

Section 10. Laboratory Procedures

10.1 Overview and General Guidance

This section contains instructions related to laboratory procedures required by HPTN 046. Some laboratory procedures will be performed in study site clinics; others will be performed in study site local laboratories.

Each study site will adhere to standards of good clinical laboratory practice (GCLP), the HPTN Network Laboratory (NL) MTN Joint Laboratory Manual and their site-specific Standard Operating Procedures, which have been approved by the HPTN NL, for proper collection, processing, labeling, and transport of specimens. All specimens will be shipped in accordance with IATA specimen shipping regulations. Storage and shipping will be documented using the HPTN Laboratory Data Management System (LDMS).

Ideally one method, test kit, and or combination of test kits will be used for each protocol specified test throughout the duration of the study. If for any reason a new or alternative method or kit must be used after study initiation, site laboratory staff must perform a validation study of the new method or test prior to changing methods. Contact the HPTN NL for further guidance on validation requirements. Similarly contact the HPTN NL in the event that the local normal range for any protocol-specified test is updated after study initiation.

Regardless of whether tests are performed in clinic or laboratory settings, study staff who perform the tests must be trained in proper testing and associated QC procedures prior to performing the tests for study purposes; training documentation should be available for inspection any time.

10.2 Overview of Laboratory Testing Locations, Specimens and Methods

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 available from the US Centers for Disease Control and Prevention and the World Health Organization can be found at the following websites:

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>

Provided in the remainder of this section is information intended to standardize laboratory procedures across sites. Adherence to the specifications of this section is essential to ensure that primary and secondary endpoint data derived from laboratory testing will be considered acceptable to drug regulatory authorities across study sites.

10.3 Labeling Specimens and Recording Laboratory Results

All samples collected at a participant visit must be labeled at the time of collection with pre-printed PTID number and collection date labels. PTID numbers are pre-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.

As a condition for study activation, each study site must establish an SOP for local specimen handling and maintenance of “chain of custody” related to testing for the primary study endpoints (safety and HIV). The HPTN NL must approve this SOP. The SOP should state how a sample is obtained, how the sample is transported from the clinic to the lab, what documentation accompanies each sample, how its departure from one place and arrival at another are documented, and how it is handled and processed once it reaches the lab. Specific information that must accompany the specimen includes: the PTID number, collection date, and visit code for each specimen. Specimen labels provided by the SDMC include this key information. Accountability for the samples must be maintained, with requirements for signatures of the involved parties (i.e. each individual who handled the specimen). The site SOP should also detail how the results are returned from the lab to the clinic as well as how problem samples are reported back to the clinic. The following sections describe the collection and processing for each type of specimen.

10.4 SCHARP Pre-printed Labels and LDMS Specimen Tracking Sheets

Prior to the start of each study, the SDMC prepares and ships an initial supply of pre-printed specimen labels along with blank label stock and a label printing macro, enabling sites to print additional specimen labels for twins, triplets and for participants enrolled later in the study. LDMS Specimen Tracking Sheets will also be shipped to each study site prior to the start of the study. Questions regarding the pre-printed labels should be directed to Diana Lynn (dlynn@scharp.org)

- **Specimen labels:** The 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 PTID 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.

- **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 10-1.

Specimens from a single participant should be packaged together. Each package should include its own LDMS Specimen Tracking Sheet. Maternal specimens must be packaged and documented separately from infant specimens.

Figure 10-1: Sample Specimen Tracking Sheet

DO NOT FAX THIS FORM TO DATAFAX

**HPTN 046
LDMS Specimen Tracking Sheet**

Group: HPTN

Visit Code (Vst)

Participant ID (PID)

.

- - -

Site Number Participant Number Chk Cohort

Specimen Collection Date

/ /

dd MMM yy

Protocol #: 046

# of TUBES (or Specimens)	PRIMARY SPECIMEN TYPE	ADDITIVE
<input type="checkbox"/>	Blood (BLD)	<input type="checkbox"/> EDTA <input type="checkbox"/> No Additive <input type="checkbox"/> Other, specify: _____
<input type="checkbox"/>	Dried Blood Spot (DWB)	<input type="checkbox"/> EDTA <input type="checkbox"/> No Additive <input type="checkbox"/> Other, specify: _____
<input type="checkbox"/>	Breastmilk (BMK)	<input type="checkbox"/> No Additive <input type="checkbox"/> Other, specify: _____
<input type="checkbox"/>	Other, specify: _____	<input type="checkbox"/> No Additive <input type="checkbox"/> Other, specify: _____

Comments: _____

Clinic Staff Initials: _____ Sending Staff LDMS Data Entry Date: / / _____ Receiving Staff

dd MMM yy

Version 1.0, 12-SEP-05

/hivnet/forms/PTN_046/forms/ldms/honDF_spec_track_ldms.fm

DRAFT

10.5 The Laboratory Data Management System (LDMS)

The LDMS must be used at all sites to track the collection, storage, and shipment of the various types of laboratory specimens tested at remote laboratories (the HPTN NL or a laboratory off-site). Detailed instructions for use of the 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 5.5. All sites should upgrade to this version as soon as possible. All sites must use the "LDMS1" label format in order to ensure that both the Specimen ID and the Global ID assigned to each specimen are printed on the LDMS generated labels. All sites should be switching to the bar-coded labels with use on the MVP-300 printer, utilizing the appropriate label size. Contact LDMS user support for further information.

