11. Laboratory and Specimen Management Procedures

11.1. Overview of Section 11

This section contains information on the laboratory procedures performed in HPTN 076.

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

- Clinics
- Local laboratories
- The HPTN Laboratory Center (LC, Baltimore, MD, USA)
- Other laboratories designated by the HPTN LC

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

Ideally, one method, test kit, and/or combination of test kits will be used for each test throughout the duration of the study. **If for any reason a new or alternative method, kit, or test must be used after study initiation, site laboratory staff must inform the HPTN LC to determine if any test kit validation is required. For international sites an updated PAL (protocol analyte list) will need to be submitted to the LC for approval by DAIDS.**

Regardless of whether tests are performed in clinic or laboratory settings, study staff that perform the tests must be trained in proper testing and associated quality control (QC) procedures before performing the tests for study purposes; documentation of training should be available for inspection at any time.

As transmission of HIV and other infectious agents can occur through contact with contaminated needles, blood, blood products, and vaginal secretions, all study staff must take appropriate precautions when collecting and handling biological specimens. Guidance on universal precautions is available from the US Centers for Disease Control and Prevention at:

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

Additional reference information can be requested from the HPTN LC. The information provided below is intended to standardize laboratory procedures for HPTN 076 across the study sites. Adherence to the specifications detailed in this section is essential to ensure that primary, secondary and exploratory endpoint data derived from laboratory testing will be considered acceptable to regulatory authorities.

11.2. Specimen Labeling

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

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

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

11.2.1. Local Specimen Processing and Storage.

For samples that are processed and stored locally, each sample will be labeled and entered into the LDMS.

11.2.2. Local Specimen Testing.

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

11.2.3. Remote Specimen Testing

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

11.2.4. Use of the LDMS

LDMS must be used at all sites to track specimens that will be tested, stored, or shipped off-site for testing. Detailed instructions for use of LDMS are available in the LDMS User Manual:

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

As of the date of this version of the SSP, the current version of LDMS is Version 9.0.1. All sites should upgrade to this version as soon as possible. All sites must use the *HPTN barcode* label format in order to ensure that both the specimen ID and the global specimen ID assigned to each specimen are printed on LDMS-generated labels.

An example of a two-dimensional LDMS-generated barcode label is below:

Row 1: LDMS Specimen ID

Row 2: Global Specimen ID

Row 3: Patient Identifier (ID1) and Study/Protocol Identifier (ID2)

Row 4: Specimen Date or Harvest Date and Specimen Collection Time

Row 5: Primary Type, Additive Type, Derivative Type, and Sub Additive/Derivative Type

Row 6: Volume/Volume Unit and Visit/Visit Unit (VID)

Row 7: Other Specimen ID

Questions related to use of LDMS for HPTN 076 should be directed to Paul Richardson (pricha18@jhmi.edu).

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

LDMS User Support at Frontier Science

Regular Hours: Monday to Friday, 12:00 AM to 6:00 PM (Eastern Standard Time)
Off-hours: See below
Email: ldmshelp@fstrf.org
Phone: +1 (716) 834-0900, extension 7311

Fax: +1 (716) 832-8448 (should be used to fax Installation Reports only)

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

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

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

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

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

1. **Your name**
2. **Your laboratory's LDMS ID number**
   This is a 3-digit number assigned by Frontier Science to uniquely identify your laboratory. It appears when you start LDMS, and can also be found in the bottom-right corner of the screen.
3. **A full explanation of the issue**
   Your explanation should include any error messages or error numbers that appeared, what you were doing in LDMS at the time the issue occurred, and steps needed to reproduce the issue. The more details that you can provide, the faster LDMS User Support can help you.
4. **How you want to be contacted**
   If you want LDMS user support to call a specific telephone number, please provide that number and extension.
5. **(If applicable) The license code or challenge code being generated by LDMS**

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

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

1. Have there been any recent changes to the computer with LDMS, such as new hardware installed, a firewall upgrade, a network name change, or another change?
2. Are you or another user able to repeat the issue?
3. If you have LDMS installed on multiple computers, does the issue occur on all of them or does it only occur on a specific computer?

