

Section 10. Laboratory Procedures

This section contains information on the laboratory procedures performed in HPTN 057 at the study sites.

Some laboratory procedures will be performed in study site clinics; others will be performed in study site local laboratories and at the HPTN Network Laboratory (NL) in Baltimore, MD, USA.

In all settings, laboratory procedures will be performed according to study site standard operating procedures (SOPs) that have been approved by the NL. Each study site will adhere to standards of good clinical laboratory practice (GCLP), the HPTN Laboratory Standard Operating Procedures (SOP) and their site-specific SOPs for proper collection, processing, labeling, and transport of specimens. Below are instructions related to specimen collection and laboratory procedures required for HPTN 057 (Sections 10.1, 10.2 and 10.3). Labeling, storage and shipping will be documented using the HPTN Laboratory Data Management System (LDMS) (Section 10.4). Instructions for the shipment of samples to the NL are included in Section 10.7. All specimens will be shipped in accordance with International Air Transport Association (IATA) specimen shipping regulations (Section 10.7.1). Quality Control and Quality Assurance Procedures (QC/QA) are described in Section 10.8. Section 10.9 describes local laboratory monitoring.

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 NL for further guidance on validation requirements. Similarly contact the 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 at any time.

When tests are performed in clinic settings, the same documentation and QC practices required in the laboratory must be undertaken in the clinic. In-clinic testing and QC procedures for in-clinic testing must be documented on log sheets that are maintained in the clinic and reviewed by the study site Laboratory Manager (or designee) at least once per month. Once the log sheets are reviewed by the Laboratory Manager (or designee) they may be stored in the local laboratory, if desired. In the event that proper QC procedures are not followed in the clinic, or that adequate QC is not maintained, the study site Laboratory Manager is responsible for ensuring that corrective action is taken and documented. Sample log sheets are available from the NL.

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:

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/research_studies/HPTN035Lab.htm

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.

10.1 Specimen Labeling

All containers into which specimens are initially collected (e.g., urine collection cups, blood collection tubes) will be labeled with SDMC-provided Participant ID (PTID) labels. PTIDs 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 site SOPs. Stored specimens will be entered into the LDMS and labeled with LDMS-generated cryovial labels. Questions about label stock and label printing computer program provided by the SDMC and pre-LDMS-entry labeling procedures for this study can be directed to the HPTN Statistical and Data Management Center (SDMC) Program Manager, **Lynda Emel**, by phone at +206-667--5803, or email at lemel@sharp.org.

10.2 Use of LDMS

LDMS must be used at all sites to track the collection, storage, and shipment of all types of specimens in HPTN 057: plasma, amniotic fluid, dried blood spots (DBS), cell pellets and breast milk. Detailed instructions for use of LDMS are available at:

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

The current version of LDMS (as of January, 2010) is Version 5.8. 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 LDMS-generated labels. Use of bar-coded labels is permitted.

Questions related to use of LDMS in HPTN 057 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 Monday through Friday. This will allow international laboratories to receive immediate support via phone or email for any LDMS issues or questions. LDMS User Support will also be available via pagers from 6 pm 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. Laboratories are encouraged to take advantage of these extended hours.

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 also be paged during off business hours 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, including the country code and city code if you are outside the United States
- A short description of the problem

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, including the country and city codes if you are outside the United States. 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 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 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 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. It is advised that the clinic and laboratory meet once a week to review and resolve any discrepancies between what the clinic has said it has collected and what the lab has received.

10.3 Specimen Testing

Sections 10.3.1 and 10.3.2 below describe requirements for local and remote specimen testing.

10.3.1 Local Specimen Testing

For samples being processed and tested locally, each site may use their own labeling and tracking system. All specimens to be stored according to the protocol after these assays are completed must be entered into the LDMS system, as these are subject to QC testing by the NL (e.g., confirmatory or duplicate 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 Data Management 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).

10.3.2 Remote Specimen Testing

Samples being sent to the NL or laboratories off-site for processing/testing (maternal and infant plasma, amniotic fluid, DBS, and breast milk) and those subject to central QC testing will be labeled and entered into the LDMS. Test results for samples shipped to the 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. No results from resistance testing will be reported to sites.

10.4 Laboratory Data Management System (LDMS)

The LDMS is used to track laboratory specimens for the time of collection through testing, storage at the local site and/or shipment for remote testing or storage (i.e., at the NL or at another laboratory off-site).

10.4.1 Pre-printed Labels and LDMS Specimen Tracking Sheets

Prior to the start of the study, the SDMC will provide each site with blank specimen labels, label software, and LDMS Specimen Tracking Sheets. The software includes a template for pre-printing specimen labels with PTID and space to write the collection date, visit code and, for PK specimens, the collection time.

Specimen Labels

All specimen labels should be pre-printed by site staff using the computer program provided by SDMC. The labels allow for the PTID number to be printed on the label and provide space to write the visit code, collection date, and time of collection for PK specimens. After the visit code and collection date are completed, labels are applied to each specimen collection tube or container in the clinic following local procedures. To ensure proper adhesion, the tube surface should be clean, dry, and at room temperature before applying the label.

Specimen Label sheets should be placed in a participant's folder once the participant has been assigned a PTID. On the day of a participant visit, the specimen labels should be removed from the participant's folder and brought to the specimen collection location.