Questions about LDMS and specimen collection, shipping and storage should be raised with the HPTN NL Representative, Paul Richardson (phone 410-502-0435, e-mail pricha18@jhmi.edu). Technical support is also available from LDMS User Support. Usual business hours from LDMS user support are 12 am - 6:00pm (ET) 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 your LDMS or are experiencing errors that prevent you from completing your LDMS lab work. To page LDMS user support, please email LDMS pager 1 (address shown below). The following information should be included in the body of your email:

1. LDMS lab number. This is a three-digit number that is different from your network-assigned clinical site number.
2. The full telephone number at which you can be reached, including the country code and city code if you are outside the U.S.*
3. A short description of the problem.

If you do not receive a response 15 minutes after emailing LDMS 1, please try LDMS 2, then finally, LDMS 3.

The pagers can also be reached by dialing directly via telephone. When paging via telephone, you will hear a voice greeting followed by three quick beeps that indicate that you are connected to the paging service. Please include the full telephone number at which you can be reached, including the country code if you are outside the United States. Please call LDMS pager 1 first (telephone number shown below). If you do not receive a response within 15 minutes after calling LDMS1, please try LDMS 2, then finally, LDMS3.

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

* You can use the following web link to look up or verify the correct country and city code for your location: <http://www.countrycallingcodes.com>

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 the LDMS with data entered on study case report forms. Any discrepancies identified during the reconciliations 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 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 the clinic and/or laboratory records as appropriate, and entered in the details section of the LDMS. The NL and SDMC will discuss and document any items that, although resolved, appear 'irresolvable' in LDMS.

10.6 Required Laboratory Assays

Tables 10-1 and 10-2 outline all laboratory assays required by the HPTN 046 protocol for mothers and infants by study 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.

10.6.1 Local Specimen Testing

For samples being processed and tested locally, such as blood for HIV DNA PCR, CD4+ cell count, CBC with differential, ALT, HIV antibody and HIV RNA PCR (in some cases), each site may use their own labeling and tracking system. However, all specimens to be stored according to the protocol must be entered into the LDMS system as these are subject to QC testing by the HPTN 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 Mother and Infant Laboratory Results Forms. See Section 12 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 AE forms only (i.e. not on the local lab results form).

Note: Labs that have passed the 20 member Virology Quality Assurance (VQA) panel and are currently enrolled and participating in the bi-monthly certification panels will be allowed to perform the plasma HIV-1 RNA viral load assay. The VQA, VQA working group and the HPTN NL will monitor the performance of the labs. If a lab's performance is rated a P (probation) by the VQA, the lab will be notified that all testing must stop until the lab's performance is rated a C (certified). If the lab's performance is rated a PC (provisionally certified), the lab may be able to repeat that panel to better their score. The HPTN NL will monitor the viral load raw data until the lab's performance is rated a C.

Table 10-1: Maternal Specimen Collection and Storage by Visit

Table abbreviations: wks = weeks; mos = months; mL = milliliter; RT = real-time; spec = specimen; CBC = complete blood count

Study Visit	Collection Tube Type or Container	Tests		Specimen Type	Specimen Amount	Test Processing Location
		RT Test	Stored Spec			
Maternal Screening ¹	EDTA	HIV EIA or rapid (if needed) ²		Whole blood	0.5 mL or finger prick	Local Lab
		Western blot		Whole blood	0.5 mL or finger prick	Local Lab
		CBC + diff		Whole blood	0.5 mL	Local Lab
		CD4+ cell count		Whole blood	0.5 mL	Local Lab
			HIV-1 RNA PCR, NVP resistance, and QA testing	Plasma DBS	4 X 1.0 mL aliquots, frozen 2-3 x 50 µL spots	See section 10.6.2
Labor and Delivery ³	EDTA	CBC + diff		Whole blood	0.5 mL	Local Lab
		CD4+ cell count		Whole blood	0.5 mL	Local Lab
			HIV-1 RNA PCR and NVP resistance	Plasma DBS	4 X 1.0 mL aliquots, frozen 2-3 x 50 µL spots	See section 10.6.2
2 wks	EDTA	CBC + diff		Whole blood	0.5 mL	Local Lab
		CD4+ cell count		Whole blood	0.5 mL	Local Lab
			HIV-1 RNA PCR and NVP resistance	Plasma DBS	4 X 1.0 mL aliquots, frozen 2-3 x 50 µL spots	See section 10.6.2
		50 mL Corning/Eppendorf tube or other sterile container		HIV-1 RNA PCR and NVP resistance	Breast milk ⁴	3 x 2.0 mL aliquots, frozen
6 wks	EDTA	CBC + diff		Whole blood	0.5 mL	Local Lab
		CD4+ cell count		Plasma	0.5 mL	Local Lab
			HIV-1 RNA PCR and NVP resistance	Plasma DBS	4 X 1.0 mL aliquots, frozen 2-3 x 50 µL spots	See section 10.6.2
		50 mL Corning/Eppendorf tube or other sterile container		HIV-1 RNA PCR and NVP resistance	Breast milk ⁴	3 x 2.0 mL aliquots, frozen
3 mos	EDTA	CBC + diff		Whole blood	0.5 mL	Local Lab
		CD4+ cell count		Whole blood	0.5 mL	Local Lab
			HIV-1 RNA PCR and NVP resistance	Plasma DBS	4 X 1.0 mL aliquots, frozen 2-3 x 50 µL spots	See section 10.6.2
		50 mL Corning/Eppendorf tube or other sterile container		HIV-1 RNA PCR and NVP resistance	Breast milk ⁴	3 x 2.0 mL aliquots, frozen
6 mos	EDTA	CBC + diff		Whole blood	0.5 mL	Local Lab
		CD4+ cell count		Whole blood	0.5 mL	Local Lab
			HIV-1 RNA PCR and NVP resistance	Plasma DBS	4 X 1.0 mL aliquots, frozen 2-3 x 50 µL spots	See section 10.6.2
		50 mL Corning/Eppendorf tube or other sterile container		HIV-1 RNA PCR and NVP resistance	Breast milk ⁴	3 x 2.0 mL aliquots, frozen
12 mos	EDTA	CBC + diff		Whole blood	0.5 mL	Local Lab
		CD4+ cell count		Whole blood	0.5 mL	Local Lab
			HIV-1 RNA PCR and NVP resistance	Plasma DBS	4 X 1.0 mL aliquots, frozen 2-3 x 50 µL spots	See section 10.6.2
		50 mL Corning/Eppendorf tube or other sterile container		HIV-1 RNA PCR and NVP resistance	Breast milk ⁴	3x 2.0 mL aliquots, frozen
18 mos	EDTA		NVP resistance	Plasma DBS	4 X 1.0 mL aliquots, frozen 2-3 x 50 µL spots	See section 11f requi.6.2

