Section 8. Lab and Specimen Management Procedures

8.1 Overview of Section 8

This section contains information on the laboratory procedures performed in HPTN 074. Laboratory procedures will be performed in a variety of settings, including:

- Clinics
- Centralized laboratories
- The HPTN Laboratory Center (LC, Baltimore, MD, USA)
- 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 appendices 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. Guidance on universal precautions is available from the US Centers for Disease Control and Prevention and the World Health Organization at:

http://www.cdc.gov/ncidod/dhqp/bp_universal_precautions.html

Additional reference information can be requested from the HPTN LC. The information provided below is intended to standardize laboratory procedures for HPTN 074 across the study sites. Adherence to the specifications detailed in this section is essential to ensure that primary and secondary endpoint data derived from laboratory testing will be considered acceptable to regulatory authorities across study sites.

8.2 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. LDMS Tracking Forms will also be provided for use if required. The staff member who collects the samples will write the visit code, specimen collection date and time as well as their initials or code on the blood tube. They will also include this information on the specimen Transport Log and Specimen LDMS tracking form.

More detailed information about the labeling procedures is provided in the Chain of Custody SOP.

When specimens are tested at the centralized 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.

8.2.1 Local Specimen Testing

For samples that are processed and tested locally, each sample will be labeled with the PTID and may be entered into the LDMS (eg. viral load testing); this labeling may be supplemented with additional labeling needed to meet local procedures. All centralized lab results must be recorded following local guidelines.

8.2.2 Remote Specimen Testing

Samples that will be sent to the HPTN LC will be labeled and entered into the LDMS. Results from HPTN LC testing will be submitted to a central database through the SCHARP Atlas portal, coordinated by SCHARP and the LC.

8.3 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. Detailed instructions for use of LDMS are available in the LDMS User Manual:

https://www.fstrf.org/apps/cfmx/apps/ldms/ldmsManual/webhelp/index.html

All sites are responsible for ensuring that they are using the most recent version of 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 example of a two-dimensional LDMS-generated barcode label is below:

500V08000009
FEQ0043F-01
999515640 057
03/Jan/2005 08:00
BLD EDT PL2 N/A
1.00 ML 0 Scr

Row 1: LDMS Specimen ID
Row 2: Global Specimen ID
Row 3: Patient Identifier (ID1) and Study/Protocol Identifier (ID2)
Row 4: Specimen Date or Harvest Date and Specimen Collection Time
Row 5: Primary Type, Additive Type, Derivative Type, and Sub Additive/Derivative Type
Row 6: Volume/Volume Unit and Visit/Visit Unit (VID)
Row 7: Other Specimen ID (if applicable)

Questions related to use of LDMS for HPTN 074 should be directed to Estelle Piwowar-Manning (epiwowa@jhmi.edu) and Paul Richardson (pricha18@jhmi.edu).

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

LDMS User Support at Frontier Science

Regular Hours: 24 hour coverage 7 days a week with the exception of Select US Holidays - Thanksgiving Day, Christmas Day, New Year's Day, Memorial Day, Independence Day. See below for contact details

Email: ldmshelp@fstrf.org
Phone: +1 (716) 834-0900, extension 7311
Fax: +1 (716) 832-8448 (should be used to fax Installation Reports only)

LDMS User Support can be contacted during off-hours on U.S. holidays, by completing the LDMS help form on the Frontier Science portal. This form can be found on the portal by clicking the “Contact LDMS User Support” link. You will need a portal account to access this form.

While it is preferred that users use the “Contact LDMS User Support” link on the portal, there may be times when you need immediately assistance during off-hours and cannot access the portal. In these situations, you can contact LDMS User Support by emailing the pager email addresses directly.

Pager 1: ldmspager1@fstrf.org
Pager 2: ldmspager2@fstrf.org
Pager 3: ldmspager3@fstrf.org

Try pager 1 first. If you do not receive a response within 15 minutes, try pager 2, and then finally pager 3.

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?

8.4 LDMS Export Back up

Each site must export its LDMS data to Frontier Science (FSTRF) at a minimum weekly or whenever changes or additions are made to the LDMS database. Exported data are used by the HPTN SDMC to generate discrepancy reports comparing the data from the LDMS with that entered onto the CRFs.

The LDMS program automatically creates a backup of your database every day and places it in C:\fstrf\backup as long as the server is up. If the server is off, the backup will try to run when Oracle is next started. To back-up your LDMS, copy the most recent files in C: fstrf\backup on your LDMS server to external media, at least once a week. The backup folder contains 7 days' worth of automatic backup files. Backing up should be a part of normal scheduled weekly or

daily tasks. Refer to the LDMS user manual for more information. Check the integrity of the back up by copying one to the auto-backup files on the server to a new location and open it with Winzip or another zip utility. Verify that the backup log concludes with a statement indicating that the backup complete successfully.

8.5 LDMS Reconciliation

All sites must follow the HPTN LC approved site specific SOP for regular reconciliation and verification of specimens that are stored; these SOPs must be followed throughout the study. All sites must also create a Specimen Log Report for site laboratory LDMS reconciliations. 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 CORE, HPTN SDMC and HPTN LC. The HPTN CORE, 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.