LDMS Specimen Tracking Sheet

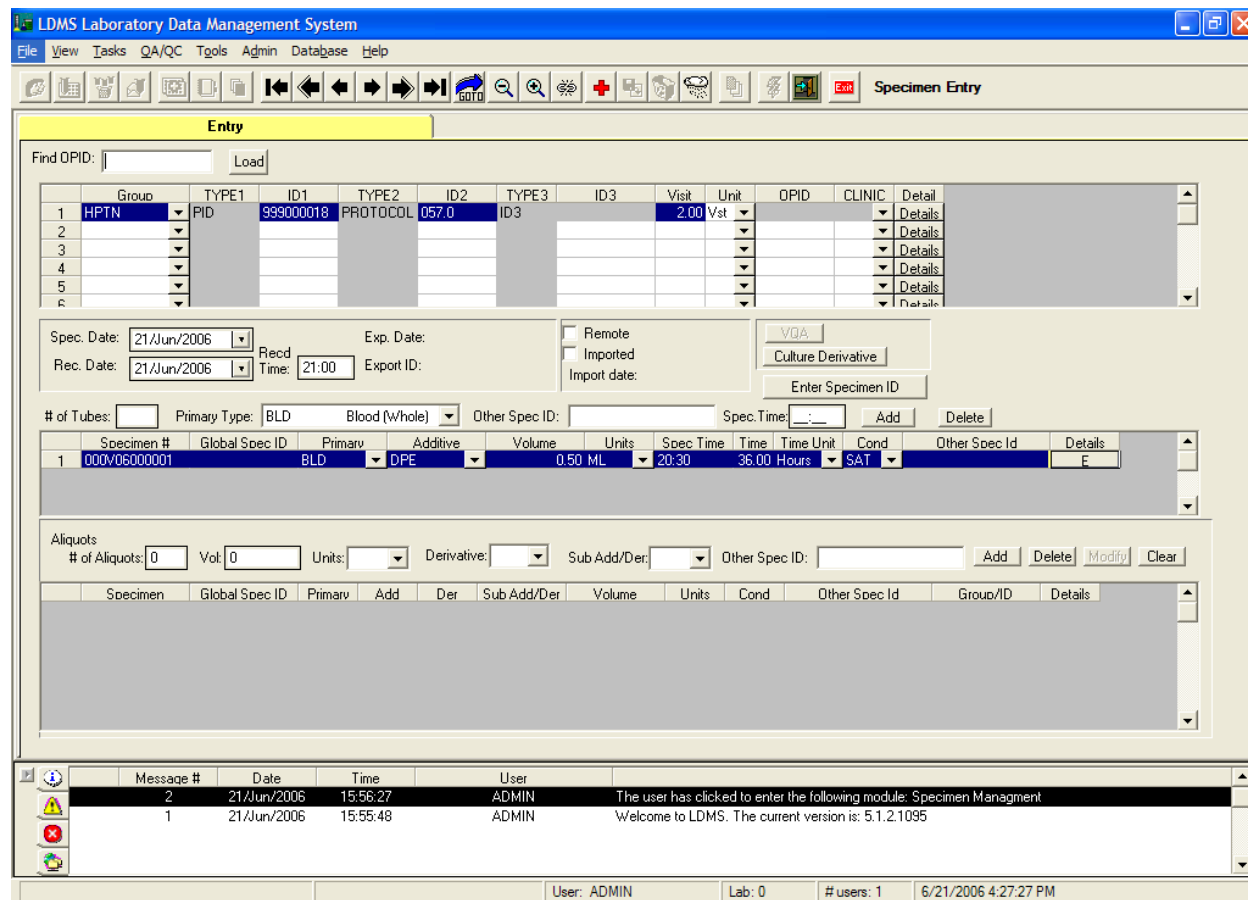
This sheet identifies those specimens that will be entered into LDMS and accompanies them to the lab. The PTID, visit code, and specimen collection date are recorded on the LDMS Specimen Tracking Sheet. A draft sample LDMS Specimen Tracking Sheet for HPTN 057 is shown in Figure 10-2.

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.

10.4.2 Logging PK Samples into LDMS

The Specimen Tracking Sheet will accompany the initial specimen(s). This Tracking Sheet should be kept near the LDMS computer to log in the remaining PK specimens. The PK specimen will be logged into LDMS as described in Chapter 3 of the HPTN – LDMS User Manual with the following exceptions.

Figure 10-1. Laboratory Data Management System Specimen Management screen



Visit Codes – Enter Visit Code from the LDMS PK Specimen Tracking Sheet. Visit Code 2.0 (Enrollment/Delivery) should be used for all maternal PK Specimens.

For Infant PK specimens (Cohorts 1 – 3) use the following Visit Codes:

Visit	Visit Code	Cohort	Specimen Collection Timepoints
Birth	2.0	1	Cord, 4 hours, 24 hours, 18-24 hours, 36-48 hours
Birth	2.0	2,3	Cord, Pre-dose, 2 hours, 10 hours, 18-24 hours
Day 3	3.0	2,3	Pre-dose, 2 hours, 10 hours
Day 5-7	4.0	2,3	Pre-dose, 2 hours, 10 hours, 18-24 hours, 36-48 hours

For Infant PK specimens (Cohort 4) use the following Visit Codes:

Visit/PK Specimen	Visit Code	Cohort	Specimen Collection Timepoints
Birth	2.0	4	Cord, Pre-dose, 2, 10, 24 hours post dose (just prior to next dose)
4 th Dose	3.0	4	Pre-dose, 2, 10, 24 hours post dose (just prior to next dose)
7 th Dose	44.0	4	Pre-dose, 2, 10, 24 hours post dose

Receive Time – Enter the Receive Date as usual in the Primary Specimen information section. For PK specimens, also the exact time that the specimen was received at the Lab using the 24-hour clock. For example, if the specimen was received at 6:00 pm, enter the Receive Time as 18:00.