1. From third trimester of pregnancy on or before 7 days postpartum
2. If documented evidence of the mother's HIV status as evidenced by 1 positive EIA or 1 positive rapid test is not available as part of standard of care at the study clinic, this testing will be performed after study informed consent has been obtained and prior to enrollment.
3. For women screened prior to labor and delivery only (on or before 7 days postpartum).
4. Only to be collected from women whose infants are still breastfeeding at each time point.

Table 10-2: Infant Specimen Collection and Storage by Visit

Table abbreviations: wks = weeks; mos = months; RT = real-time; spec = specimen; CBC = complete blood count; NVP = nevirapine						
Study Visit	Collection Tube Type or Container	Tests		Specimen Type	Specimen Amount	Test Processing Location
		RT Test	Stored Spec			
Birth – on or before 7 days after birth (Peripheral Blood)	EDTA	CBC + diff		Whole blood	0.5 mL	Local lab
		HIV-1 DNA PCR (or RNA PCR)	HIV-1 DNA pellet storage	Whole blood	1 x 0.5 mL aliquot 3 x 0.1 mL cell pellet ¹	Local lab
			HIV-1 RNA PCR, NVP resistance, NVP concentration, and QC	Plasma DBS	4-5 x 0.3 mL aliquots, frozen ² 2-3 x 50 µL spots	See section 10.6.2
	Clotted blood ⁴	ALT ⁴		Serum ⁴	0.5 mL	Local lab
2 wks	EDTA	CBC + diff		Whole blood	0.5 mL	Local lab
		CD4+ cell count ³		Whole blood		
		HIV-1 DNA PCR (or RNA PCR)	HIV-1 DNA pellet storage	Whole blood	1 x 0.5 mL aliquot 3 x 0.1 mL cell pellet ¹	Local lab
		HIV-1 RNA PCR, NVP resistance, NVP concentration, and QC	Plasma DBS	4-5 x 0.3 mL aliquots, frozen ² 2-3 x 50 µL spots	See section 10.6.2	
Clotted blood ⁴	ALT ⁴		Serum ⁴	0.5 mL	Local lab	
5 wks	EDTA	CBC + diff		Whole blood	0.5 mL	Local lab
		HIV-1 DNA PCR (or RNA PCR)	HIV-1 DNA pellet storage	Whole blood	1 x 0.5 mL aliquot 3 x 0.1 mL cell pellet ¹	Local lab
			HIV-1 RNA PCR, NVP resistance, NVP concentration, and QC	Plasma DBS	4-5 x 0.3 mL aliquots, frozen ² 2-3 x 50 µL spots	See section 10.6.2
	Clotted blood ⁴	ALT ⁴		Serum ⁴	0.5 mL	Local lab
6 wks	EDTA	CBC + diff		Whole blood	0.5 mL	Local lab
		CD4+ cell count ³		Whole blood		
			HIV-1 DNA pellet storage	Whole blood	1 x 0.5 mL aliquot 3 x 0.1 mL cell pellet ¹	Local lab
		HIV-1 RNA PCR, NVP resistance, NVP concentration and QC	Plasma DBS	4-5 x 0.3 mL aliquots, frozen ² 2-3 x 50 µL spots	See section 10.6.2	
Clotted blood ⁴	ALT ⁴		Serum ⁴	0.5 mL	Local lab	
8 wks	EDTA		HIV-1 DNA pellet storage	Whole blood	1 x 0.5 mL aliquot 3 x 0.1 mL cell pellet ¹	Local lab
			HIV-1 RNA PCR, NVP resistance, NVP concentration and QC	Plasma DBS	4-5 x 0.3 mL aliquots, frozen ² 2-3 x 50 µL spots	See section 10.6.2
	Clotted blood ⁴	ALT ⁴		Serum ⁴	0.5 mL	Local lab
3 mos	EDTA	CBC + diff		Whole blood	0.5 mL	Local lab
		CD4+ cell count ³		Whole blood		
		HIV-1 DNA PCR (or RNA PCR)	HIV-1 DNA pellet storage	Whole blood	1 x 0.5 mL aliquot 3 x 0.1 mL cell pellet ¹	Local lab
		HIV-1 RNA PCR, NVP resistance, NVP concentration and QC	Plasma DBS	4-5 x 0.3 mL aliquots, frozen ² 2-3 x 50 µL spots	See section 10.6.2	
Clotted blood ⁴	ALT ⁴		Serum ⁴	0.5 mL	Local lab	
6 mos	EDTA	CBC + diff		Whole blood	0.5 mL	Local lab
		CD4+ cell count ³		Whole blood		
		HIV-1 DNA PCR (or RNA PCR)	HIV-1 DNA pellet storage	Whole blood	1 x 0.5 mL aliquot 3 x 0.1 mL cell pellet ¹	Local lab
		HIV-1 RNA PCR, NVP resistance, NVP concentration and QC	Plasma DBS	4-5 x 0.3 mL aliquots, frozen ² 2-3 x 50 µL spots	See section 10.6.2	
Clotted blood ⁴	ALT ⁴		Serum ⁴	0.5 mL	Local lab	
9 mos	EDTA	HIV-1 DNA PCR (or RNA PCR)	HIV-1 DNA pellet storage	Whole blood DBS	1 x 0.5 mL aliquot 3 x 0.1 mL cell pellet ¹ 2-3 x 50 µL spots	Local lab
12mos	EDTA	CBC + diff		Whole blood	0.5 mL	Local lab
		CD4+ cell count ³		Whole blood		
		HIV-1 DNA PCR (or RNA PCR)	HIV-1 DNA pellet storage	Whole blood	1 x 0.5 mL aliquot 3 x 0.1 mL cell pellet ¹	Local lab