Any discrepancies identified during the reconciliation between the CRF and LDMS data that SCHARP provides are included in a discrepancy report for each site. Sites are expected to resolve all discrepancies within one week of receipt of the report. The HPTN LC is responsible for reminding sites to adhere to the one-week timeframe and for following up with sites that do not resolve discrepancies within one week. The HPTN SDMC reviews the discrepancy reports for critical samples (e.g., plasma needed for confirmatory HIV testing) that appear to be missing, and works with the 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 'unresolvable' in LDMS. Any corrections to the LDMS need to be made following guidelines provided by FSTRF and by the HPTN LC.

8.6 Protocol related testing and sample collection

The following samples will be collected and processed at the screening, enrollment, and follow up visits as indicated in tables 8.2 – 8.5

- In-country HIV testing using local standard of care
- Plasma archive/storage.
- In country testing for substances of abuse using local standard testing procedures.
- Urine archive/storage.
- Dried urine process and archive.

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

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

Table 8.2 Schedule of Study Visits and Specimen Collection – Index Participants

	Screening	Enrollment	Week 4	Week 13	Week 26	Week 39	Week 52	Week 65	Week 78	Week 91	Week 104	Exit visit
HIV testing	X[1]	X[1]										
HIV viral load	X[2]											
CD4 cell count	X[2]				X		X		X		X	X[3]
Urine testing for substances of abuse		X	X	X	X	X	X	X	X	X	X	X[3]
Plasma storage[4]	X 10ml	X 20ml	X 10ml	X 20ml	X 20ml	X 20ml	X 20ml	X 20ml	X 20ml	X 20ml	X 20ml	X[3] 20ml
Frozen urine storage[5]		X	X	X	X	X	X	X	X	X	X	X[3]
Dried urine storage[6]		X			X		X					X[3]

1. Refer to HIV testing algorithm for screening and enrollment of index participants. Figure 8A.1
2. To be performed on the sample collected at screening if any of the HIV tests at screening is reactive or positive.
3. Must be performed at exit visit irrespective of when that occurs.
4. See section 8.8.1 for plasma processing and storage instructions. Blood collection noted is minimum volume.
5. See section 8.9.3 for frozen urine preparation and storage instructions.
6. See section 8.9.4 for dried urine preparation and storage instructions.

Table 8.3 Schedule of Study Visits and Specimen Collection – Index Participants during extension beyond original exit

	Re-enrollment	Quarterly	Exit
HIV testing	X[1]		
CD4 cell count		X[2]	X[2]
Urine testing for substances of abuse	X	X	X
Plasma storage[4]	X 20ml	X 20ml	X 10ml
Frozen urine storage[5]	X	X	X

1. Refer to HIV testing algorithm for screening and enrollment of index participants. Figure 8A.1
2. Must be performed at exit visit irrespective of when that occurs.
3. CD4 cell count testing will be performed 3 months and 9 months after re-Enrollment.
4. See section 8.8.1 for plasma processing and storage instructions. Blood collection noted is minimum volume.
5. See section 8.9.3 for frozen urine preparation and storage instructions

Table 8.4 Schedule of Study Visits and Specimen Collection – Network Injection Partners

NOTE: ALL network injection partners will attend the screening, enrollment and week 4 visits. After the week 4 visit, the partner will adopt their index visit schedule.

	Screening	Enrollment	Week 4	Week 13	Week 26	Week 39	Week 52	Week 65	Week 78	Week 91	Week 104	Exit visit
HIV testing	X[1]	X[1]	X[2]	X[2]	X[2]	X[2]	X[2]	X[2]	X[2]	X[2]	X[2]	X[2, 3]
Urine testing for substances of abuse		X	X	X	X	X	X	X	X	X	X	X[3]

Plasma storage[4]	X[7] 10ml	X[7] 10ml	X 10ml	X 10ml	X 10ml	X 10ml	X 10ml	X 10ml	X 10ml	X 10ml	X 10ml	X[3] 10ml
Frozen urine storage[5]		X	X	X	X	X	X	X	X	X	X	X[3]
Dried urine storage[6]		X			X		X					X[3]

1. Refer to HIV testing algorithm for screening and enrollment of network Injection partners. Figure 8A.2
2. Refer to HIV testing algorithm for follow up of network injection partners. Figure 8A.3. HIV testing is not required if HIV infection was confirmed at a previous visit.
3. Must be performed at exit visit irrespective of when that occurs.
4. See section 8.8.1 for plasma processing and storage instructions. Blood collection noted is minimum volume.
5. See section 8.9.3 for frozen urine preparation and storage instructions.
6. See section 8.9.4 for dried urine preparation and storage instructions.
7. It is not necessary to continue to store plasma at screening for non HIV infected Network Injection Partners who do not enroll.

8.7 HIV Testing

HIV testing will be performed using blood (no oral fluid testing) at participant visits in accordance with the testing algorithms described in Figures 8A.1 – 8A.4.

For further help on implementing the HIV testing algorithm seek guidance from the HPTN LC.

All tests and associated QC procedures must be documented on local laboratory log sheets or other laboratory source documents.

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 and verified within the specified time frame. 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 verification times. These must be recorded on testing log sheets.

8.7.1 HIV Testing for Index Participants

Participants with one or more reactive HIV test results at screening may be eligible for enrollment as an Index Participant. In all cases HIV infection status will be confirmed using local HIV testing guidelines.

In addition to having positive or reactive HIV test results at the screening visit, all participants must have a positive or reactive HIV test result at the enrollment visit before they are allowed to enroll as an index participant.