Specimen Time – Enter a value in the # of Tubes edit field, select CRD for cord blood sample or BLD for whole blood sample from the Primary Type combo box and click the Add button in the usual manner. In the Primary grid, select the additive (DPE for spray dried potassium EDTA) from the drop down box, enter the volume, and select the unit from the drop down box. In the Specimen Time field (Spec Time), enter the exact time that the specimen was drawn, again using the 24 hour clock.

of Tubes – This applies to the number of tubes at one given PK time point. If the lab tech is entering all of the PK specimens for a participant at one time, a new screen must be used for each PK time point.

Specimen Time/Time Unit – Enter the specimen time (Time) and Time Unit. This is not the actual time the specimen was drawn – it is the sample collection time point as indicated in tables 10-5a and b, 10-6 and 10-7a and b.

Only the following should be entered into these fields for HPTN 057 PK cord blood specimens:
0:00 Hours (For Cord Blood)

PK collection Tables 10-5a, 5b, 6, 7a, 7b, 8a and 8b, indicate time collection windows. At this stage these windows should be disregarded. Only the following combinations should be entered into these fields for HPTN 057 PK whole blood specimens:

0:00 Pre Dose
0:00 Hours (For Delivery)
1:00 Hours
2:00 Hours
4:00 Hours

8:00 Hours
10:00 Hours
12:00 Hours
18:00 Hours
36:00 Hours

IMPORTANT – Use the “18:00 Hours” combination to indicate the specimen collected between 18 – 24 hours post delivery or post dose. Use the “36:00 Hours” combination to indicate the specimen collected between 36 – 48 hours post delivery or post dose.

For Cohort 4 infants, enter 24:00 Hours into the LDMS for the 24Hr pk time point.

Condition – Enter SAT in the drop down box if the sample is received in a satisfactory condition. If the sample is received clotted the sample should be processed as usual but enter CLT into this drop down box.

Other Spec ID – This free text box allows for additional information about the sample to be entered. For the following sample collections, the following information should be entered:

For Cord Blood specimen enter “Cord Blood”
For Delivery specimen enter “Delivery”

Figure 10-2. HPTN 057 LDMS Specimen Tracking Sheet sample

DO NOT FAX THIS FORM TO DATAFAX

HPTN 057
LDMS Specimen Tracking Sheet

Group: HPTN

Visit Code (Vst)

Participant ID

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- - -

Site Number Participant Number Chk Type

Specimen Collection Date

/ /

dd MMM yy

Protocol #: 057

# 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"/>	Amniotic fluid (AMN)	<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: _____ / LDMS Data Entry Date: / / _____
Sending Staff dd MMM yy Receiving Staff

Version 1.0, 20-OCT-05
 hivnet/forms/HPTN_057/forms/p057_norDF_specimen_tracking_sheet_ldms/

10.4.3 LDMS Specimen Processing and Storage

Upon receipt of the specimen shipment, the local laboratory inspects each package in the shipment to ensure the PTID on all the specimens in the package match the PTID 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. 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 PK). The 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 is documented, and how it is handled and processed once it reaches the lab. Specific information that must accompany the specimen includes – the PTID, collection date and time for PK specimens, and visit code for each specimen. Specimen labels and LDMS Specimen Tracking Sheets 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 and how problem samples are reported to the clinic. The following sections describe the collection and processing for each type of specimen.

10.5. Blood: Non-PK

Blood will be collected for HIV-related testing, safety laboratory assessments and storage throughout this study. After blood collection, all collection tubes should be labeled with the PTID, collection date, collection time, and visit code. The labels will be white for all non-PK specimens. Blood will be collected according to local procedures and sent to the local lab for analysis and/or entry into LDMS, if applicable. See Tables 10.1 and 10.2 for maternal and infant non-PK specimen collection and storage requirements by visit, for all cohorts.

Maternal blood samples will be collected in ethylenediaminetetraacetic acid (EDTA) tubes for complete blood count (CBC) with differential, CD4+ cell counts, HIV RNA PCR, and plasma storage; clotted tubes will be used for chemistries. Infant blood samples will be collected in small, EDTA tubes for CBC with differential, HIV testing and plasma storage; clotted tubes will be used for the chemistries.

If maternal or infant blood sample volume is limited, priority should be completion of safety labs, followed by storage for PK testing, and then DNA/RNA PCR and plasma storage. Chemistries are prioritized as follows: creatinine, calcium/albumin, phosphate, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, alkaline phosphatase.