Table abbreviations: wks = weeks; mos = months; RT = real-time; spec = specimen; CBC = complete blood count; NVP = nevirapine

Study Visit	Collection Tube Type or Container	Tests		Specimen Type	Specimen Amount	Test Processing Location
		RT Test	Stored Spec			
			HIV-1 RNA PCR, NVP resistance, NVP concentration and QC	Plasma DBS	4-5 x 0.3 mL aliquot, frozen ² 2-3 x 50 µL spots	See section 10.6.2
18 mos	EDTA	CBC + diff		Whole blood	0.5 mL	Local lab
		CD4+ cell count ³		Whole blood		
		HIV EIA or rapid HIV test		Whole blood	1 x 0.5 mL aliquot	Local lab
			HIV-1 RNA PCR, NVP resistance, NVP concentration and QC	Plasma DBS	4-5 x 0.3 mL aliquots, frozen ² 2-3 x 50 µL spots	See section 10.6.2

1. If DNA PCR is performed, use 1 Pellet for real-time testing at the local lab, and store 2 pellets for possible future confirmatory testing/QA testing. If DNA PCR is not performed in real-time, store all aliquots for possible future confirmatory testing/QA testing.
2. All plasma, even if less than 0.3 mL, should be stored.
3. To be done on infants confirmed to be HIV-infected.
4. Some methodologies allow the use of EDTA plasma for ALT analysis. Please check with your local lab if this option has been validated.

10.6.2 Remote Specimen Testing

Samples indicated as “Stored Spec” in Tables 10-1, 10-2, and as “Plasma Storage” and “Dried Blood Spots” in Table 10-3 will be stored on-site until the site receives a request for further testing and / or shipping. Sites will receive specific instructions indicating which samples should be tested and / or shipped. Testing will only be performed on selected samples. However, since it will not be known which infants are HIV-1 infected, or at which visit infant HIV-1 infection is confirmed, specimens (maternal and infant plasma, and breast milk) will be stored at every study visit indicated.

HIV-1 RNA PCR assays for maternal and infant plasma will be performed locally (on-site), provided that the site is certified. The sites will **only** perform HIV-1 RNA PCR assays in real time if used for clinical management of the patient. These results can then be submitted to SCHARP via Laboratory Results Forms. Otherwise, this assay will be performed at the end of the study on samples determined by the protocol virologist. HIV-1 RNA PCR assays for breast milk will be performed at the HPTN NL. Assays for NVP concentration and NVP resistance will be performed at the HPTN NL. Quality Control (QC) testing for HIV diagnostic assays will be performed at the HPTN NL.

All samples stored on-site will be labeled and entered into the Laboratory Data Management System (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 for QA HIV diagnostic testing. If there are discrepant results, additional samples may be requested to resolve the discrepancy. No results from NVP concentration or NVP resistance testing will be reported to sites.

10.6.3 LDMS Specimen Processing and Storage

When the specimen is received at the local laboratory, each package (specimens and tracking sheet) will be checked following the local sites chain of custody SOP. If discrepancies are noted, the clinic staff should be contacted and local procedures for QC and correction followed.

Each type of specimen will be logged in, and the appropriate number of labels will be generated for that specimen type. If the dried blood spot card is made in the laboratory from a tube of blood, it is logged into LDMS as BLD. All non-blood specimens should be entered into LDMS

with the “No additive” type. LDMS-generated labels will be prepared using the LDMS system and printed on the LDMS printer using label stock certified for long term freezer storage and dry ice shipping. The LDMS-generated labels will be applied to each cryovial using standard procedures, which will be placed into a freezer box. For details on using the LDMS barcode labels, please refer to memo issued to sites on 18th March 2008. For Dried Blood Spots use BLD (blood) for the primary and then DBS (dried blood spot) for the derivative with each spot being an aliquot, so for example one card with 3 spots that was collected in an EDTA tube would be BLD/EDT/DBS with three aliquots of 50 µL each of DBS (no sub/add/der).

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.

10.7 Specimen Collection

This section describes how specimens should be collected for immediate laboratory analysis or long-term storage.

10.7.1 Blood

Blood will be collected for testing and storage 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.

Table 10-3 shows the blood tests to be conducted at specific visits.

Maternal blood samples will be collected in EDTA tubes. Approximately 7-10 mL of blood should be collected at each maternal blood draw. Infant blood samples will be collected in smaller EDTA tubes (for CBC with differential, CD4+ cell counts, HIV testing and plasma storage) and clotted tubes that will be used for the ALT. Up to 4 mL of blood should be drawn from infants through 9 months of age, and up to 5 mL can be drawn thereafter.

If infant blood is limited, priority should be completion of HIV assays first followed by clinical assays (CBC, CD4+, and ALT) and then, if possible, processing of plasma and DBS samples for storage.

10.7.2 Dried Blood Spot (DBS) Preparation and Storage

Dried blood spots are to be prepared from mothers at the following time points: 3rd trimester screening or on or before day 7 pp, 2 and 6 weeks, and 3, 6, 12 and 18 months.