All cases of HIV infection must be confirmed using two independent samples collected on different days. (These different days would be the screening visit and the enrollment visit.) Plasma storage is required at every visit at which HIV testing is performed.

HIV testing will not be necessary during a re-screen visit for participants previously confirmed as HIV infected using two separate samples collected on different days.

8.7.1.1 HIV Testing for Index Participants Who Initially Screened as Network Injection Partners But Had a Reactive or Positive HIV Test Result

Referred network injection partners who have one or more reactive/positive HIV tests at study entry (Screening and/or Enrollment) may re-screen as index participants if the initial referring index does not enroll.
These participants should be retested for HIV infection when they re-screen as an index participant. If this re-screen is on a different day from the initial screen, no further HIV testing is required at the enrollment visit because HIV infection would have been confirmed using two independent samples collected on different days.

Figure 8.A.1 HIV Testing Algorithms for Index Participants:

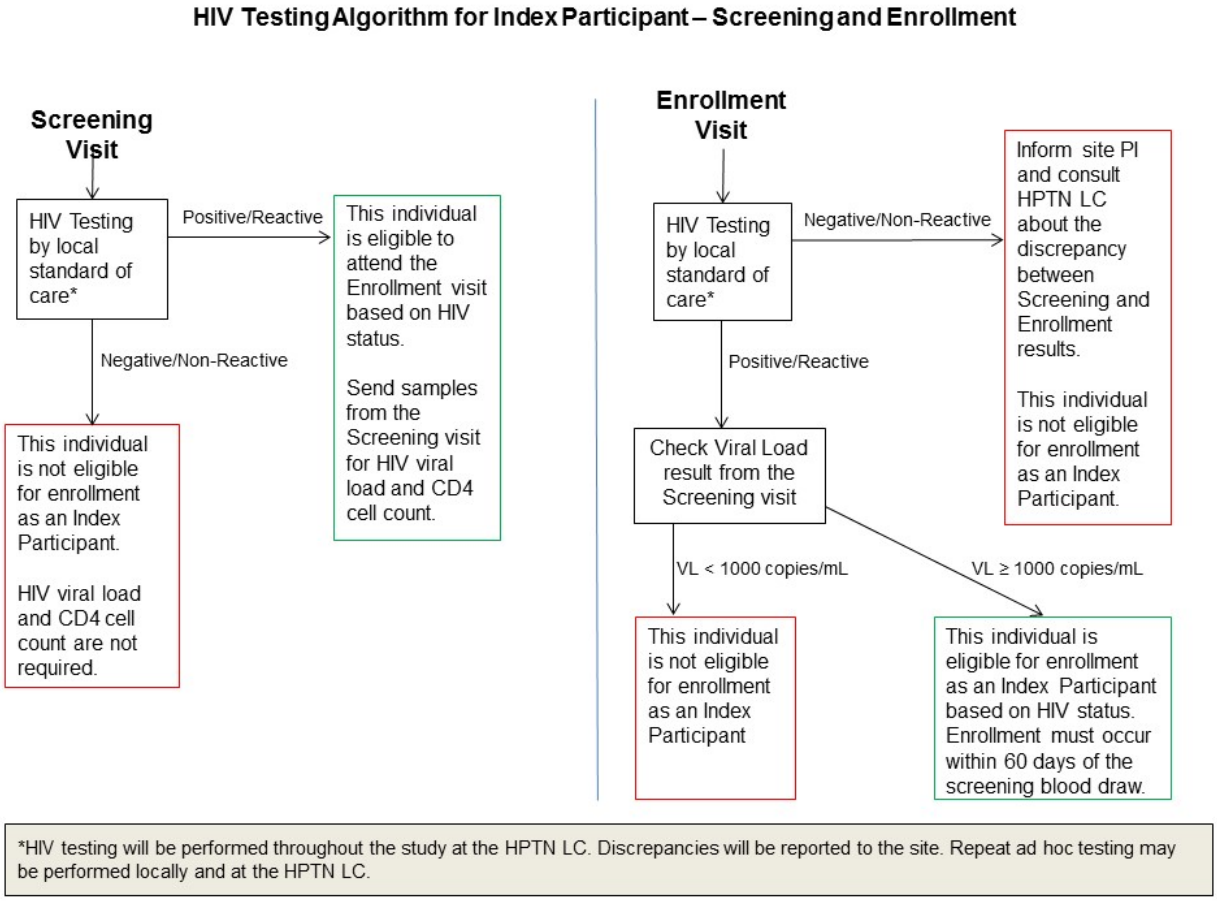
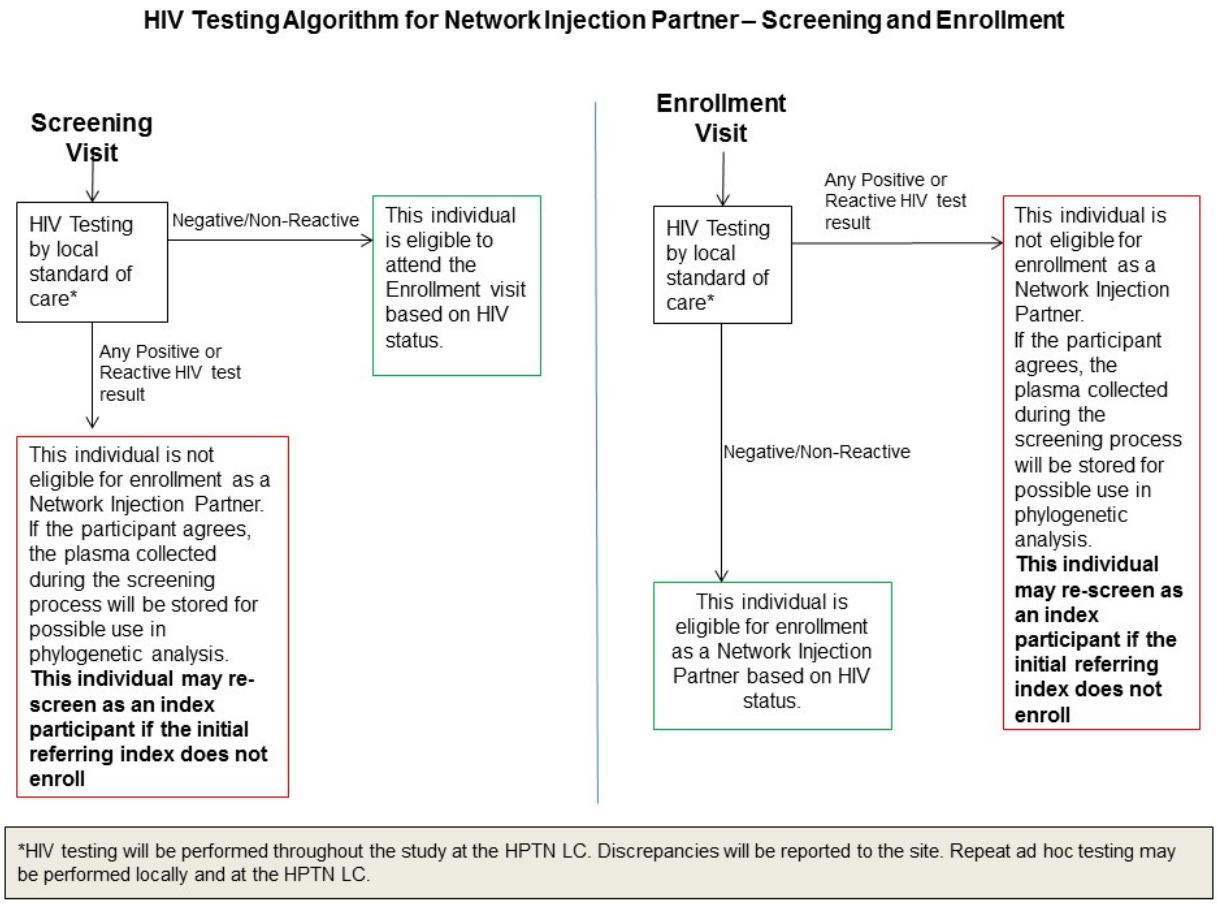