11.2.5. LDMS exportation, discrepancies and backup

Each site must export its LDMS data to Frontier Science (FSTRF) at a minimum on a weekly basis or whenever changes or additions are made to the LDMS database. Exported data are used by the HPTN SDMC to generate a discrepancy reports comparing the data from the LDMS with that entered onto the CRFs. Any discrepancies identified during the reconciliation are included in a discrepancy report for each site. Sites are expected to resolve all discrepancies within one week of receipt of the report. The HPTN LC is responsible for reminding sites to adhere to the one-week timeframe and for following up with sites that do not resolve discrepancies within one week. The HPTN SDMC reviews the discrepancy reports for critical samples (e.g., plasma needed for confirmatory HIV testing) that appear to be missing, and works with the LC and site staff to undertake appropriate corrective action. All corrective action should be documented in paper-based clinic and/or laboratory records as appropriate, and entered in the details section of LDMS. The LC and SDMC will discuss and document any items that, although resolved, appear 'unresolvable' in LDMS. Any corrections to the LDMS need to be made following guidelines provided by FSTRF. The LDMS system will back up the LDMS data daily. It is the site's responsibility to transfer the backup file to an external device following procedures outlined by LDMS user support.

11.2.6. LDMS Reconciliation

All sites must follow the HPTN LC approved site specific SOP for regular reconciliation and verification of specimens that are stored; these SOPs must be followed throughout the study. In the event that the required volume or number of sample aliquots is not obtained at any time point, designated site clinic and lab staff must immediately inform the HPTN CORE, HPTN SDMC and LC. The HPTN CORE, SDMC, and LC will provide guidance on how to respond to the problem. In addition to following this guidance, designated site and lab staff will work together to document the problem, take appropriate corrective and preventive action, and document all action taken. Reconciliation must be performed for all specimen types that are received by the laboratory and stored in the LDMS. The lab staff should provide the HPTN LC with a detailed accounting of these issues in a regulatory Note to File or a specific agreed upon format.

11.3. Protocol Related Testing and Sample Collection

Samples will be collected and processed at the screening, enrollment, and follow up visits as indicated in tables 11.1. and 11.2.

Collect specimens and label tubes according to local regulations and the specimen collection SOPs.

Blood collection tubes must be filled to the appropriate fill level as indicated by the tube manufacturer and must be collected according to the order of draw dictated by your local institution. Please contact the HPTN LC for advice if your tube type is not listed below or if the order of draw differs from the following:

- Citrate Tube (coagulation)
- Serum Tubes
- EDTA (Ethylenediaminetetraacetic acid) Tubes
- Fluoride (glucose) Tube

After collection:

- EDTA tubes (Lavender top) and fluoride tubes (grey top) should be gently inverted at least 8 times (or as specified by manufacturer) after specimen collection to prevent clotting.
- Citrate tubes (Light blue top) should be gently inverted at least 4 times (or as specified by manufacturer) after specimen collection to prevent clotting.
- For plasma storage 20 ml of whole blood should be collected into spray dried EDTA tubes eg BD 366643 or other to yield 5 x 1.8mL plasma.