10.5.1 Procedure for Calculation of Corrected Calcium

For this protocol the severity of hyper- or hypo calcemia will be graded and corrected for the albumin level, as described in Section 4.6 of Protocol Version 2.0 and copied below:

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE- THREATENING
Calcium, serum, high (corrected for albumin)				
Adult and Pediatric ≥ 7 days	10.6 – 11.5 mg/dL 2.65 – 2.88 mmol/L	11.6 – 12.5 mg/dL 2.89 – 3.13 mmol/L	12.6 – 13.5 mg/dL 3.14 – 3.38 mmol/L	> 13.5 mg/dL > 3.38 mmol/L
Infant*†, < 7 days	11.5 – 12.4 mg/dL 2.88 – 3.10 mmol/L	12.5 – 12.9 mg/dL 3.11 – 3.23 mmol/L	13.0 – 13.5 mg/dL 3.245 – 3.38 mmol/L	> 13.5 mg/dL > 3.38 mmol/L
Calcium, serum, low (corrected for albumin)				
Adult and Pediatric ≥ 7 days	7.8 – 8.4 mg/dL 1.95 – 2.10 mmol/L	7.0 – 7.7 mg/dL 1.75 – 1.94 mmol/L	6.1 – 6.9 mg/dL 1.53 – 1.74 mmol/L	< 6.1 mg/dL < 1.53 mmol/L
Infant*†, < 7 days	6.5 – 7.5 mg/dL 1.63 – 1.88 mmol/L	6.0 – 6.4 mg/dL 1.50 – 1.62 mmol/L	5.50 – 5.90 mg/dL 1.38 – 1.51 mmol/L	< 5.50 mg/dL < 1.38 mmol/L

Note: Albumin is measured only to determine of the severity grade for hyper- or hypocalcemia; this requires correction for albumin. Corrected calcium levels will be monitored for safety; therefore, reporting of AEs for albumin is not required.

Laboratories will need to measure total calcium and albumin and use that data to calculate the corrected calcium. This calculation should be performed on all samples that have both total calcium and albumin results. Corrected calcium results should be reported in mg/dL on the Laboratory Results case report forms.

For these calculations, assume a normal albumin level is 4.0 g/dL.

The effect of correcting the calcium is:

Correct the total calcium upward by 0.08 mg/dL for every 0.1 g/dL fall in albumin.

Correct the total calcium downward by 0.08 mg/dL for every 0.1 g/dL rise in albumin.

For example, if the albumin level is 3.3 g/dL (decreased by 0.7), and the calcium level is 9.8 mg/dL, the corrected calcium would be 10.4 mg/dL.

This formula for calculation is:

$$\text{Corrected Ca} = [(4.0 - \text{measured albumin}) \times 0.8] + \text{Measured Ca}$$

(when calcium is expressed in mg/dl and albumin is expressed in g/dL)

When calculating corrected calcium values, you must use the values recorded on the Mother or Infant Laboratory Results CRF, not the values from the laboratory source documentation, especially if the values were converted to different units or rounded before they were on the CRF.

10.5.2 Dried Blood Spot (DBS) Preparation and Storage

Dried blood spots are to be prepared from infant blood at the following time points: within 24 hours of birth, 5 – 7 days, 6 and 12 weeks and 6, 9 and 12 months.

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

It is important to label both sections of the filter paper with an LDMS label, so that when the section containing the three spots is removed for shipment to the NL the section containing the remaining two spots (which will remain on-site) will also have a label. Filter papers designed for the collection of blood can vary between manufacturers; refer to Laboratory SOP for further information.

Allow filter paper cards to air dry at room temperature for as 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. Dry filter paper should be placed in a ziploc bag with desiccant. Store samples at room temperature, not exposed to sun or heat.

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

Table abbreviations: wks = weeks; mos = months; pp=postpartum mL = milliliter; RT = real-time; spec = specimen						
Amniotic Fluid is to be collected only on those women enrolled in Cohorts 1, 3 and 4 (who received study drug and delivered by C-section)						
Study Visit	Collection Tube or Container	Tests		Specimen Type	Specimen Amount	Test Processing Location
		RT Test	Stored Spec			
Maternal Screening ¹		HIV Confirmatory Test (HIV EIA, Rapid test or Western Blot) ²		Depends on test	Depends on test	Local Lab
Maternal Screening ³	EDTA	CBC w/ diff and platelet count		Whole blood	0.5 mL	Local Lab
			HIV-1 RNA PCR	Plasma	0.5 mL	Local Lab
		Resistance Testing	Plasma	Up to 4 X 1.0 mL aliquots, frozen	Network Lab	
	No Preservative	Chemistries ⁴		Serum	1.0 mL	Local Lab
Labor and Delivery ¹⁰	EDTA	CBC w/ diff and platelet count		Whole blood	0.5 mL	Local Lab
		CD4+ cell count		Whole blood	0.5 mL	Local Lab
			HIV-1 RNA PCR ⁵	Plasma	0.5 mL	Local Lab
		Resistance testing ⁵	Plasma	Up to 4 X 1.0 mL aliquots, frozen	Network Lab	
	No Preservative (Cohorts 1 and 3 only)	Chemistries ⁴		Serum	1.0 mL	Local Lab
		TDF Concentration ⁶	Amniotic Fluid	2 X 2.0 mL	Network Lab	
24 – 48 hrs pp	EDTA	CBC w/ diff and platelet count		Whole blood	0.5 mL	Local Lab
	No Preservative	Chemistries ⁴		Serum	1.0 mL	Local Lab
	Corning/Eppendorf tube or other sterile container		TDF Concentration ^{7,9}	Breast milk	5 x 1.0 mL aliquots, frozen	Network Lab
5 to 7 days pp	EDTA	CBC w/ diff and platelet count		Whole blood	0.5 mL	Local Lab
			HIV-1 RNA PCR	Plasma	0.5 mL	Local Lab
			Resistance testing	Plasma	Up to 4 X 1.0 mL aliquots, frozen	Network Lab
	No Preservative	Chemistries ⁴		Serum	1.0 mL	Local Lab
	Corning/Eppendorf tube or other sterile container		TDF Concentration ^{7,9}	Breast milk	5 x 1.0 mL aliquots, frozen	Network Lab
6 wks	EDTA	CBC w/ diff and platelet count		Whole blood	0.5 mL	Local Lab
			HIV-1 RNA PCR	Plasma	0.5 mL	Local Lab
			Resistance testing	Plasma	Up to 4 X 1.0 mL aliquots, frozen	Network Lab
	No Preservative	Chemistries ⁴		Serum	1.0 mL	Local Lab
	Corning/Eppendorf tube or other sterile container		TDF Concentration ^{7,9}	Breast milk	5 x 1.0 mL aliquots, frozen	Network Lab
12 wks	EDTA		HIV-1 RNA PCR	Plasma		Local Lab ⁸
			Resistance testing	Plasma	Up to 4 X 1.0 mL aliquots, frozen	Network Lab
	Corning/Eppendorf tube or other sterile container		TDF Concentration ^{7,9}	Breast milk	5 x 1.0 mL aliquots, frozen	Network Lab
6 mos pp	EDTA		HIV-1 RNA PCR	Plasma		Local Lab ⁸
			Resistance testing	Plasma	Up to 4 X 1.0 mL aliquots, frozen	Network Lab
12 mos pp	EDTA		HIV-1 RNA PCR	Plasma		Local Lab ⁸
			Resistance testing	Plasma	Up to 4 X 1.0 mL aliquots, frozen	Network Lab