Figure 8.A.2 HIV Testing Algorithms for Injection Partners at Screening and Enrollment:



Participants with one or more reactive HIV test results at either the screening or enrollment visit will not be eligible for enrollment **as a network injection partner,** regardless of subsequent test results. In those cases, HIV infection status will be confirmed using local HIV testing guidelines.

An individual who has been screened to be a Network Injection Partner but has been confirmed as HIV infected is eligible to **re-screen as an index participant if the initial referring index does not enroll.**

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

Figure 8.A.3 HIV Testing Algorithm for Index Participants Who Originally Screened as Network Injection Partners.

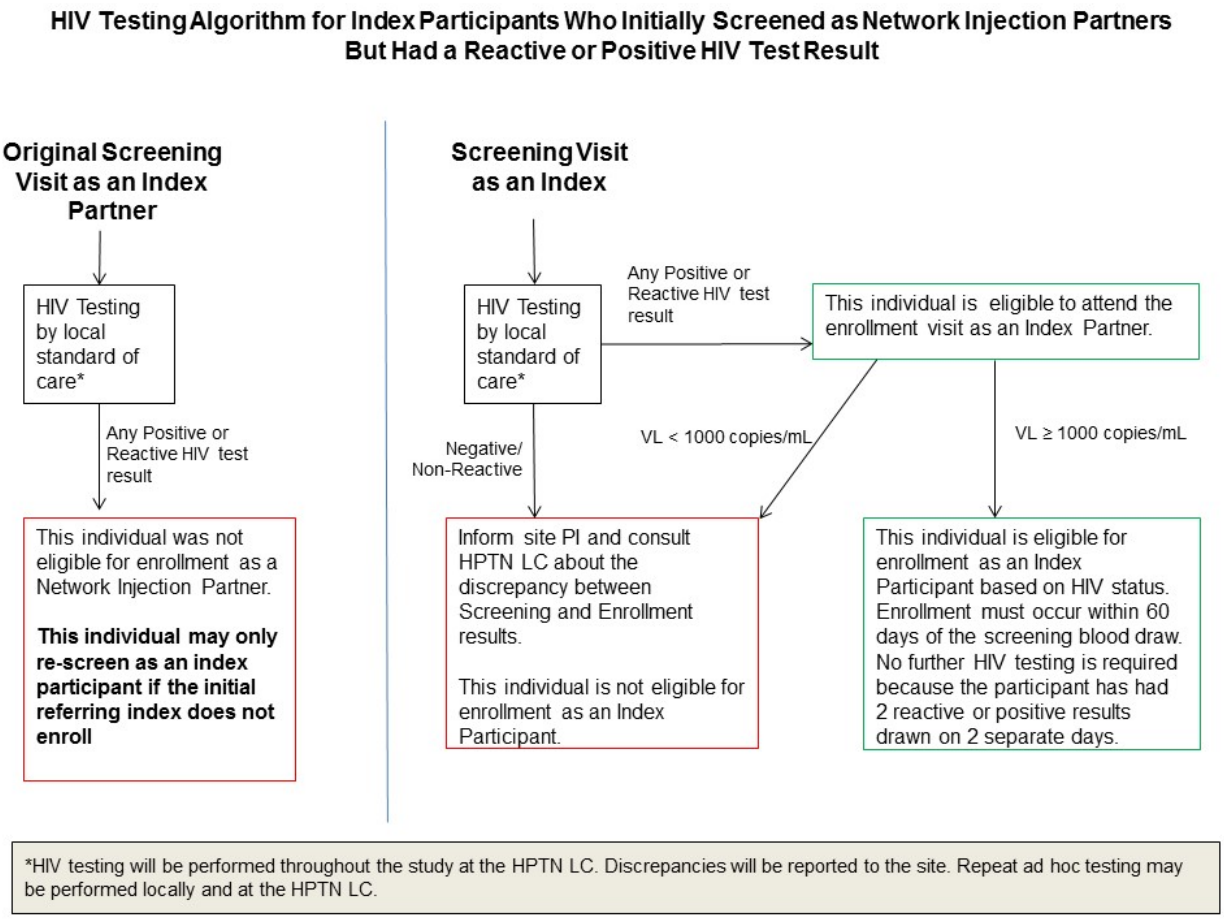
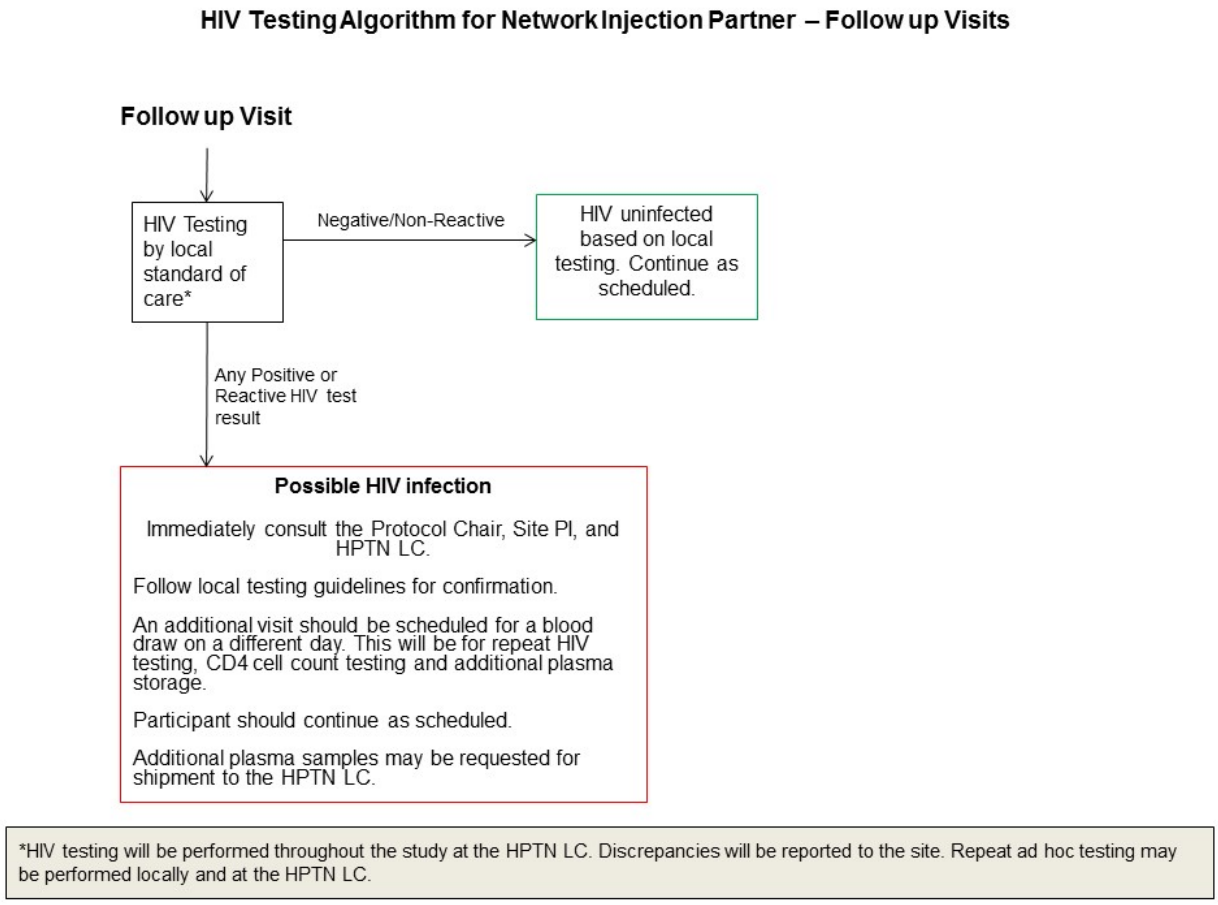


Figure 8.A.4 HIV Testing Algorithm for Injection Partners-Follow up visit:



If an injection partner has a reactive or positive HIV test result at any time after the enrollment visit, the participant should be scheduled for an extra visit to occur within 14 days of the initial reactive or positive test result.