Table 11.1 Schedule of Study Visits and Specimen Collection – All Participants

	Oral Phase						Injection and Tail Phase Follow Up – Weeks										
	Screening	Day 0, Enrollment	DOT Visits 1 -2	DOT Visit 3	DOT Visit 4	Week 2, Oral Run-in Safety Visit	Week 4, First Injection	Week 6, Safety	Week 8, Safety	Week 12, Second Injection	Week 14	Week 20, Third Injection	Week 28, Fourth Injection	Week 36, Fifth Injection	Week 44, Sixth Injection	Week 52, Primary Outcome Visit	Week 64, Tail Phase	Week 76, Tail Phase
Visit Code	1.0	2.0		3.0		4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.0	15.0	16.0
HIV testing[1]	X	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X
Hepatitis testing[2]	X																	
Syphilis testing	X																	
Hematology (CBC with Differential and platelets)	X	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X
Chemistry[3]	X	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X
Potassium and Magnesium	X																	
Urine pregnancy testing[4]	X	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X
Urinalysis[5]	X	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X
Urine or vaginal swab GC/CT testing[6]	X																	
Cervicovaginal and rectal fluid storage[7]														X				
Plasma Storage[8]	X	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X
Plasma for PK testing[8]		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X
Whole blood storage for Pharmacogenomic testing[9]		X																

1. Following the HIV algorithms described in SSP section 11.3.1. HIV testing does not need to be performed after confirmation of HIV infection (based on results from samples collected on two separate dates). At the oral dosing visit (Day 0) and injection visits (Weeks 4, 12, 20, 28, 36, and 44), at least one same day negative or non-reactive HIV test result must be obtained PRIOR to administering the study product.

2. Hepatitis testing includes hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (HBsAb), and hepatitis C antibody (HCAb).

3. Chemistry testing includes: total bilirubin, CPK, alkaline phosphatase, creatinine, AST, ALT, total protein, glucose, calcium and phosphorous.

4. Pregnancy testing may be performed in the clinic or the laboratory at all visits where this testing is indicated. Testing may be performed using a urine, plasma, or serum sample. The assay used for pregnancy testing must have a limit of detection of 25 mIU/mL or lower. Women not of childbearing potential are excluded from pregnancy testing throughout their participation in this study. Pregnancy testing is not required at subsequent visits if a positive result is obtained. At the first oral dosing visit (Day 0) and injection visits (Weeks 4, 12, 20, 28, 36 and 44), a pregnancy test must be performed and pregnancy must be ruled out PRIOR to administering the study product. Urine pregnancy testing may be performed in the clinic or the laboratory. Pregnancy testing is not required if a positive result was obtained at a prior visit.

5. Urinalysis includes protein and glucose; this testing may be performed in the clinic or the laboratory.

6. Urine or vaginal swab for GC/CT, will be performed at Screening. The choice of sample type is site dependant.

7. Cervicovaginal and rectal fluid collection can be done at week 36 (preferred) or week 44. For participants in the Tissue Subset, fluid collection and storage must be performed at the same visit as the vaginal tissue biopsy (week36 (preferred) or week 44). See Table 11.2. All samples for PK (plasma, vaginal tissue, cervicovaginal fluid and rectal fluid) should be collected within a 4-hour window.

8. Plasma for storage and for PK can be obtained from 20mL of EDTA whole blood that has been drawn at the same time and date as the sample for HIV testing. See SSP Section 11.4.1 for specimen processing details. At Enrollment (day 0) and visits (DOT visit#3, Weeks 2, 4, 12, 20, 28, 36 and 44), blood for PK must be drawn prior to the administration of study product.

9. Store 4 mL of EDTA whole blood for pharmacogenomics analysis. The whole blood specimen must be entered into LDMS and stored frozen. See section 11.4.2 for specimen processing details.

Table 11.2 Additional Procedures for Participants in the Tissue Subset. US Sites Only.