1. Anytime during pregnancy
2. If required to confirm HIV status
3. ≥ 34 weeks gestation
4. Tests includes bilirubin, AST, ALT [SGPT], creatinine, calcium, phosphorous, alkaline phosphatase, albumin
5. Blood Sample collected in active labor PRIOR to TDF Dosing (Cohorts 1, 3 and 4)
6. Only in Mother's who deliver by C-section (Cohorts 1, 3 and 4)
7. Only in Mother's who are currently breastfeeding
8. Stored for later assay if necessary
9. To be tested on a subset of participants as requested by the NL
10. Labor and Delivery blood should be collected before the TDF dose is given. Specimens should be collected within 48 hours of delivery.

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

Table abbreviations: wks = weeks; mos = months; RT = real-time; spec = specimen						
Study Visit	Collection Tube or Container	Tests		Specimen Type	Specimen Amount	Test Processing Location
		RT Test	Stored Spec			
Within 24 hours of birth	EDTA	CBC w/ diff and platelet count		Whole blood	0.5 mL	Local lab
		HIV-1 DNA or RNA PCR ¹		Whole blood or Plasma	2 x 0.1 mL aliquots ²	Local lab
			Resistance Testing	Plasma	Up to 5 x 0.3 mL aliquot, frozen ³	Network Lab
	No Preservative	Chemistries ⁴		Serum	1.0 mL	Local Lab
	Dried Blood Spot ⁶		Back-up	Whole Blood	0.25ml	Local Lab
Day 5 – 7	EDTA	CBC w/ diff and platelet count		Whole blood	0.5 mL	Local lab
		HIV-1 DNA or RNA PCR ¹		Whole blood or Plasma	2 x 0.1 mL aliquots ²	Local lab
			Resistance Testing	Plasma	Up to 5 x 0.3 mL aliquot, frozen ³	Network Lab
	No Preservative	Chemistries ⁴		Serum	1.0 mL	Local Lab
	Dried Blood Spot ⁶		Back-up	Whole Blood	0.25ml	Local Lab
6 wks	EDTA	CBC w/ diff and platelet count		Whole blood	0.5 mL	Local lab
		HIV-1 DNA or RNA PCR ¹		Whole blood or Plasma	2 x 0.1 mL aliquots ²	Local lab
			Resistance Testing	Plasma	Up to 5 x 0.3 mL aliquot, frozen ³	Network Lab
	No Preservative	Chemistries ⁴		Serum	1.0 mL	Local Lab
	Dried Blood Spot ⁶		Back-up	Whole Blood	0.25ml	Local Lab
12 wks	EDTA	CBC w/ diff and platelet count		Whole blood	0.5 mL	Local lab
		HIV-1 DNA or RNA PCR ¹		Whole blood or Plasma	2 x 0.1 mL aliquots ²	Local lab
			Resistance Testing	Plasma	Up to 5 x 0.3 mL aliquot, frozen ³	Network Lab
	No Preservative	Chemistries ⁴		Serum	1.0 mL	Local Lab
	Dried Blood Spot ⁶		Back-up	Whole Blood	0.25ml	Local Lab
6 mos	EDTA	HIV-1 DNA or RNA PCR ^{1,5}		Whole blood or Plasma	2 x 0.1 mL aliquots ²	Local lab
			Resistance Testing	Plasma	Up to 5 x 0.3 mL aliquot, frozen ²	Network Lab
	Dried Blood Spot ⁶		Back-up	Whole Blood	0.25ml	Local Lab
12mos	EDTA	HIV-1 DNA or RNA PCR ^{1,5}		Whole blood or Plasma	2 x 0.1 mL aliquots ²	Local lab
			Resistance Testing	Plasma	Up to 5 x 0.3 mL aliquot, frozen ³	Network Lab
	Dried Blood Spot ⁶		Back-up	Whole Blood	0.25ml	Local Lab

1. If positive confirm with a DNA PCR or RNA PCR on a different specimen
2. Use 1 aliquot for RT test and store 1 aliquot for future confirmatory testing (if needed).
3. All plasma, even if less than 0.3 mL, should be stored.
4. Tests includes bilirubin, AST, ALT [SGPT], creatinine, calcium, phosphorous, alkaline phosphatase, albumin
5. Breastfeeding infants only.
6. To be made in the Laboratory.