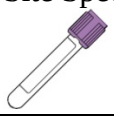
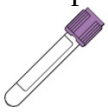
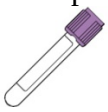
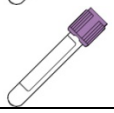
At this extra visit the participant will have blood drawn for repeat HIV testing, CD4 cell count, and additional plasma storage as indicated in table 8.4.

Table 8.5. Additional Procedures for Network Injection Partners who have a Reactive or Positive HIV test at any Time After Enrollment

	HIV confirmation visit following a reactive or positive HIV result.	Weeks 26, 52, 78 and 104 (if HIV infection has been confirmed at a prior visit)
HIV testing	X	
CD4 cell count	X	X
Additional Plasma storage from 10mL of EDTA whole blood.	X[1]	

1. See section 8.8.1 for plasma processing and storage instructions.

Table 8.6 Testing and Specimen Types

Tests		Specimen Type	Specimen Amount and tube type	Test Processing Location
Real Time Testing	**Stored Specimen for future test**			
HIV testing		Whole Blood	Site Specific 	Centralized lab or Clinic
CD4		Whole Blood	Site specific 	Centralized Lab
RNA		Whole Blood	Site specific 	Centralized Lab
	Plasma Storage	Whole Blood	1 to 2 x 10 ml EDTA*  	Processing Lab
Testing for substances of abuse		Urine	 10 mL Urine	Centralized Lab or Clinic
	Urine Storage	Urine		Processing Lab
	Dried Urine Storage	Urine		Processing Lab

*Check Tables 8.2 and 8.3 for collection requirements

8.8 Blood Collection and Processing

8.8.1 Plasma Processing and Storage

Five aliquots of plasma will be prepared from the 20 mL blood tube and a minimum of 2 aliquots of plasma will be prepared from the 10 mL blood tube.

10 mL or 20 mL of EDTA whole blood will be drawn for plasma storage at each time point at which HIV testing is performed as indicated in Tables 8.2. and 8.3.. HPTN LC should be informed at any time that two or less aliquots of 1.8mL are stored.

An additional 10 mL of EDTA whole blood will be drawn for plasma storage for network injection partner participants with a reactive or positive HIV test at any time after enrollment as indicated in Table 8.4. This additional plasma will be stored in aliquots of 1.8 mL. Store all plasma in as many aliquots as possible.

Sites will 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. Size and number of collection tubes may vary depending on local lab requirements.
- Deliver this to the local LDMS laboratory along with the LDMS Specimen Tracking Sheet or site specific requisition.
- 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. Cryovial size may vary, but 2.0 mL external thread 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.
- Blood processing and plasma storage should be performed within 6 hours of sample collection.
- The exact volume of blood collected should be entered into the LDMS. If 16 mL of blood is collected, the volume for the primary tube should state 16 mL.
- 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 another sterile centrifuge tube.
- Centrifuge plasma again at 800 - 1000 x g for 10 minutes to remove any contaminating debris, cells, or platelets.
- Log samples into LDMS and generate LDMS labels. (PL2) Each aliquot will have its own individual identification number (Global Specimen ID).
- Store plasma in aliquot number order. For example if there is only 3 mL of

plasma for archive, store 1.8 mL in aliquot 1. Store the remaining 1.2 mL in aliquot 2 and adjust the aliquot volume in LDMS to indicate 1.2 mL.

- For aliquots that have less than 1.8 mL, change the individual condition code of that aliquot to SHV.
- For aliquots that have a volume of 0.0ml, do not delete the aliquot. Change the aliquot condition code to QNS.
- 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 permission has been received from the LC to destroy the aliquots.. Note that some testing will be performed after study visits have been completed. Study sites should plan to store specimens for at least 5 years after the last study visit.

LDMS Entry:

LDMS Specimen Code for Plasma Storage

Test	Primary LDMS Code	Additive	Derivative	Sub Add/Deriv
Plasma Storage	**BLD**	**EDT**	**PL2**	**N/A**

Codes used in table:

BLD	Blood
EDT	EDTA
PL2	Plasma, Double Spun
N/A	Not Applicable
Other Spec ID:	Not Applicable