Dried blood spots are to be prepared from infant blood at the following time points: within 7 days of birth, 2, 5, 6 and 8 weeks, and 3, 6, 9, 12 and 18 months. Prepare DBS for infants only if it appears there will be enough blood for all other testing required from the EDTA tubes.

Dried blood spots should be prepared from an EDTA blood tube received in the laboratory. The EDTA tube should be well mixed before preparing the DBS. Pipette 50ul of whole blood directly onto the centre of each spot on the filter paper so that it fills up the circle. Two or three blood spots should be collected. .

We recommend using the **903 Protein Saver™ card by Whatman, formerly S & S.**

Allow filter paper cards to air dry at room temperature (18-25° C) for at least 2 hours (do not place filter paper in heat or sun; spread cards in one layer – they should not be placed on top of one another). They can be hung by clothespins to a line to air dry completely. Place each dried card in its own ziplock bag with its own individual desiccant bag for storage. Store samples at room temperature, not exposed to sun or heat. Dried blood spots need to be entered into the LDMS and stored in an appropriate location so they can be easily retrieved when necessary.

Table 10-3: HPTN 046 Required Laboratory Blood Assays Per Participant Visit

	Screening 3rd trimester or on or before day 7 pp	Labor / Delivery (on or before day 7 pp) ¹	2 wk	5 wk	6 wk	8 wk	3 mo	6 mo	9 mo	12 mo	18 mo
Maternal Evaluations											
HIV EIA or rapid test (if needed) ²	X										
Western Blot	X										
CBC with differential	X	X	X		X		X	X		X	
CD4+ cell count	X	X	X		X		X	X		X	
Dried Blood Spots ⁷	X	X	X		X		X	X		X	X
Plasma Storage ³ NVP resistance HIV-1 RNA PCR	X	X	X		X		X	X		X	X
Infant Evaluations		Enrollment (on or before day 7 post birth)									
CBC with differential		X	X	x	X		X	X		X	X ⁴
CD4+ cell count ⁴			X		X		X	X		X	X
ALT		X	X	x	X	x	X	X			
Dried Blood Spots ⁷		X	X	x	X	X	X	X	X	X	x
Roche Amplicor HIV-1 DNA PCR ⁵		X	X	x			X	X	X	X	
Cell Pellet Storage		X	X	X	X	X	X	X	X	X	
HIV EIA or rapid HIV test ⁶											X
Plasma Storage ³ NVP resistance HIV-1 RNA PCR NVP concentration		X	X	x	X	X	X	X		X	X

1. For women screened prior to labor and delivery only (on or before day 7 postpartum)
2. If documented evidence of the mother's HIV status as evidenced by 1 positive EIA or 1 positive rapid test is not available as part of standard of care at the study clinic, this testing will be performed after study informed consent has been obtained and prior to enrollment.
3. See section 10.6.2.
4. To be done on infants with confirmed HIV infection only
5. If DNA PCR is not available, quantitative RNA PCR may be used. If PCR positive, confirm with a repeat PCR on a second sample obtained on or before the participant's next scheduled visit
6. If reactive, confirm with a Western Blot on a second sample obtained on or before the participant's next scheduled visit
7. 2-3 Dried Blood Spots are made if there is extra blood only. NO additional blood is taken. These will be used for possibly DNA/RNA PCR and NVP resistance testing.

10.7.3 Breast Milk Collection

Breast milk for HIV-1 RNA and HIV-1 genotyping and testing will be collected on scheduled 2 and 6 week visits and 3, 6, and 12 month visits from mothers as long as the infant is breastfeeding or up to 12 months, whichever is sooner. Specimens will be stored on site and shipped to the NL for testing. The SDMC will identify the samples needed for testing and request from the sites that these samples be shipped to the NL. See specimen-shipping instructions in Section 10.9. Following are the supplies and procedures for the collection of breast milk.

Supplies

- Clean water and soap
- Sterile gauze pads
- One sterile 50 mL Corning or Eppendorf centrifuge tube, labeled with the SCHARP pre-printed PTID number and date labels.
- Sterile urine cup or other sterile container (optional, if preferred for the collection of the sample), labeled with the SCHARP pre-printed PTID number and date labels.
- Specimen Tracking Sheet

Procedure for Collecting Samples

Before collection of milk, the woman and nurse should wash both hands with soap and water and dry their hands. The woman should be seated during the collection of breast milk. If a nurse is to assist in the collection, the nurse should also be seated.

Gently clean the breasts with soap and water. Rinse soap away with clean water and dry the breast with sterile gauze pads.

The collection of milk can begin. The collection may be obtained from one or both breasts. If milk is collected from both breasts, the milk from both breasts can be collected in a single container (combined).

Ask the woman not to touch the nipple, to keep it as clean as possible. She should position her hand on the breast by placing the fingers below and the thumb above the areola. If the breast size is very large, it may be easier to place one hand above and one hand below the breast. A sterile 50 mL centrifuge tube labeled with the SCHARP pre-printed labels should be placed in front of the nipple. A urine cup or other sterile container may also be used for collection, if preferred. It may be easiest if the nurse holds the collection container.

The woman should express the milk in a manner that is comfortable for her. The woman may prefer to compress her thumb and fingers together while moving the hand away from the chest wall in a 'milking' action towards the nipple. Continue to express the milk at a comfortable rate until at least 10 mL of breast milk have been collected. If 10 mL of breast milk cannot be collected from one breast, then the procedure should be repeated with the other breast.