10.5.3 Amniotic Fluid Collection and Processing

Amniotic fluid is to be collected for PK studies from mothers who deliver by caesarean in Cohorts 1, 3 and 4 only.

Specimens should be collected into a suitable sterile specimen container according to local procedures and labeled with the PTID, the specimen type, the date and the time (24-hour clock format). Specimens and all appropriate documents should be transported to the laboratory so they can be processed within 1 hour of collection.

Laboratory: Note the appearance of the amniotic fluid (e.g., clear or blood stained). Centrifuge the specimen at 200g for 5 minutes. Aliquot the supernatant into two (2) 2.0 mL cryovials. Label all specimens with LDMS generated labels.

Store the samples frozen (-20°C or -70°C). One aliquot is to be shipped to NL upon request and one aliquot is to be held on site for later shipment if necessary.

10.5.4 Breast Milk Collection and Processing

Breast milk will be collected only from mothers who are currently breastfeeding. See Table 10-1 for the schedule of visits that include breast milk collection.

Using local procedures, the specimens should be collected into a suitable sterile specimen container and labeled with the PTID, the specimen type, the date and the time collected (24-hour clock format).

The breast milk samples should be transported to the laboratory within 1 hour of collection, placed in a refrigerator within 4 hours of collection, and processed within 24 hours after being received in the laboratory.

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

If samples have been refrigerated, allow them to come to room temperature before processing. Gently vortex the milk (slow speed) in the capped container.

Remove a 1 mL aliquot of milk with a serological pipette and transfer to a cryovial marked with the PTID, date, and 'WH' to denote whole milk. Repeat to prepare at least 5 aliquots of 1 mL each.

Log the samples into the LDMS system, and label the cryovials with the LDMS generated labels (BMK/NON/BMW). The vials should then be stored frozen (at -20°C or -70°C).

10.5.5 Maternal HIV Testing

HIV testing will be performed during the first maternal screening visit for women who do not have documented HIV-1 infection. A confirmatory HIV test will be performed if documented confirmation is not available.

The following are considered to be “documented HIV-1 infection” or “documented confirmation” for the purposes of this study:

“A Western Blot or IFA confirmed, HIV positive result that has been reported during a time that the reporting lab has demonstrated proficiency in HIV testing as defined by the Network Lab.”

If this documented HIV infection is not available, maternal screening should be performed by rapid HIV or EIA followed by a confirmatory WB or IFA using the same sample.

The final confirmatory result must be performed using an FDA approved assay.

Please contact the NL at networklab@hptn.org for further clarification if required.

10.5.6 Infant HIV Testing

Blood (drawn in an EDTA collection tube) will be collected for infant HIV testing by real-time HIV-1 DNA PCR or RNA PCR within 24 hours of birth, at 5-7 days, at 6 and 12 weeks, and at 6 and 12 months in breastfeeding infants.

HIV testing for infants 15 months of age or younger should be completed according to the algorithm below.

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. The NL should be consulted.
- ➔ Positive: This confirms HIV infection.

In summary, at each scheduled testing time point:

- If the first assay is negative, the participant is considered to be HIV-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 to be HIV-infected.

- If this second assay is negative or indeterminate, additional testing may be required. The NL 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 in real time. Blood should continue to be collected according to the schedule in table 10.2, and plasma should be stored for viral load and genotyping.

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 the SDMC via Laboratory Results Forms. Otherwise, the HIV RNA assay will be performed at the end of the study on samples selected by the protocol virologist.

10.5.7 Resistance Testing

HIV genotyping will be done on a subset of plasma and breast milk samples collected throughout the study to determine the frequency and duration of drug-resistant HIV. The NL is responsible for identifying which samples will undergo HIV genotyping. Sites should not send samples for HIV genotyping until instructed to do so by the SDMC.

Once the NL and SDMC indicate that samples should be shipped, only the following aliquots should be shipped: Maternal plasma: 1 aliquot of 1.0 mL. Maternal breast milk: 2 aliquots of 1.0 mL. Infant plasma: 1 aliquot of 0.3 mL. This will ensure that back-up samples exist if the samples in the first shipment are lost or degraded in shipment.

10.6 Blood: PK

Cord blood and maternal PK specimens will be collected from all women enrolled in Cohorts 1, 3 and 4. PK specimens will be drawn as indicated in tables 10-4, 10-5a and b, 10-6, 10 – 7a and b, and 10-8a and b. These tables may be copied and included in participant charts to ensure the collection of the correct specimens.

All PK samples must be delivered to the laboratory in a timely manner to allow for specimen processing and freezing within 1 hour of collection.

All PK samples will have a green label adhered to them so that they may be readily identified as a PK specimen in the lab.

In discussion with the participants, sites may elect to draw the PK samples by phlebotomy or via the use of an indwelling catheter. Heel sticks should be sufficient for the collection of these samples in infants.

There is a higher probability that samples will develop a clot when using spray dried potassium EDTA tubes. To help avoid this, it is important to mix the sample immediately after collection. Once at the lab, the sample processor should look for the presence of a clot before the sample is centrifuged. If there is a clot, it should be documented in the comments section of the Mother and Infant DataFax forms. It should also be documented in LDMS as described in 10.4.2

Important - these samples should not be discarded if they are clotted, but it is important that the presence of a clot is noted.

