipment as indicated in the following pages, for Johns Hopkins Hospital (urine and plasma) or the University of Colorado (DBS). For some shipments, an alternate address may be provided at the time of request.

Notify the HPTN LC via email (epiwowa@jhmi.edu) when the shipment has been picked up from the site by the courier/shipping company. Attach an electronic copy of the shipping manifest and LDMS batch to the email notification, and include the following information in the notification:

- Name of courier/shipping company
- Shipment tracking number
- Number of boxes shipped
- Date of shipment
- Expected date of arrival

Upon request, urine plasma and serum samples will be shipped to:

Estelle Piwowar-Manning
Johns Hopkins University Hospital
Department of Pathology

Pathology Building, Room 313
600 North Wolfe Street
Baltimore, MD 21287, USA
Phone: 410-502-0752
LDMS Number 300

Other samples, such as those from seroconverters, will also be requested on an ad-hoc basis and may be included in quarterly shipments. Separate shipping instructions will be provided at that time by LC non-protocol team members.

Separate LDMS batches are required for the quarterly shipments, any QA requested samples, and seroconverter samples if they are sent in the same shipment. Urine and serum aliquots should also be included on a separate LDMS batch, as they will be passed to a different testing laboratory.

DBS Shipping

DBS sample lists for shipment will be posted on the SCHARP Atlas website for each site. An Email will be sent one week before the quarterly shipments are scheduled to notify each site that the up-to-date shipping list is posted. This should allow each site to have more than one week for DBS shipment preparation (and approximately one week for plasma shipment preparation).

Storing DBS by individual participant will simplify the shipment process.

Sites should ship the DBS cards directly to:

Lane Bushman
C/O Pete Anderson
University of Colorado at Denver
Skaggs School of Pharmacy and Pharmaceutical Sciences
CAVP Laboratory
C-238-V20, Rm V20-4410
12850 East Montview Blvd
Aurora, CO 80045 USA
Phone: 303-724-6132
LDMS Number 533

When shipping DBS, make sure specimens are shipped on dry ice. Check the desiccant packs and humidity indicators before shipping, and replace if needed. Boxes should be placed in a water tight secondary containers (Tyvek bags) to protect from humidity while in transit. Make sure to generate an LDMS shipping manifest with each shipment including all requested information.

11.9.1 HIV QA Testing

Selected plasma aliquots will be shipped to the HPTN LC for HIV QA testing according to the HPTN Manual of Operations; additional testing may be performed (e.g. ABO typing).

When samples are received at the HPTN LC, the LC will perform additional QA and HIV testing. This will include:

- Quality assurance testing to confirm results of in-country testing
- Testing to confirm seroconversion events

Data from the HPTN LC will be submitted to the SDMC.

11.9.2 Pharmacology and Toxicology Testing

Plasma and DBS samples for ARV drug levels will be collected throughout the study. These samples will be collected from participants at time points indicated in Tables 11-1, 11-2 and 11-3. Analysis at the HPTN LC may be limited to a subset of the samples.

Pharmacology testing will be performed at the HPTN LC or at an outside laboratory designated by the HPTN LC. The primary pharmacologic assessments will be performed using assays that have been validated and approved by the Clinical Pharmacology Quality Assurance (CPQA) Committee. Results will not be returned to the sites or study participants.

Stored plasma may also be tested for the presence of other substances and drugs used for HIV or hepatitis treatment. Stored urine may be tested for the presence of medications used to treat substance use, other concomitant medications, and substances of abuse.

11.9.3 Other Testing

The HPTN LC will perform QA testing, including testing to determine HIV infection status in selected cases. Additional assays may be performed at the HPTN LC or a laboratory designated by the HPTN LC. This testing may include the following tests for participants who acquire HIV infection: HIV viral load, HIV resistance testing, HIV subtyping, HIV superinfection, and other tests to characterize HIV viruses and/or the host response to HIV infection. Phylogenetic analysis of HIV sequences may also be performed to study viral dynamics (how viruses spread in the community). Results will not be returned to the sites or study participants, with the exception of HIV testing (if results obtained at the HPTN LC do not agree with site results) and the exception for resistance test results, noted below.
Resistance testing will be performed at the HPTN LC or a laboratory designated by the HPTN LC. This testing will be performed retrospectively at the end of the study. If real-time resistance testing is needed for clinical management, that testing should be arranged by the site outside of the study; separate specimens should be collected for that testing. Results from specialized resistance testing (e.g., minority variants analysis, if performed) will not be returned to study sites.

Stored samples may also be used characterize HCV strains, the host response to HCV infection, and the relationship between HIV and HCV infections. This may include phylogenetic analysis.

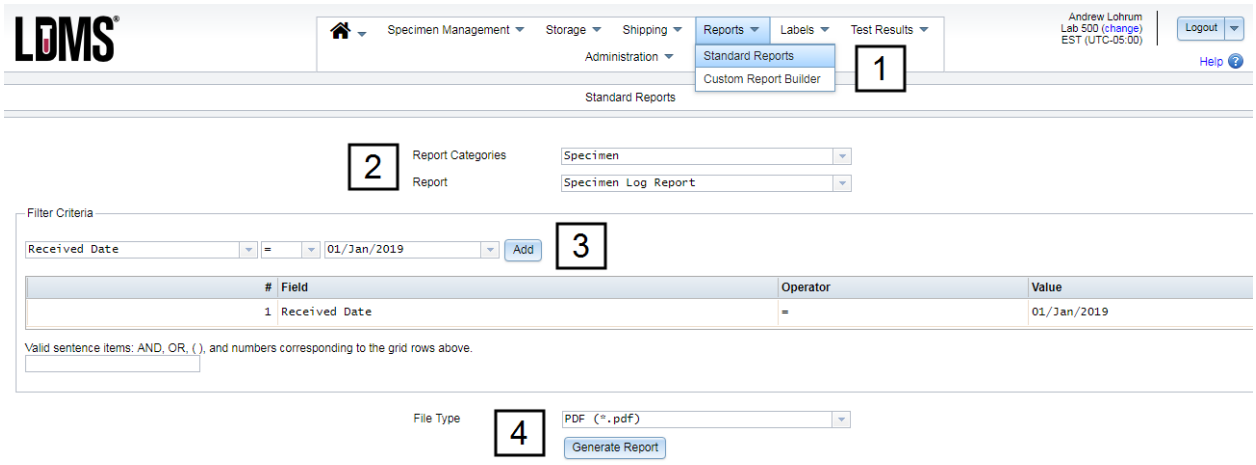
11.10 Laboratory Monitoring

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

11.9 Webs: Specimen Log Report

Upon request the HPTN LC may request to see a Specimen Log Report.

This report provides the user with a specific set of information for each of their logged specimens. The report will provide the user with the participant, primary, and aliquot information for each of their specimens. The report also provides the user with the condition codes, comments, and shipping information (if available) for the given specimens. Using the search criteria below will provide the user with a list of all specimens received by the lab on a particular date.



1. On the LDMS menu bar, hover over **Reports** and click **Standard Reports**.
2. Select the following:
    a. Report Categories: **Specimen**
    b. Report: **Specimen Log Report**
3. In **Filter Criteria**:
    a. **Field**: Received Date
    b. **Operator**: '='
    c. **Value**=Current Date
4. Set **File Type** to PDF; Click **Generate Report**