If the breast milk was collected in a container other than the labeled 50 mL centrifuge tube (e.g., urine cup), the breast milk should be transferred to a sterile 50 mL tube labeled with the SCHARP pre-printed labels immediately after collection. The 50 mL centrifuge tube containing the sample should be capped for storage. The nurse should document which breasts were used for collection (left, right, or both) on study source documents.

10.8 Infant HIV Testing

Blood will be collected for infant HIV testing by real-time Roche Amplicor HIV-1 DNA PCR at 2 and 5 weeks and 3, 6, 9 and 12 months. HIV EIA/rapid testing will be performed at 18 months only. HIV testing should be completed as described in section 5.6 of this SSP manual and according to one of the two algorithms included as Figures 10-2 and 10-3. *Note: Quantitative RNA PCR may be used if DNA PCR is not available.*

If the initial PCR is negative the participant will be considered uninfected at that time point. If the initial test is indeterminate, then a second aliquot will be tested. If the initial test is positive or the second test is still indeterminate, a second specimen will be collected on a different day as soon as possible, but no later than the next scheduled visit, to be tested by PCR for confirmation. If the second sample is positive after the initial positive, the infant will be confirmed to be HIV infected. If the second sample is negative, indeterminate or positive following an initial indeterminate result, additional testing may be required at or before the next scheduled visit. In this situation please contact the HPTN NL for further advice. An infant will be considered HIV-infected only if two separate blood specimens drawn on different days are each positive by HIV DNA PCR. To summarize:

Sample 1: PCR (DNA or RNA)

Negative: Report as HIV uninfected.

Positive: Collect Sample 2 as soon as possible; no later than next scheduled visit.

Indeterminate DNA: Repeat testing of sample 1 in duplicate. If result is still indeterminate, collect sample 2 as soon as possible; no later than next scheduled visit.

Sample 2: PCR (DNA or RNA)

Indeterminate or Negative: May require additional testing at or before next scheduled visit. Consult the HPTN NL.

Positive (if sample 1 was indeterminate): Consult the HPTN NL.

Positive (if sample 1 was positive): This confirms HIV infection.

In summary, at each scheduled testing time point:

- If the first assay is negative, the participant is considered uninfected.
- If the first DNA assay is indeterminate, sample 1 will be retested in duplicate. If the result is still indeterminate, another assay is done on a different specimen drawn on a different day no later than the next scheduled visit and sooner if possible.
- If the first assay is positive, another assay is done on a different specimen drawn on a different day no later than the next scheduled visit and sooner if possible.
- If this second assay is positive the participant is considered infected.
- If this second assay is negative or indeterminate, additional testing may be required. Network Laboratory at JHU should be consulted.

If it is confirmed that an infant has become infected with HIV it is not necessary to perform subsequent RNA or DNA assays real time.

The site may continue to perform the RNA or DNA assay if results are required for clinical management. These results can then be submitted to SCHARP via Laboratory Results Forms. Otherwise, the HIV-1 RNA assay will be performed at the end of the study on samples selected by the protocol virologist.