		Oral Phase				Injection and Tail Phase Follow Up – Weeks										
	Screening	Day 0, Enrollment	DOT Visits 1 -4	Week 2, Oral Run-in Safety Visit	Week 4, First Injection	Week 6, Safety	Week 8, Safety	Week 12, Second Injection	Week 14	Week 20, Third Injection	Week 28, Fourth Injection	Week 36, Fifth Injection	Week 44, Sixth Injection	Week 52, Primary Outcome Visit	Week 64, Tail Phase	Week 76, Tail Phase
Visit Code	1.0	2.0		4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.0	15.0	16.0
Pap Test, if indicated[1,2]							X									
Coagulation testing (PT/INR, aPTT) [2,]						X										
Vaginal tissue processing/storage[3]												X				

1. To be offered if a satisfactory Pap smear is not documented within the last 12 calendar months and is indicated. See Protocol Section 3.1.1.
2. Coagulation and Pap testing can also be done at later visits, but results must be available for enrollment into the Tissue Subset.
3. Vaginal tissue will be used for pharmacologic assessments. Vaginal tissue will be collected only at US sites that have this capacity. Collection at Week 36 is preferred but tissue may be collected Week 44. Vaginal tissue must be collected at the same visit as cervicovaginal and rectal fluid collection. All samples for PK (plasma, vaginal tissue, cervicovaginal fluid and rectal fluid) should be collected within a 4-hour window.

11.3.1. HIV Testing

HIV testing will be performed using blood collected by phlebotomy (no finger stick or oral fluid testing) at participant visits in accordance with the testing algorithms described in Figures 11.1 – 11.3.

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

Whole blood will be collected according to site-specific procedures.

Participants with one or more reactive HIV test results at either the screening or enrollment visit will not be eligible for enrollment, regardless of subsequent test results.

RNA testing for acute HIV infection must be performed within 28 days prior to Enrollment.

The Protocol Chair, Site PI, CMC and HPTN LC must be notified immediately if one or more reactive HIV test results are obtained at any follow up visit after enrollment. This includes the result of the 4th generation EIA test that is collected at the enrollment visit.

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

HIV infection must be confirmed using two independent samples collected on different days. Plasma storage is required at every visit at which HIV testing is performed.

All tests and associated QC procedures must be documented on local laboratory log sheets or other laboratory source documents. Kit lot numbers and expiry dates must also be documented.

All staff involved in HIV testing and verification of HIV test results should be aware of the testing time frame for the HIV test, so that all tests are performed and verified within the specified time frame. Place appropriate timekeeping devices in all test settings to ensure that each test is read and verified at appropriate time points. Documentation is required for the testing start and stop times, as well as, result verification times. These must be recorded on testing log sheets.
If a participant has a reactive or positive HIV test at any time after enrollment, additional blood draw and testing is required as detailed in Table 11-3.

