Section 11 Laboratory Procedures

Section 11 Laboratory Procedures

This section contains instructions related to laboratory procedures required by HPTN 058. Each study site will adhere to standards of good laboratory practice (GLP), the HPTN Laboratory Standard Operating Procedures and their site-specific Standard Operating Procedures (SOPs) for proper collection, processing, labeling, and transport of specimens. All specimens will be shipped in accordance with International Air Transport Association (IATA) specimen shipping regulations. Storage and shipping will be documented using the HPTN Laboratory Data Management System (LDMS).

11.1 Biohazard Containment

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, all study personnel will employ appropriate blood and secretion precautions in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the US Centers for Disease Control and Prevention (CDC). A copy of the CDC's guidelines entitled "Universal Precautions for Prevention of Transmission of HIV and Other Bloodborne Infections" can be found at: http://www.cdc.gov/ncidod/dhqp/bp_universal_precautions.html

Additional laboratory reference information can be found in the joint HPTN-MTN Laboratory Manual, which is available at:

http://www.hptn.org/web%20documents/CentralLab/HPTN-MTNLABMANUALVersion1.0.pdf

11.2 Specimen Labeling

All containers into which specimens are initially collected (e.g., urine collection cups, blood collection tubes) will be labeled with PTID labels. SCHARP will provide a label template, and label stock, if needed. Study PTIDs are to be printed on these labels; however, study staff must write the specimen collection date on each label. The visit code also may be written on the label. When specimens are tested at the local lab, any additional labeling required for on-site specimen management and chain of custody will be performed in accordance with the site SOPs.

- **Specimen labels:** The optional initial supply of specimen labels are pre-printed with the PTID number and space for clinical staff to write the visit code and specimen collection date. Additional site-generated labels will also contain a pre-printed PTID number with space to write the visit code and collection date. After the visit code and collection date are completed, labels will be applied to each specimen collection tube or container in the clinic. To ensure proper adhesion, the tube surface should be clean, dry, and at room temperature before applying the label.

All Specimen Label sheets should be placed in a participant's folder once the participant has been assigned an ID number. On the day of a participant visit, the Specimen Labels should be taken out of the participant's folder and brought to the specimen collection location.

11.3 Use of LDMS

LDMS must be used at all sites to track the collection, storage, and shipment of plasma. Detailed instructions for use of LDMS are available at:

http://www.fstrf.org/ldms/manual/5.0/manual5.0.html

As of the date of this section, the current version of LDMS is Version 5.6. All sites should upgrade to this version as soon as possible. All sites must use HPTN Barcode label format for bar-coded labels in order to ensure that both the Specimen ID and the Global ID assigned to each specimen are printed on LDMS-generated labels. All sites must be using the bar-coded labels for use on the MVP-300 printer, utilizing the appropriate label size. Contact LDMS user support for further information.
Questions related to use of LDMS in HPTN 058 should be directed to Paul Richardson (pricha18@jhmi.edu + 410-502-0435). Technical support also is available from LDMS User Support. Usual business hours for LDMS User Support are 12 am to 6:00 pm ET on Monday through Friday. During business hours, please contact LDMS User Support as follows:

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

LDMS User Support can be paged during off business hours (6pm to 12 am Monday through Friday and on weekends) if you are locked out of LDMS or experience errors that prevent you from completing LDMS lab work. To page LDMS User Support, email LDMS pager 1 (address shown in table below) and include the following information in the body of your email:

- LDMS lab number (this is a three-digit number that is different from your network assigned clinical site number)
- The full telephone number at which you can be reached
- A short description of the problem

If a response is not received within 15 minutes after emailing LDMS 1, try emailing LDMS 2, then finally, LDMS 3.

The pagers also can be reached via telephone. When paging via telephone, after dialing you will hear a voice greeting followed by three quick beeps that indicate you are connected to the paging service. Please include the full telephone number at which you can be reached. Please call LDMS pager 1 first (telephone number shown in table below). If you do not receive a response within 15 minutes after calling LDMS 1, please try LDMS 2, then finally, LDMS 3.

LDMS User Support Paging Details		
Pager	**Email Address**	**Telephone Number**
LDMS 1	ldmspager1@fstrf.org	+716-556-0583
LDMS 2	ldmspager2@fstrf.org	+716-556-0584
LDMS 3	ldmspager3@fstrf.org	+716-556-0585

Each site must export its LDMS data to Frontier Science (FSTRF) on a weekly basis. Exported data are used by the HPTN SDMC to generate a monthly specimen repository report and to reconcile data entered in LDMS with data entered on study case report forms. Any discrepancies identified during the reconciliation are included in a monthly discrepancy report for each site. Sites are expected to resolve all discrepancies within two weeks of receipt of the report. The HPTN NL is responsible for reminding sites to adhere to the two-week timeframe and for following up with sites that do not resolve discrepancies within two weeks. The 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 NL 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 NL and SDMC will discuss and document any items that, although resolved, appear 'unresolvable' in LDMS.