Table 10-4. Maternal and Infant Specimen Collection, Processing and Storage Procedures

Amount Collected	Collection Tube or Container	Immediate Specimen Handling	Specimen Processing	Specimen Storage and Shipping
Cord Blood and Maternal PK - 2.0 mL Whole Blood	Potassium EDTA spray dried (Do not use collection tubes with liquid anticoagulant)	Samples should be processed and frozen within 1 hour of collection. If this is not achieved the time from collection to separation and freezing must be noted.	Samples should be centrifuged for 10 min at 1500 x g. Contact NL for further advice if this is not achievable.	Plasma aliquots should be stored at -70°C or -20°C
Infant PK - 0.5 mL Whole blood			If the plasma yield is <150 µL, all of the plasma should be aliquotted into one labeled cryovial. 150 µL of plasma should be removed and aliquotted into a labeled cryovial. Any excess plasma should be aliquotted into a second labeled cryovial.	One cryovial containing 150µL of plasma (or <150 µL if yield is poor) is to be shipped to NL. The cryovial containing the excess plasma is to be stored on site and may be shipped to the NL if requested.

Table 10-5a. COHORT 1: Maternal PK Specimens

Cohort 1 – Maternal PK Specimens							
Delivery²							
Pre – Dose¹	+1 hrs (+/- 30 mins) Post – Dose	+2 hrs (+/- 30 mins) Post - Dose³	+4 hrs (+/- 1 hr) Post- Dose	+8 hrs (+/- 2 hrs) Post- Dose	+12 hrs (+/- 2 hrs) Post- Dose⁴	18-24 hrs Post - Dose	36-48 hrs Post - Dose

1. Pre dose needs to be collected < 1hr before dose
2. If collection of delivery specimen falls within 1 hour of another scheduled PK specimen collection time point, the previously scheduled specimen need not be obtained.
3. There must be at least 1 hour between the collections of the +1hr and the +2hrs sample.
4. There must be at least 4 hours between the collections of the +8hr and the +12hrs sample.

Note:

If the mother vomits within 1 hour of dosing, only the cord blood specimen will be collected.

If the mother does not deliver within 24 hours of dosing, only the cord blood specimen will be collected.

Table 10 – 5b. COHORT 1: Infant PK Specimens

Cohort 1 - Infant PK Specimens				
Cord Blood	+4 hrs (+/- 1 hr) Post Delivery	+12 hrs (+/- 2 hrs) Post- Delivery	18-24 hrs Post - Delivery	36-48 hrs Post – Delivery

Note:

If the mother vomits within 1 hour of dosing, only the cord blood specimen will be collected.

If the mother does not deliver within 24 hours of dosing, only the cord blood specimen will be collected.

If an infant PK specimen is missed, no further infant PK sampling will be done

Table 10 – 6 COHORT 2: Infant PK Specimens

No maternal PK specimens to be collected

Cohort 2 - Infant PK Specimens						
	Cord Blood	Pre-Dose	+2 hrs (+/- 30 mins) Post - Dose	+10 hrs (+/- 1 hr) Post- Dose	18-24 hrs Post - Dose	36-48 hrs Post - Dose
Birth	X	X ¹	X	X	X	
Day 3		X ¹	X	X		
Day 5		X ¹	X	X	X	X

1. To be collected < 1 hour before dosing.

Note:

If an infant PK specimen is missed, no further infant PK sampling will be done.

If an infant misses a dose of TDF for any reason, the PK specimens associated with (following)that dose should not be obtained and subsequent dosing and PK sampling will not be done.

Table 10-7a. COHORT 3 Maternal PK Specimens

NOTE: If 600 mg dose of TDF is given then ONLY the delivery specimen is collected.

Cohort 3 - Maternal PK Specimens (900 mg dose at labor)							
Delivery²							
Pre – Dose¹	+1 hrs (+/- 30 mins) Post – Dose	+2 hrs (+/- 30 mins) Post - Dose³	+4 hrs (+/- 1 hr) Post- Dose	+8 hrs (+/- 2 hrs) Post- Dose	+12 hrs (+/- 2 hrs) Post- Dose⁴	18-24 hrs Post - Dose	36-48 hrs Post - Dose

1. Pre dose needs to be collected < 1hr before dose
2. If collection of delivery specimen falls within 1 hour of another scheduled PK specimen collection time point, the previously scheduled specimen need not be obtained.
3. There must be at least 1 hr between the collections of the +1 hr and the +2 hrs sample.
4. There must be at least 4 hrs between the collections of the +8 hrs and the +12 hrs sample.

Note:

If the mother vomits within 1 hour of dosing, only the cord blood specimen will be collected.

If the mother does not deliver within 24 hours of dosing, only the cord blood specimen will be collected.

Table 10-7b. COHORT 3 Infant PK Specimens

Cohort 3 - Infant PK Specimens						
	Cord - Blood	Pre-Dose	+2 hrs (+/- 30 mins) Post - Dose	+10 hrs (+/- 1 hr) Post- Dose	18-24 hrs Post - Dose	36-48 hrs Post - Dose
Birth	X	X¹	X	X	X	
Day 3		X²	X	X		
Day 5		X²	X	X	X	X

1. If cord blood is collected and infant receives the initial dose of TDF ≤ 2 hrs after birth then this specimen can be omitted. If the initial TDF dose will be given > 2 hrs after birth, then a separate pre-dose specimen must be collected ≤ 1 hr before dosing. If the cord blood sample is NOT obtained, then the pre-dose sample should be collected < 1 hr before dosing.
2. To be collected < 1 hr before dosing.