Figure 10-2: HIV Testing Algorithm For Infants 15 Months Of Age or Younger

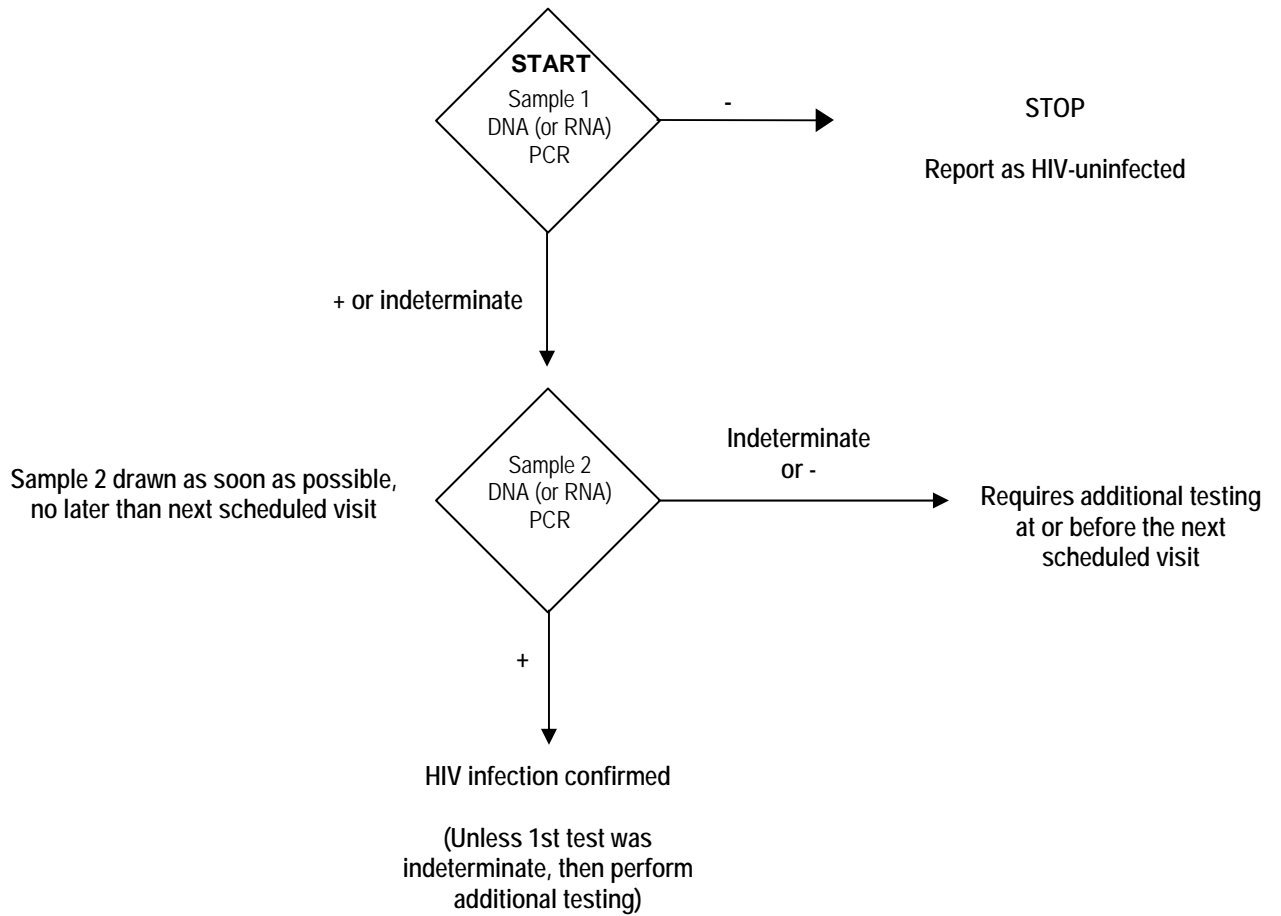
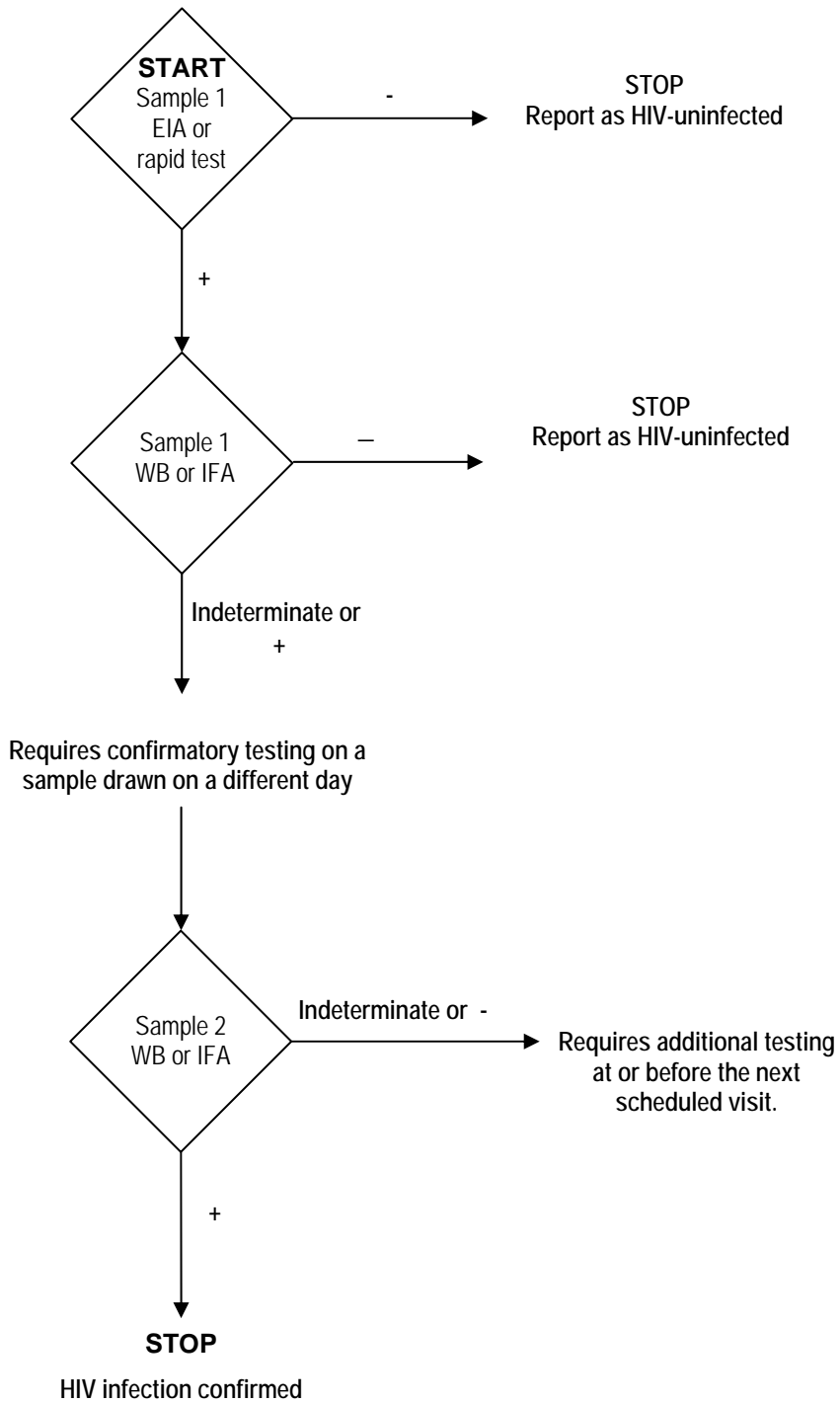


Figure 10-3: HIV Antibody Testing Algorithm for Infants Older Than 15 Months of Age



10.9 Required Stored Specimens

Throughout the course of HPTN 046, breast milk, dried blood spots, and plasma will be stored for later protocol-specified testing. In addition, participants at some sites will be asked to provide informed consent for long-term storage of specimens remaining after the study is over for possible future testing. Based on information reported on the CRFs, the SDMC will provide each site a list of participants who consented for long-term specimen storage. Before specimens from these participants are shipped or used for any additional testing, site staff must confirm that written informed consent for each is on file. For participants who do not consent for long-term storage of leftover specimens and possible future research testing, all archived samples will be destroyed after all protocol-required and quality assurance testing has been completed at the end of the trial. Destroyed specimens must be deleted from LDMS following the site approved Sample Destruction SOP. The following sections describe the processing and storage for each type of specimen.