11.3.1 LDMS Specimen Tracking Sheets

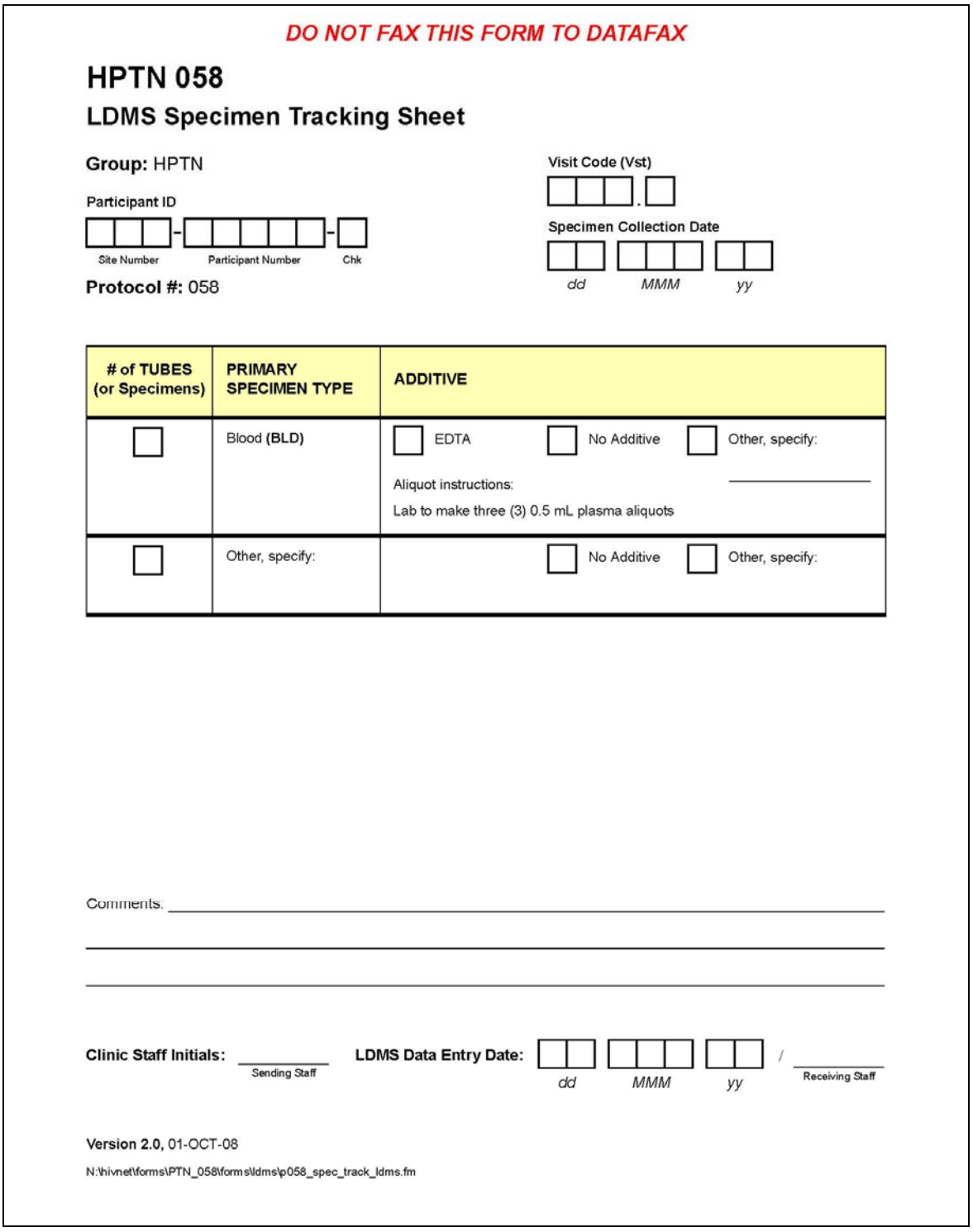
LDMS Specimen Tracking Sheet: This sheet identifies those specimens that will be entered into LDMS and accompanies them to the lab. The PTID number, visit code, and specimen collection date are recorded on the LDMS Specimen Tracking Sheet. A draft sample LDMS Specimen Tracking Sheet is shown in Figure 11-1. The use of this tracking sheet is optional.

Specimens from a single participant should be packaged together. Each package should include its own LDMS Specimen Tracking Sheet.

LDMS Specimen Tracking Sheets will be prepared and shipped to each study site prior to the start of the study

Specimens to be stored according to the protocol after assays are completed must be entered into the LDMS system as these are subject to quality control (QC) testing by the Network Lab (NL) (e.g., specimens for HIV testing) and may be needed for later confirmation or backup. All local lab results will be transcribed onto the Laboratory Results Forms. See Section 7 for detailed forms completion instructions. If a test not specifically required by the protocol is performed for clinical care or diagnostic purposes, the results will be recorded in the participant's source documents and, if appropriate, on the adverse event (AE) forms only (i.e. not on the local lab results form).

Figure 11-1 Sample Specimen Tracking Sheet



DO NOT FAX THIS FORM TO DATAFAX

HPTN 058

LDMS Specimen Tracking Sheet

Group: HPTN

Participant ID

☐☐☐-☐☐☐☐-☐
Site Number Participant Number Chk

Protocol #: 058

Visit Code (Vst)

☐☐☐.☐

Specimen Collection Date

☐☐ ☐☐☐ ☐☐
dd *MMM* *yy*

# of TUBES (or Specimens)	PRIMARY SPECIMEN TYPE	ADDITIVE
☐	Blood **(BLD)**	☐ EDTA ☐ No Additive ☐ Other, specify: _____ Aliquot instructions: Lab to make three (3) 0.5 mL plasma aliquots
☐	Other, specify:	☐ No Additive ☐ Other, specify:

Comments: _____

_____

_____

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

Version 2.0, 01-OCT-08

N:\hivnet\forms\PTN_058\forms\ldms\p058_spec_track_ldms.fm

11.3.2 LDMS Specimen Processing and Storage

When the specimen shipment is received at the local laboratory, each package in the shipment will be checked to ensure that all the specimens in the package are for the same PTID as on the Specimen Tracking Sheet and that the type and number of each specimen marked on the Specimen Tracking Sheet are correct. If discrepancies are noted, the clinic staff should be contacted and local procedures for QC and correction followed.