Note:

If the mother vomits within 1 hour of dosing, only the cord blood specimen will be collected.

If the mother does not deliver within 24 hours of dosing, only the cord blood specimen will be collected.

If an infant PK specimen is missed, no further infant PK sampling will be done.

If an infant misses a dose of TDF for any reason, the PK specimens associated with (following) that dose should not be obtained. Subsequent dosing and PK sampling will not be done.

Table 10-8a. COHORT 4 Maternal PK Specimens

NOTE: 600 mg dose of TDF is given and ONLY the delivery specimen is collected.

Cohort 4 - Maternal PK Specimens (600 mg dose at labor)
Delivery sampling only

Note:

If the mother vomits within 1 hour of dosing, only the cord blood specimen will be collected. Infant will not be dosed

If the mother does not deliver within 24 hours of dosing, only the cord blood specimen will be collected. Infant will not be dosed

Table 10-8b. COHORT 4 Infant PK Specimens

Cohort 4 - Infant PK Specimens					
	Cord - Blood	Pre-Dose	+2 hrs (+/- 30 mins) Post - Dose	+10 hrs (+/- 1 hr) Post- Dose	+ 24 hrs (+/- 2 hrs, must be drawn just prior to next dose
Birth	X	X¹	X	X	X
4th Dose		X²	X	X	X
7th Dose		X²	X	X	

1. If cord blood is collected and infant receives the initial dose of TDF ≤ 2 hrs after birth, then this specimen can be omitted. If the initial TDF dose will be given > 2 hrs after birth, then a separate pre-dose specimen needs to be collected ≤ 1 hr before dosing. If the cord blood sample is NOT obtained, then the pre-dose sample should be collected < 1 hr before dosing.

2. To be collected < 1 hr before dosing.

Note:

If the mother vomits within 1 hour of dosing, only the cord blood specimen will be collected.

If the mother does not deliver within 24 hours of dosing, only the cord blood specimen will be collected.

If an infant PK specimen is missed, no further infant PK sampling will be done.

If an infant's birth, 4th or 7th dose of TDF is missed for any reason or vomits, the PK specimens associated with (following) that dose should not be obtained. Subsequent dosing and PK sampling will not be done

If an infant vomits the 2nd, 3rd, 5th or 6th dose of TDF and the infant is not re-dosed then subsequent dosing and PK sampling will not be done.

10.7 Shipping to the HPTN Network Laboratory

Throughout the course of HPTN 057, plasma, cell pellets/DBS, breast milk and amniotic fluid samples will be shipped to the NL for evaluation of TDF concentration, HIV-1 RNA PCR, resistance testing and quality assurance. PK samples should be sent to the NL after 5 mother/infant pairs have had a full set of PK samples collected or when notified by the NL. The NL will request that certain samples from infants and mothers for HIV-1 RNA PCR and resistance testing be sent to the NL. The SDMC will notify the sites when additional samples for quality assurance should be sent. Different types of samples can be sent in the same shipment to the NL. All specimens sent to the 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.

The frozen samples should be placed in the cryovial box in the order of the shipping manifest for shipments of 15 vials or more. If fewer than 15 vials are to be sent, they can be placed in plastic shipping baggies inside the orange topped shipping canister. A photocopy of the Specimen Tracking Sheet should accompany the specimen to the laboratory as well as the LDMS shipping manifest, box report, and LDMS diskette. If shipping more than 15 vials, they should be placed in a 9 x 9 storage box following the order on the box map.

The orange-topped canister should have absorbent material included. This is then placed in its cardboard box, which is placed inside the diagnostic shipping box and the box is filled with sufficient carbon dioxide (dry ice) to last at least 48 hours. If shipping more than 15 vials, wrap the box in absorbent material, place it inside a shipping bag, seal, and place in the shipping container. Ship samples as diagnostics packing code 650, UN 3373.

Note: When shipping dangerous goods the following is to be clearly labeled on shipping boxes and written on the shipper's declaration of dangerous goods:

- Emergency contact: Telephone 1.800.424.9300
- Reference: HPTN and assigned Chemtrec code

Prior to shipment, please contact Estelle Piwowar-Manning at 410.614.6736 (phone), 410.614.0430 (fax) or epiwowa@jhmi.edu to set up the shipment with World Courier. Any questions relating to specimen handling, shipping, or identification should be directed to Estelle Piwowar-Manning at 410.614.6736.

Shipping Address:

The Johns Hopkins University Hospital
c/o Estelle Piwowar-Manning
Department of Pathology
Pathology Building -Room 313
600 North Wolfe Street
Baltimore, Maryland 21287

10.7.1 International 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 (supplied by the NL annually) and at least one person certified in IATA regulations. This certification must be renewed every two years.

10.8 Quality Control and Quality Assurance Procedures

The 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 quality control procedures, including proper maintenance of laboratory testing equipment, use of appropriate reagents, etc. NL staff will follow up directly with site staff to resolve any quality control or quality assurance problems identified through proficiency testing and/or on-site assessments.

Plasma and or cell pellet/DBS samples from all infants identified as HIV-infected and an equal number of randomly selected HIV-uninfected infants will be retested by the NL.

The SDMC will inform site staff of the samples selected for quality assurance testing, and 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.

Each site must submit monthly laboratory QC reports to the NL for all safety laboratory tests (Hematology, CD4 cell count, 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 your site's report.

10.9 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, PPD monitors will also 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.