Figure 11.1 HIV Testing Algorithm at the Screening Visit

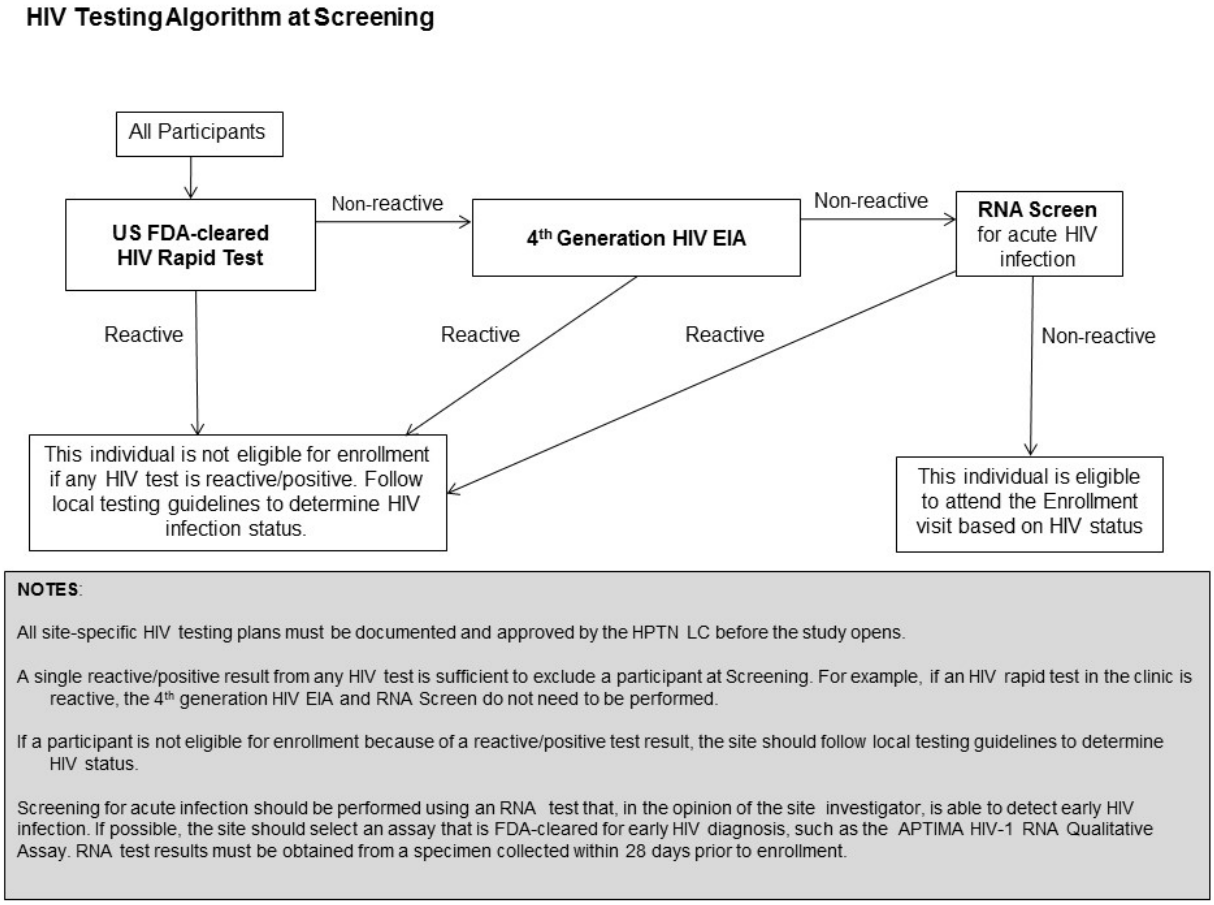
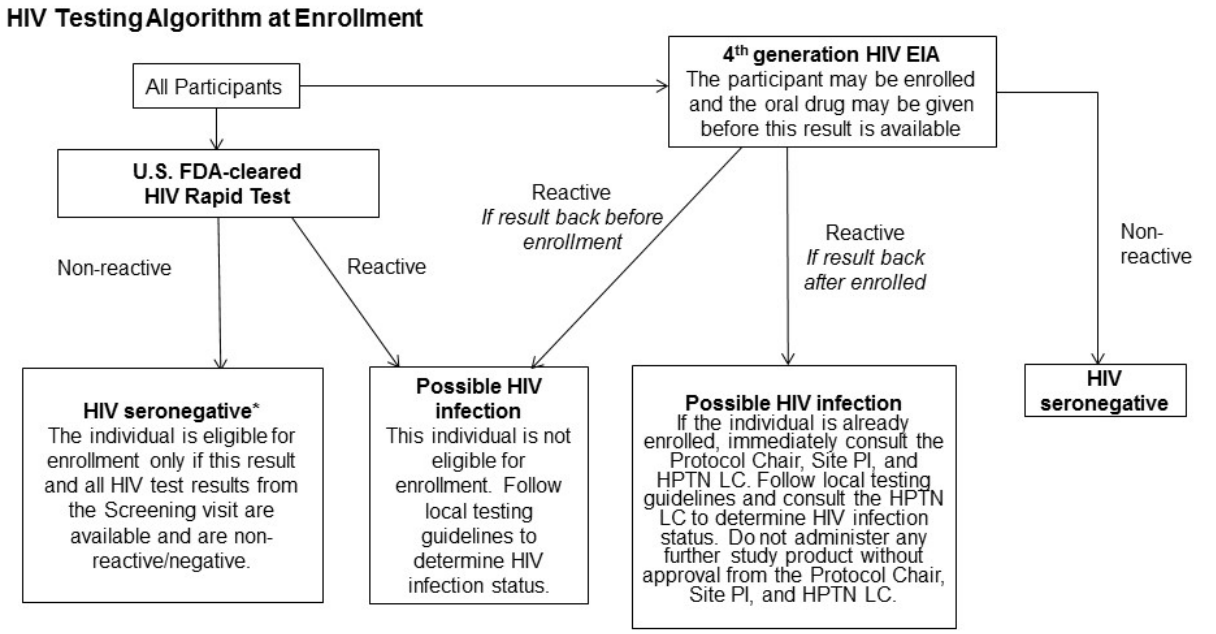