The laboratory tech will open the LDMS Specimen Management window and position the cursor at the Group box. The LDMS laboratory tech will enter the Group (HPTN), the PTID, and the protocol number from the Specimen Tracking Sheet. At this point the cursor should be in the Visit box. The visit code and type of visit ('VST') will be entered.

This finishes entry of participant information and begins primary specimen information entry. The collection and receipt dates are entered next. The dates may be entered by hand or by using the pull down calendar. The rest of the information will be entered following the HPTN LDMS User Manual (Section 3, Specimen Management). Plasma specimens will then be logged in, and three labels will be generated using label stock certified for long term freezer storage and dry ice shipping. The NL will specify all label stock to be used.
The LDMS-generated labels will be applied to each cryovial using standard procedures, the specimens will be aliquoted and then placed into a freezer box.

If three aliquots of 0.5mL are not obtained per participant visit, adjustments must be made in the LDMS to reflect the actual volume of specimen stored.

Each aliquot will be entered into the LDMS system using the Storage Management screen. They will be given assigned locations in identified containers and those containers will be added to the previously prepared storage structures in LDMS. Each container will then be placed into the LDMS-assigned freezer location according to LDMS specifications.

If specimens are designated by the protocol to be shipped to the HPTN NL or off-site laboratory, the specimens will be removed from the local freezer storage and placed into an appropriate shipping container using the LDMS system. The NL will define the shipping containers and packing and labeling procedures appropriate to the type of specimen. Instructions for shipping samples to the NL are included in section 11.7.

An electronic shipping manifest will be created for each shipment by the LDMS system. The electronic manifest disk will be placed in the shipping container along with the shipping manifest hardcopy and box report prior to final packing and pickup. After pickup, the electronic shipping manifest will also be sent as an email attachment to the intended recipient of the shipment (see specific specimen shipping instructions for exceptions). The email will include the name of the shipper and the shipment tracking number, so that the receiving laboratory can contact the shipper if the shipment does not arrive on schedule.

11.4 Required Laboratory Assays

Table 11-1 outlines all laboratory assays required by the HPTN 058 protocol for participants by visit. The tables identify the type of collection tube, the amount and type of specimen to be processed and stored, required tests at specific visits, and the processing lab. Specimen volumes are given only as a guide and site should consult with the local lab to determine volume requirements. Total blood volume at each visit must not exceed that stated in the consent forms.

All specimen collection tubes must be labeled with a SCHARP-provided PTID label. Labeling should take place in the presence of the participant. Collect specimens and label tubes according to local regulations and site-specific SOPs. Additional site-specific labeling is allowed. After collection:

At time points when rapid HIV testing is performed, pipette blood from the EDTA tube for the rapid test(s). Remember to send the remainder of the blood to the local lab for plasma storage.

Blood will be collected for testing and plasma storage at various time points throughout this study. All tubes should be labeled with the PTID number and collection date after blood collection. Blood will be collected according to local procedures and sent to the local lab for entry into LDMS if applicable and analysis.
Blood samples will be collected in EDTA or Serum Collection tubes. Lavender top tubes (usually additive = EDTA) require no additional processing prior to testing, but should be gently inverted at least eight times (or as specified by manufacturer) after specimen collection to prevent clotting.

If blood is limited, priority should be completion of HIV assay first followed by clinical assays (ALT, bilirubin, creatinine, CBC, platelet count) and then, if possible, processing of plasma samples for storage.

It is important for all site staff to observe the correct order of blood draw to prevent sample contamination:

The blood tube for chemistry and LFT must be drawn before the EDTA tube.

Table 11-1 Specimen Collection and Storage by Visit in HPTN 058.

Abbreviations: wk = week; wks = weeks; ml = milliliter; RT = real-time; spec = specimen; CBC = complete blood count