For infant samples, store 4-5 x 0.3 mL plasma aliquots using labeled (LDMS generated label) cryovials and 3 x 0.1 ml DNA cell pellets according to local procedures. Store any remaining plasma, even if less than 0.3 mL. Store plasma aliquots in a -70°C freezer at the local repository.

For maternal samples, store 4 x 1.0 mL plasma aliquots and 3 x 2.0 mL of breast milk using labeled (LDMS generated label) cryovials according to local procedures. Breast milk is only to be collected from women whose infants are still breastfeeding at each time point.

Dried Blood Spots will be stored on infants and mothers according to the schedule in tables 10-1 and 10-2. 2-3 (50 μL each spot) DBS will be stored for each participant. DBS are stored only if there is enough specimen. NO additional blood will be drawn to enable storage of DBS.

The HPTN NL will contact you and give you instructions if the stored samples are required for analysis.

10.10 Breast Milk Processing and Storage

Milk samples should be placed in a refrigerator within 4 hours of collection and processed within 24 hours after being received in the laboratory.

Equipment and supplies

Vortexer
Pipettor for serological pipettes
Sterile 1 ml serological pipettes
Cryovials, 2 mL
LDMS Labels

Procedure for aliquoting milk samples

Even though the milk samples are not sterile, sterile technique should be used throughout their processing.

If samples have been refrigerated, allow them to come to room temperature before processing. Gently vortex the milk (slow speed) in the capped 50 mL Corning or Eppendorf tube.

Remove a 2 mL aliquot of milk with a serological pipette and transfer to a 2 mL cryovial marked with the PTID number, date, and 'WH' to denote whole milk. Repeat to prepare at least 3 aliquots of 2 mL each.

Log the samples into the LDMS system, and label the cryovials with the LDMS generated labels (BMK/NON/BMW). The vials should be frozen and stored at -70°C.

10.11 Resistance Testing

HIV genotyping (NVP resistance testing) will be done on a subset of plasma and breast milk samples collected throughout the study to determine the frequency and duration of NVP-resistant HIV strains and their relationship to MTCT. Additional samples may be requested / tested in the future. The HPTN NL and SCHARP are responsible for notifying sites which samples must be shipped to the HPTN NL for NVP resistance testing. Samples should not be sent for NVP resistance testing until instructed to do so by the HPTN NL and SCHARP.

Once the HPTN NL indicates that samples should be shipped, only two aliquots from the same sample should be sent per shipment. This will insure that back-up samples exist if the samples in the first shipment are ruined.

10.12 Shipping Samples to the HPTN NL

Throughout the course of HPTN 046, plasma and breast milk samples will be shipped to the HPTN NL for evaluation of NVP concentration, NVP resistance, HIV RNA (breast milk samples and samples from sites without certification), and HIV quality assurance. SCHARP will provide sites with shipping lists.

Different types of samples can be sent in the same shipment to the HPTN NL. All specimens sent to the HPTN NL or a laboratory off-site for analysis will be transported in accordance with IATA specimen shipping regulations and individual carrier guidelines. All shipments will be documented using the HPTN LDMS.

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. For non-US sites, the HPTN NL will work with the site to arrange for shipping via World Courier.
- 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 aliquot for each PTID and date) from the freezer and confirm the PTID number, 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.
 - 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. (Please check with manufacturer of your LN2 shipper for appropriate internal packaging)Fill the box with sufficient carbon dioxide (dry ice) to last at least 48 hours. World Courier will replenish dry ice as necessary. LN2 shippers are manufactured to keep temperature 7 – 14 days. World Courier should deliver the LN2 shipper within this time frame.

- Include a copy of the shipping manifest, box map, LDMS diskette as well as CDC import permit in the shipment. For dry ice shipments and LN2 shipments, use diagnostics packing code 650, UN 3373,(include Non flammable gas label, Keep Upright, and Do Not Drop – Handle with Care stickers) and address the shipment to:

Estelle Piwowa-Manning/Dr. Brooks Jackson
Johns Hopkins University Hospital
Department of Pathology
Pathology Building, Room 313
600 North Wolfe Street
Baltimore, Maryland 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 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.

10.12.1 IATA Shipping Regulations

The International Air Transport Association (IATA) Dangerous Goods Regulations are the worldwide gold standard for shipping. Each lab should have the current IATA manual and anyone participating in shipping and receiving certified in IATA regulations. This certification needs to be renewed every two years.

10.12.2 Local 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 inspection of laboratory facilities and documentation as well as 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.

10.13 Quality Control and Quality Assurance Procedures

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

Throughout the course of the study, plasma and/or cell pellet samples from all HIV infected infants and an equal number of randomly selected uninfected infants will be retested by the HPTN NL using FDA-licensed tests (i.e., HIV antibody, HIV Western blot, HIV DNA PCR or HIV RNA). In the event of false positive or false negative HIV result that changes the endpoint infection status of the subject, a sample from the last visit from all subjects will be retested. In addition, 10% of women enrolled will be retested by the HPTN NL for HIV antibody using FDA licensed tests. In the event of a false positive result that

changes the infection status of the subject, an additional 100 or 20% of samples (whichever is greater) from enrolled 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 site staff will ship the selected specimens to the HPTN NL. All specimens will be shipped in accordance with the HPTN Manual of Laboratory Operations and IATA specimen shipping regulations.

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

All sites must submit monthly laboratory QC reports to the HPTN NL for all safety laboratory tests (Hematology, CD4s, and Chemistry). These reports should contain monthly Levy-Jennings plots and corrective actions, if applicable. Please contact Paul Richardson (pricha18@jhmi.edu, +410-502-0435) to determine the required content and format of the report.