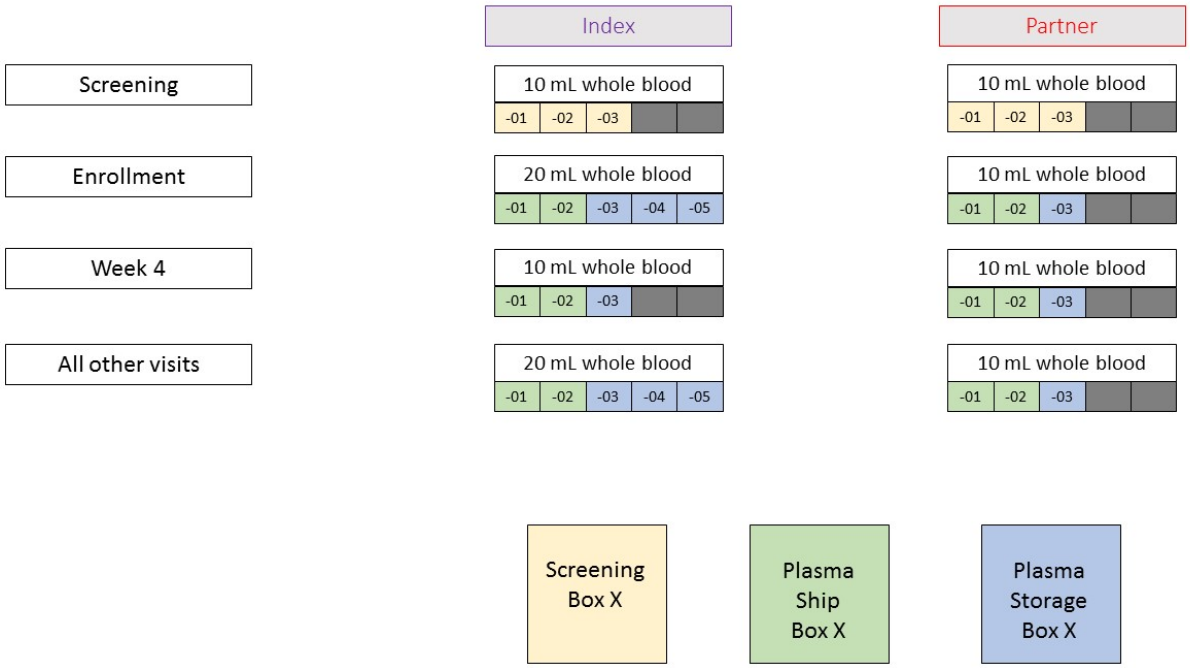
Primary Condition Code	Aliquot Condition Code
SAT	**SHV**
SNC	**QNS**
DSR	
HEM	

LIP	
ICT	
CLT	
PST	

- 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:
- **Screening Visit**
  - All Global Spec ID (-01, 02 and -03 if prepared): Store all screening visit aliquots from all participants in a box for **Screening (label Screening box x).**
- **All other visits**
  - Global Spec ID -01 and -02 - Store all global ID-01s and 02s from all participants from subsequent visits in a box for **LC Plasma designated to-be-shipped (label Plasma ship box x)**
  - Global Spec ID -03, 04, and 05 if prepared will be stored together in a **Storage** box. (**label Plasma storage box x)**
  - All plasma vials are stored in the LDMS and in a -70°C to -90°C freezer. Specimens in the "Plasma ship" box will be shipped to HPTN Laboratory Center (LC) when requested (quarterly). These boxes should be marked to ship in the LDMS.
  - In certain circumstances, the HPTN LC may request additional aliquots of the remaining 3 vials from the storage box.

An example of plasma aliquot storage can be seen in Figure 8.A.4.

Figure 8.A.5 Plasma Storage Graphic:



All enrolled study participants must consent to collection and storage of their plasma 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.

It is not necessary to continue to store plasma at screening for non HIV infected Network Injection Partners who do not enroll.

8.8.2 QA for HIV Testing

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 and resolve

discrepancies)

- Testing to determine the HIV status of all samples that have reactive results from in-country testing.
- Testing to confirm the HIV status of samples that have non-reactive results from in-country testing
- Testing to confirm seroconversion events

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

8.9 Urine Collection for Substances of Abuse Testing.

Urine for storage should be collected into a clean sterile urine collection container. The container must be labeled appropriately with the PTID and date and time of collection in the presence of the participant in order to avoid specimen mix ups.

Urine should be sent immediately for local testing and/or processing for storage. If this is not possible, urine must be held refrigerated until it can be sent. Urine can be stored refrigerated up to a maximum of 7 days.

The specimen must be received at the processing laboratory along with appropriate fully completed documentation such as a requisition or LDMS tracking sheet.

8.9.1 On Site Urine Testing for Substances of Abuse.

Study sites will test urine for substances of abuse at the study site clinic or designated laboratory using test kits and procedures that are currently in use as standard of care at that study site. This is to assess the reliability of such real life testing in the field. As such this testing should not be subject to additional QA procedures or GCLP requirements that would not routinely be used at the site outside of the study. Adherence to good practice standards is however recommended.

Results of this testing will be reported on the relevant CRF.

8.9.2 Urine Testing for Substances of Abuse at the LC.

Frozen urine and dried urine will be stored at the study sites until requested by the LC for shipment.

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

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

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.
- 1 additional aliquot label is required per dried urine method required, at visits when dried urine storage is required.

LDMS Entry:

LDMS Specimen Codes used For Urine.

Note: Storage of dried urine cartridges will only be performed at the VietNam site for the enrollment of Index Participants..

Test	Global ID	Primary LDMS Code	Additive	Derivative	Sub Add/Deriv	Volume	Unit
Urine Storage	-01	URN	NON	URN	N/A	1.8	ML
Urine Storage	-02	URN	NON	URN	N/A	1.8	ML
Dried Urine Storage – Filter Paper	-03	URN	NON	DUR	FPR	200	uL
Dried Urine Storage – Cartridge	-04	URN	NON	DUR	UCT	50	uL

Codes used in table:

URN	Urine
NON	NONE
DUR	Dried Urine
N/A	Not Applicable
FPR	Filter Paper
UCT	Urine Cartridge
ML	milliliter
uL	microliter

8.9.3 Frozen Urine

Transfer approximately 1.8 ml into each of two 2 mL cryovials, labeled with a LDMS label (Global Spec ID -01 and 02).