Study Visit	Collection Tube Type or Container	Tests	Specimen Type	Specimen Volume Guide[12]
		RT Test		
Screening	Urine Cup	Drug Screen	Urine	30 mL
		Pregnancy Test[2]	Urine	[2]
	EDTA	CBC + Platelets	Blood	3.0 mL
		Rapid HIV tests[3]	Blood	3.0 mL
		Plasma Storage[13]		
		Hep B Sag[11]	Site dependant	2.0 mL
		HCV Antibody[4,11]	Site dependant	2.0 mL
	Serum Tube[1]	Chemistry[5] + LFT[6]	Serum	4.0 mL
Enrollment / Randomization	Urine Cup	Pregnancy Test[2 + 7]	Urine	[2]
Safety Phase Wk 1	Urine Cup	Drug Screen	Urine	30 mL
	EDTA	CBC + Platelets	Blood	3.0 mL
	Serum Tube[1]	Chemistry[5] + LFT[6]	Serum	4.0 mL
Safety Phase Wk 2	Urine Cup	Drug Screen	Urine	30 mL
	EDTA	CBC + Platelets	Blood	3.0 mL
	Serum Tube[1]	Chemistry[5] + LFT[6]	Serum	4.0 mL
Safety Phase Wk 3	Urine Cup	Drug Screen	Urine	30 mL
	EDTA	CBC + Platelets	Blood	3.0 mL
	Serum Tube[1]	Chemistry[5] + LFT[6]	Serum	4.0 mL
Safety Phase Wk 4	Urine Cup	Drug Screen	Urine	30 mL
		Pregnancy Test[2]	Urine	[2]
	EDTA	CBC + Platelets	Blood	3.0 mL
	Serum Tube[1]	Chemistry[5] + LFT[6]	Serum	4.0 mL
InterventionVisit Wk 4,8	Urine Cup	Drug Screen	Urine	30 mL
		Pregnancy Test[2]	Urine	[2]
Wk 12	Urine Cup	Drug Screen	Urine	30 mL
		Pregnancy Test[2 + 8]	Urine	[2]
	Serum Tube[1]	LFT[6]	Serum	4.0 mL
Wk 16	Urine Cup	Drug Screen	Urine	30 mL
		Pregnancy Test[2 + 8]	Urine	[2]
Wks 20, 24	Urine Cup	Drug Screen	Urine	30 mLl
		Pregnancy Test[2 + 8]	Urine	[2]
Wk 26	Urine Cup	Drug Screen	Urine	30 mL
		Pregnancy Test[2 + 8]	Urine	[2]
	EDTA	CBC + Platelets	Blood	3.0 mL
		Rapid HIV test[3 + 14]	Blood	3.0 mL
		Plasma Storage[13]		
		Hep B Sag[11]	Site dependant	2.0 mL
		HCV Antibody[4, 11]	Site dependant	2.0 mL
	Serum Tube[1]	Chemistry[5] + LFT[6]	Serum	4.0 mL
Wk 28	Urine Cup	Drug Screen	Urine	30 mL
		Pregnancy Test[2 + 8]	Urine	[2]
Wks 32, 36	Urine Cup	Drug Screen	Urine	30 mL
		Pregnancy Test[2 + 8]	Urine	[2]
Wk 40	Urine Cup	Drug Screen	Urine	30 mL
		Pregnancy Test[2 + 8]	Urine	[2]
	Serum Tube[1]	LFT[6]		4.0 mL
Wks 44, 48	Urine Cup	Drug Screen	Urine	30 mL
		Pregnancy Test[2 + 8]	Urine	[2]
Wk 52	Urine Cup	Drug Screen	Urine	30 mL
		Pregnancy Test[2 + 8]	Urine	[2]
	EDTA	CBC + Platelets	Blood	3.0 mL
		Rapid HIV test[3 + 14]	Blood	3.0 mL
	Serum Tube[1]	Plasma Storage[13]		
		Chemistry[5] + LFT[6]	Serum	4.0 mL
Wk 78, 104, 130, 156 [10]	Urine Cup	Drug Screen	Urine	30 mL
		Rapid HIV test[3 + 14]	Blood	3.0 mL
	EDTA	Plasma Storage[13]		

Table 11-1 Specimen Collection and Storage by Visit notes.

1. Serum Tube – blood collection tube with no anticoagulant or preservative.
2. Female patients only. Can be done from same sample collected for drug screen.
3. If either rapid HIV test is positive, a Western Blot or Immunofluorescent Assay will be performed using the same sample for confirmation.
4. Reactive HCV Antibody tests will be repeated using a different HCV antibody enzyme immunoassay, HCV RNA assay, or HCV RIBA. Hepatitis testing may be performed at any point during first year of participation if clinically indicated.
5. Chemistry is just creatinine
6. LFT is ALT and total bilirubin.
7. For confirmation participant is not pregnant at time of first dosing.
8. Substitution treatment arm only, except for those participants in the detoxification arm who undergo a second detoxification at week 26.
9. If confirmed subsequent HIV tests not performed.
10. Participants will be followed for a minimum of 24 months and a maximum of 36 months depending on when they are enrolled.
11. Plasma obtained from EDTA can also be used. Sites should follow local procedures.
12. Specimen volumes here are for guidance only. Total blood volume must not exceed that stated in the consent form.
13. Store 3 x 0.5 mL aliquots of plasma. If three aliquots of 0.5mL are not obtained per participant visit, adjustments must be made in the LDMS to reflect the actual volume of specimen stored.
14. Follow algorithm for HIV testing at follow up. If subsequent samples are drawn for confirmation, plasma storage is also required.

11.4.1 Remote Specimen Testing

None of the routine laboratory tests for this study will be conducted at the HPTN NL. However, a sample of specimens will be retested by the NL at the site or at the HPTN NL for quality assurance purposes. Samples being sent to the HPTN NL or laboratories off-site for processing will be labeled and entered into the LDMS. Test results for samples shipped to the HPTN NL will not be recorded on a DataFax form. Individual results of NL testing will not be returned to the site; however, the site will be notified of any discrepant test results. If there are discrepant results, additional samples may be requested to resolve the discrepancy.

11.4.2 HIV Testing

Blood will be tested for evidence of HIV infection using tests that have been validated at the study site. All tests and associated QC procedures, must be documented on local laboratory log sheets or other laboratory source documents.

Perform all rapid tests according to site SOPs and package inserts. All staff involved in HIV testing and verification of HIV test results should be aware of the different testing timeframes for each rapid test, so that all tests are performed and verified within the specified timeframe. Place appropriate timekeeping devices in all test settings to ensure that each test is read and verified at appropriate time points. Document the testing start and stop times, as well as result verification times, on testing log sheets.

At all sites, when Western blot testing is required, an FDA-approved Western blot kit must be used. Perform this test according to site SOPs and the package insert. Interpret results based on the pattern of bands present, as follows:

Positive: At least two of the major bands — gp160/gp120, gp41, p24 — must be present and must be at least as intense as the low positive control gp120 band. The gp41 band must be broad and diffuse. (NOTE if any sites use the Cambridge HIV Western blot kit please inform the HPTN NL. The Cambridge Western blot kit uses a different control band for comparison)

Indeterminate: One or more bands are present, but the blot does not meet the criteria for a positive result as described above.

Negative: No bands are present.

All laboratory staff that read and interpret Western blot results for the study participants are required to complete proficiency testing approximately every six months. The HPTN NL will post an image of an actual Western blot run on the HPTN 058 web page for this purpose. Relevant laboratory staff from each site can review these images and submit their interpretations of the images to the HPTN NL via the web page. After each proficiency testing cycle, the HPTN NL will report results back to each site Laboratory Manager and specify any corrective action that may be needed. Contact the HPTN NL for additional information and guidance on performing and documenting the proficiency testing. Also contact the HPTN NL when new laboratory staff are hired, so that proficiency testing can take place prior to such staff interpreting Western blots for study purposes.

All participants who receive a negative HIV result by Western blot are to be considered HIV seronegative. No further testing is necessary until the next scheduled visit.

All participants who receive an indeterminate or positive Western blot should have a repeat blood draw. This repeat blood draw should be sent to the local laboratory for a repeat HIV Western blot analysis. For sample volumes, please refer to local laboratory requirements.

All participants who receive a positive HIV result by Western blot on this second sample are to be considered confirmed HIV seropositive.

If the laboratory reports a negative or indeterminate Western blot on this repeat testing, the site should consult the HPTN NL for guidance. Additional sample collection and testing may be needed.

HIV testing - screening.
Figure 11-2 depicts the testing algorithm for screening. Site staff should draw blood for HIV testing via two rapid HIV tests. If the initial rapid HIV tests are negative, the participant will be considered uninfected. If either of the initial tests are positive, a Western Blot (WB) or IFA should be performed on the first sample. If the WB or IFA result is negative, the participant will be considered uninfected but will not be eligible for enrollment at this time. If the WB or IFA result is positive or indeterminate, the participant will not be eligible for enrolment and will be referred to support services. The participant may be screened again (only once) for enrollment after 30 days from the initial blood draw

HIV testing – follow up
Figure 11-3 depicts the testing algorithm for follow-up visits. Site staff should draw blood for HIV testing via rapid HIV tests. If both of the initial rapid HIV tests are negative, the participant will be considered uninfected. If either of the initial tests are positive, a Western Blot (WB) or IFA should be performed on the first sample. If the WB or IFA result is negative, the participant will be considered uninfected. If the WB or IFA result is positive, a second sample will be collected on a different day for confirmatory testing with a WB or IFA. If the second confirmatory test is positive, the participant will be determined to be HIV infected. If the WB or IFA is negative or indeterminate, additional testing may be required at or before the next scheduled visit. In this case please consult the Network Lab for further advice. Blood drawn for confirmatory testing should also be sent for plasma storage.

Figure 11-2 HIV Antibody Testing Algorithm -- Screening
Taken from Protocol V 2.0 APPENDIX II-A: HIV Antibody Testing Algorithm – Screening