- 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 -01 - Store all global ID-01s from all participants in a box labelled **LC Urine Designated to be Shipped**.

    - Global Spec ID -02 - Store all global ID-02s from all participants in a box labelled **Stored Frozen Urine** .

Transfer labeled cryovials to the freezer locations assigned in the LDMS, store at -70° to -90°C

Samples in the box labelled LC Urine Designated to be Shipped will be sent to the LC testing laboratory upon request.

Samples in the box labelled Stored Frozen Urine will be stored on site until a request for shipment to the LC, or permission to destroy has been given.

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.

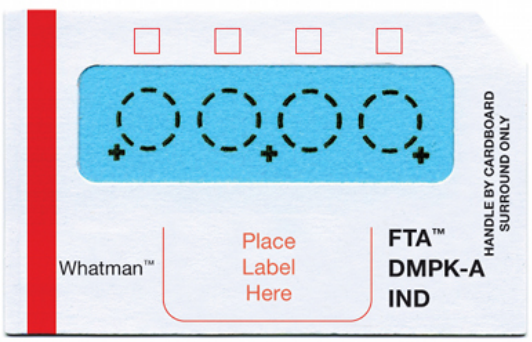
8.9.4 Dried Urine

At certain visits dried urine will also be stored and labeled with one or two LDMS labels (Global Spec ID -03 and 04)
One label will be used for each dried urine spot as follows:

8.9.4.1 Preparation of Dried Urine Filter Paper

Description:

Indicating FTA DMPK Cards have been designed for use with colorless samples such as urine. They contain a dye that will clearly show the location of the urine sample spots.



Using a disposable transfer pipette, transfer one drop of urine (estimated drop size 50uL) onto each of the four circles on the card. Hold the pipette tip a few mm above the card, **DO NOT** allow the pipette to touch the card.

It is important to have the sample spread evenly on the card surface as it quickly soaks in, giving a spot area directly proportional in area to the sample volume.

Label the card with LDMS label Global Specimen ID **-03.**

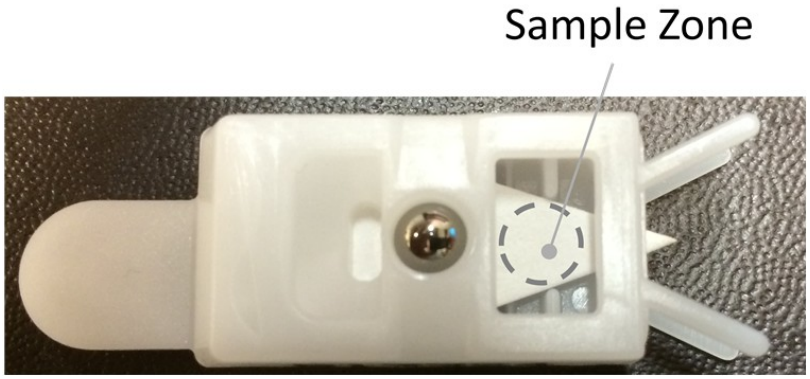
Dry the card thoroughly (2–3 hours) at room temperature.

Store each card at ambient temperature in its own zip lock bag containing desiccant.

Samples will be bulk shipped to the HPTN LC upon request.

8.9.4.2 Preparation of Dried Urine Cartridge (VIETNAM SITE ONLY, INDEX ENROLLMENT)

Using a transfer pipette one drop (approximate size 50 uL) of urine in sample zone of the cartridge.



Paper spray cartridge. The sample zone is indicated by the dotted full circle.

Label the card with LDMS label Global Specimen ID -**04.**

Allow the cartridge to dry for approximately 15 minutes at room temperature.
Store each cartridge at ambient temperature in its own zip lock bag containing desiccant.

Samples will be bulk shipped to the HPTN LC upon request.

8.10 Shipping of Samples to the HPTN Laboratory Center

The following types of specimens will be shipped to the HPTN LC for testing:

HIV QA testing

At least one aliquot from all study participants at each visit will be shipped to the HPTN LC for HIV QA testing. QA testing will be performed according to the HPTN Manual of Operations; additional testing may be performed. e.g. ABO typing.

Other testing

The HPTN may perform viral load and resistance testing. The HPTN LC may also perform specialized assays to characterize HIV viruses and the immune response to HIV infection in participants who become HIV-infected during the study. This testing may include HIV genotyping, HIV phenotyping, HIV subtyping, minority variants assays, phylogenetic/linkage testing, or other tests to characterize HIV viruses and/or the host response to HIV infection,

including assays to evaluate HIV incidence. In addition, testing may be performed for detection of antiretroviral drugs or other substances in study samples. In some cases, assays may be performed at an outside laboratory designated by the HPTN LC. Results from testing performed at the HPTN LC or at an outside laboratory designated by the HPTN LC will not be returned to study sites or study participants.

Shipping of Samples to the HPTN LC:

Each site will ship plasma samples to the LC upon request. 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 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 Lab 300 as the ship to lab ID number. The site should export the data to FSTRF after a batch has been made and notify SCHARP and HPTN LC with the batch 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, box map, LDMS diskette. For dry ice shipments, use diagnostics packing code 650, UN 3373, and address the shipment to:

Estelle Piwowar-Manning/
Johns Hopkins University Hospital
Department of Pathology
Pathology Building, Room 311
600 North Wolfe Street
Baltimore, MD 21287
USA

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, and expected date of arrival.

8.11 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.