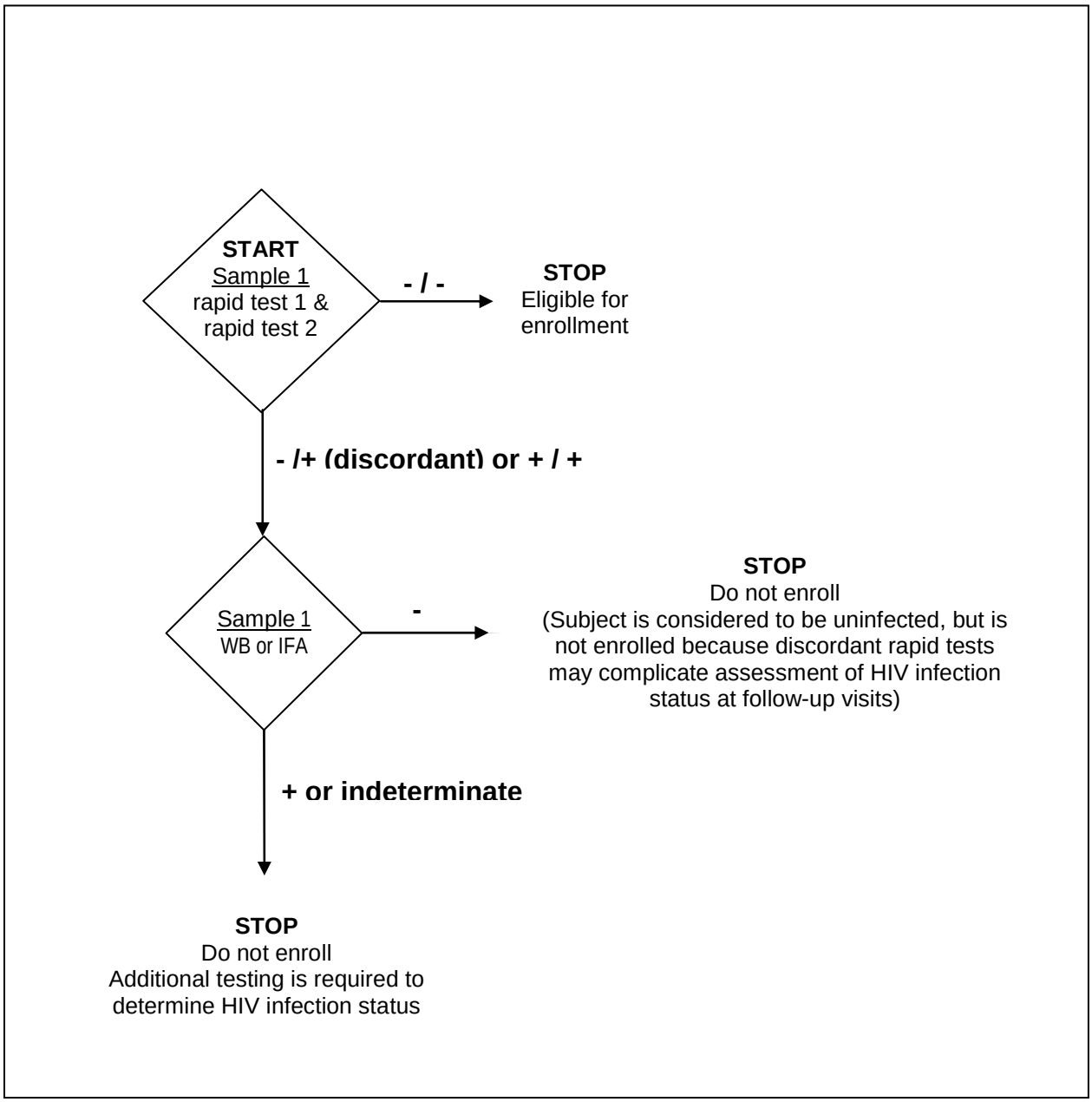
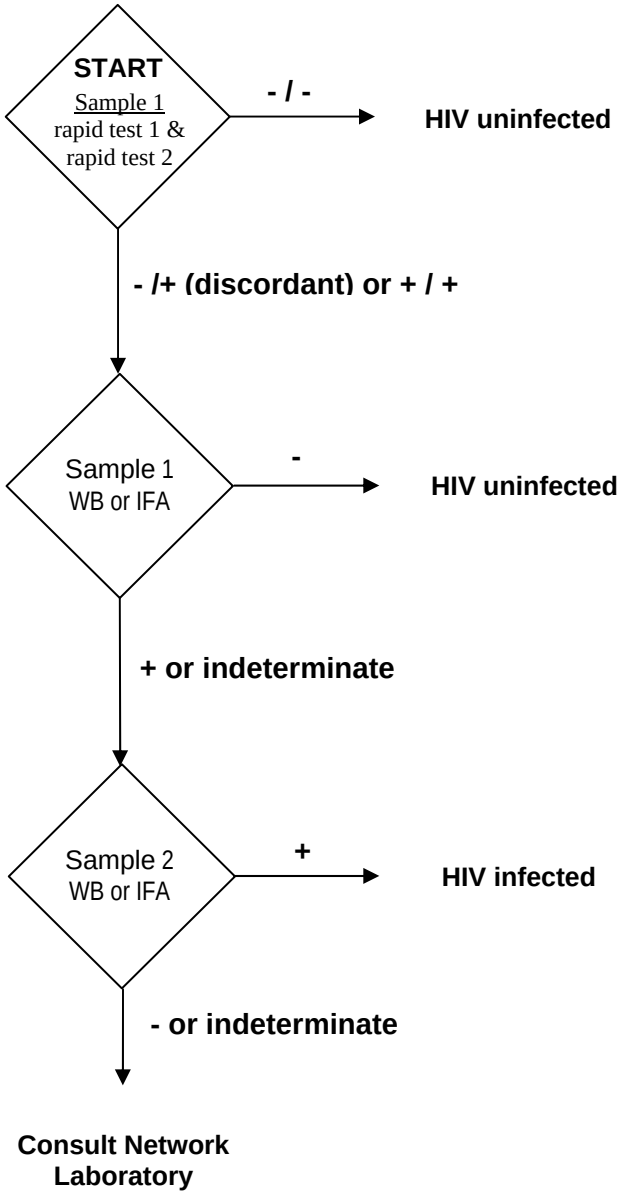


Figure 11-3 HIV Antibody Testing Algorithm – Follow-up
Taken from Protocol V 2.0 APPENDIX II-B: HIV Antibody Testing Algorithm – Follow-up



11.4.3 HCV Testing

Blood will be tested for evidence of HCV infection using tests that have been validated at the study site. All tests and associated QC procedures, must be documented on local laboratory log sheets or other laboratory source documents.

Perform all HCV assays according to site SOPs and package inserts.

Testing at the site can follow either of the algorithms shown in Figure 11-4 or 11-5.

Figure 11-5 describes sequential HCV testing:
Specimens which produce a single non reactive HCV EIA test result from method one should be reported as non reactive..

Specimens which produce a single reactive HCV EIA result from method one must be re tested using the second method. This second method could be either a different HCV EIA method, HCV RNA, or HCV RIBA. If this second test is also reactive the specimen should be reported as reactive.

If this second result is non reactive please consult with the HPTN Network Lab at NetworkLab@HPTN.org

Figure 11-6 describes HCV testing in parallel:
If both methods produce non reactive results, the specimen should be reported as non reactive.

If both methods produce reactive results, the specimen should be reported as reactive.

If both methods produce different results please consult the HPTN Network Lab for further advice.

Figure 11-4 HCV Antibody Testing Algorithm – Sequential Analysis

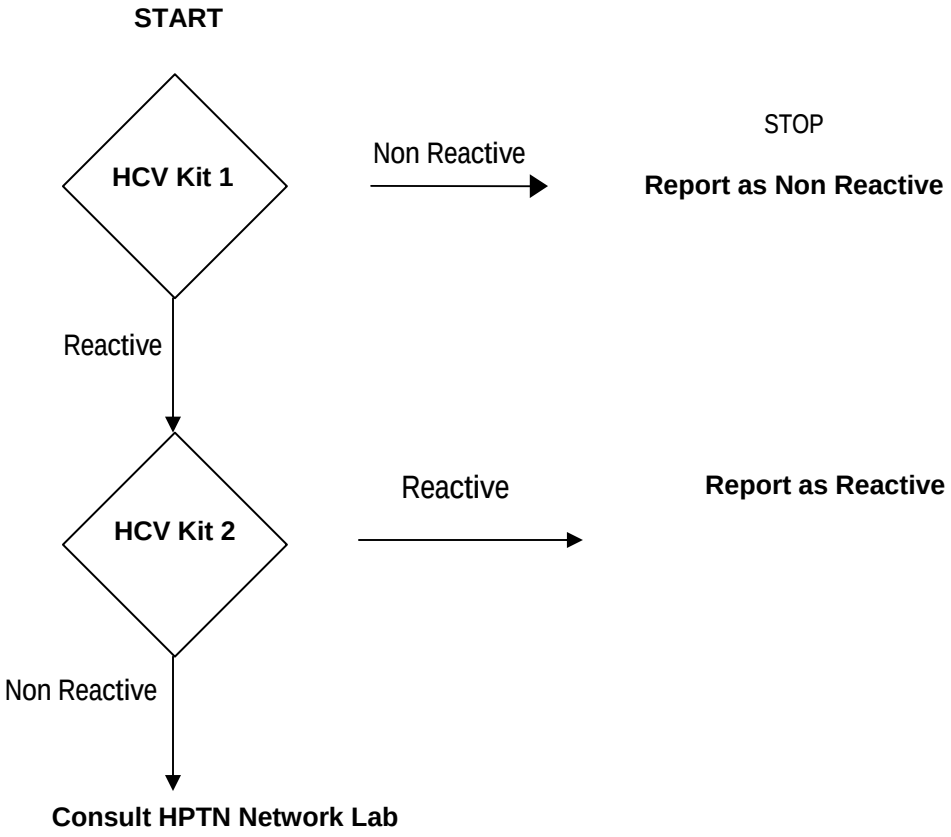
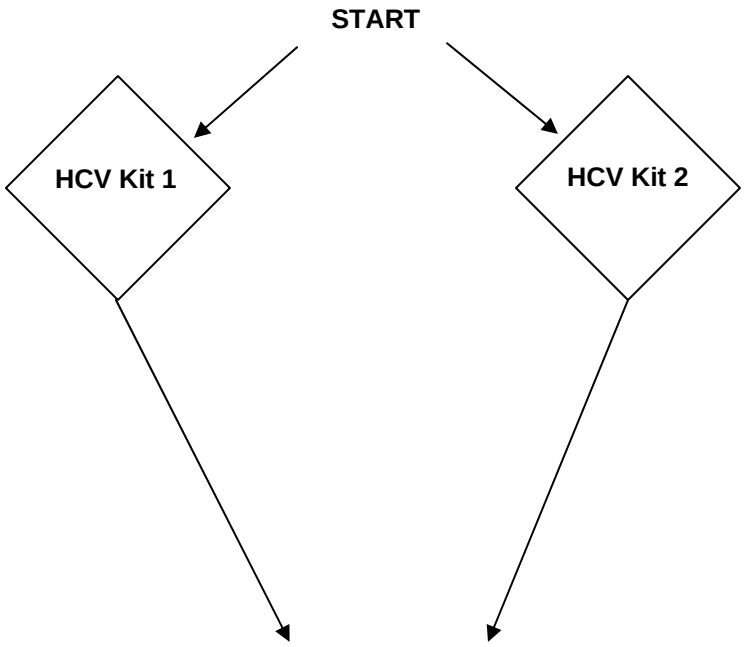


Figure 11-5 HCV Antibody Testing Algorithm – Parallel Analysis



If both reactive report as reactive.

If both non reactive report as non reactive.

If results are discordant
consult HPTN Network Lab.

11.4.4 Hepatitis B Testing

Blood will be tested for evidence of Hepatitis B infection using Hepatitis B Surface Antigen assays that have been validated at the study site. All tests and associated QC procedures, must be documented on local laboratory log sheets or other laboratory source documents.

Perform all Hepatitis B assays according to site SOPs and package inserts.

11.4.5 Hematology and Chemistry Testing

Blood will be collected for hematology and chemistry testing using tests that have been validated at the study site. All tests and associated QC procedures, must be documented on local laboratory log sheets or other laboratory source documents.

Perform all assays according to site SOPs and package inserts.

11.4.6 Plasma Archive

Throughout the course of HPTN 058, plasma from enrolled participants will be stored for later protocol-specified testing or future analysis. Plasma will be stored for all study participants, even those that have not agreed to long term storage.

Stored plasma will be used for the following purposes:

Quality Assurance (QA) HIV testing by the HPTN NL either on site or at the HPTN NL.

Incidence Testing or testing related to estimation of HIV incidence

Possible future research testing, if the participant provides written informed consent for such testing

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 HIV testing has been completed, this includes testing related to estimation of HIV incidence at selected sites. 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. Therefore, 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 the study is completed, the 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 NL will provide detailed instructions for specimen destruction and documentation thereof.

For blood samples, 3 x 0.5 mL plasma aliquots using labeled [LDMS generated label] cryovials according to local procedures. Store plasma aliquots in a –70°C freezer at the local repository.

A comment should be made in the LDMS whenever three aliquots are not stored.

The following codes should be used when logging plasma into the LDMS:

Specimen Type	LDMS Code
Primary Sample Whole Blood	BLD
Additive EDTA	EDT
Derivative Code Plasma (single spun)	PL1
Derivative Code Plasma (double spun)	PL2

All sites must have established SOPs for weekly reconciliation and verification of plasma archive specimens; these SOPs must be followed throughout the study. In the event that the required volume or number of plasma aliquots is not obtained at any time point, designated site clinic and lab staff must immediately inform the HPTN CORE, SDMC and NL. The HPTN CORE, SDMC, and NL will provide guidance on how to respond to the problem. In addition to following this guidance, designated site clinic and lab staff will work together to document the problem, take appropriate corrective and preventive action, and document all action taken.

11.4.7 Urine Testing

Urine will be tested for evidence of pregnancy and drug use using tests that have been validated at the study site. All tests and associated QC procedures must be documented on local laboratory log sheets or other laboratory source documents.

Urine will not be stored and should be discarded after use in accordance with local regulations.

Only staff who have been trained to perform the analysis and interpret the results of the urine tests should perform these procedures.

All staff who perform this testing are required to participate in the regular analysis of proficiency testing samples that will be distributed by the laboratory management.

11.5 Quality Control and Quality Assurance Procedures

The HPTN NL has established a proficiency-testing program at each study site. NL staff also will conduct periodic visits to each site to assess the implementation of on-site laboratory QC procedures, including proper maintenance of laboratory testing equipment, use of appropriate reagents, etc. NL staffs will follow up directly with site staff to resolve any QC or QA problems identified through proficiency testing and/or on-site assessments.

Throughout the course of the study, plasma samples from all HIV infected participants and an equal number of randomly selected uninfected participants will be retested by the NL. In the event of false positive or false negative HIV result, which changes the endpoint infection status of the subject, a sample from the last visit from all subjects will be retested. Site laboratory inspections will be done to check for adequate and appropriate collection, handling, storage and shipping of specimens, and general site lab QA.

The SDMC will inform site staff of the samples selected for quality assurance testing, and if necessary site staff will ship the selected specimens to the NL. All specimens will be shipped in accordance with the HPTN Manual of Laboratory Operations and IATA specimen shipping regulations.

The NL will test the specimens for HIV antibody and compare the results of their tests with the results obtained by the local labs. NL staff will follow up directly with site staff to resolve any quality assurance problems identified through this process.

11.6 Shipping Samples to the HPTN Network Laboratory

Throughout the course of HPTN 058, plasma samples may be shipped from selected sites to the HPTN NL for quality assurance or for further testing. When shipping:

The SDMC will provide a listing of samples (by PTID and specimen collection date) to be included in each shipment.

Upon receipt of each listing from the SDMC:

- Contact the HPTN NL at Johns Hopkins University (Estelle Piwowar-Manning: epiwowa@jhmi.edu, +410-614-6736) to coordinate the timing and logistics of the shipment. US sites may ship to the HPTN NL via Federal Express Monday through Thursday, with 24-hour fax notification.

- Working from the SDMC list of specimens to be shipped, use LDMS to generate a shipping manifest, box map, and LDMS shipping diskette for the selected samples.

- Obtain the selected specimens (one or more aliquots for each PTID and date as instructed) from the freezer and confirm the PTID, global ID, and date on the cryovial labels.

- Place the aliquots in a 5x5 or 9x9 cryovial box in the order of the shipping manifest.

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

- When shipping on carbon dioxide and/or liquid nitrogen (LN2), wrap the cryovial box in absorbent material and place it inside a shipping bag. Seal the bag and then place it in a shipping box. Fill the box with sufficient carbon dioxide (dry ice) to last at least 48 hours. World Courier will replenish dry ice as necessary. Please check with the manufacturer of the LN2 shipper for appropriate internal packaging. LN2 shippers are manufactured to maintain temperatures for 7-14 days, and World Courier should deliver the LN2 shipper within this time frame.

- Include a copy of the shipping manifest, box map, LDMS diskette, and CDC import permit in the shipment. For dry ice shipments and LN2 shipments, use diagnostics packing code 650, UN 3373. Use Non-Flammable Gas labels, Keep Upright stickers, and Do Not Drop – Handle With Care stickers, and address the shipment to:

  Estelle Piwowar-Manning/
  Johns Hopkins University Hospital
  Department of Pathology
  Pathology Building, Room 313
  600 North Wolfe Street
  Baltimore, MD 21287
  USA

- Notify the HPTN NL 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.

11.7 Laboratory Monitoring

The DAIDS Clinical Site Monitoring Group (PPD) conducts quarterly monitoring visits to HPTN study sites with ongoing studies (see also Section 16 of the HPTN Manual of Operations). In addition to performing monitoring tasks specified by the Division of AIDS (DAIDS) in study clinics and administrative locations, monitors also will perform monitoring tasks specified by DAIDS in each site's local laboratory or laboratories. Laboratory monitoring tasks may include confirmation of the use of LDMS and verification of specimen storage as recorded in LDMS. Specimens selected for on-site verification generally will not be pre-announced to site staff.

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