Figure 11.2 HIV Testing Algorithm at the Enrollment Visit:

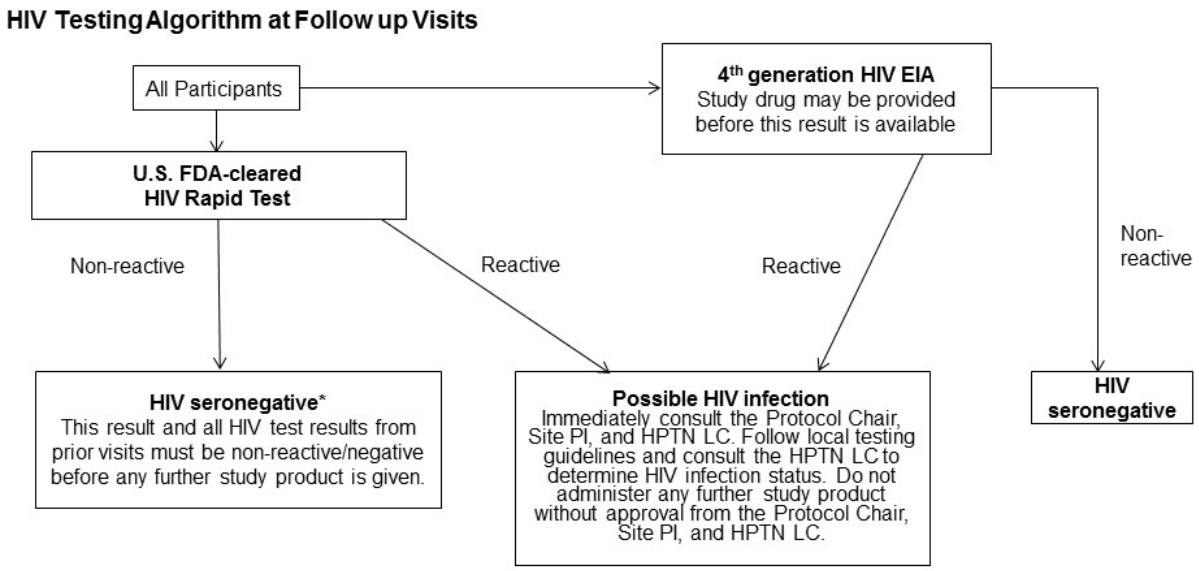


NOTES:

Before providing study drug, the site must ensure that the HIV rapid test from the Enrollment visit, and all HIV results from the Screening visit are available and are negative or non-reactive.

*If acute HIV infection is suspected, the participant is not eligible for Enrollment at this time. In this case, the site should follow the algorithm, but should also send sample for an RNA test that, in the opinion of the site investigator, is able to detect early HIV infection. If possible, the site should select an assay that is FDA-cleared for early HIV diagnosis, such as the APTIMA HIV-1 RNA Qualitative Assay. Contact the HPTN LC, Site PI, and Protocol Chair for additional guidance.

Figure 11.3 HIV Testing Algorithm at Follow Up Visits:



NOTES:

At any visit where study product will be given, the site must ensure that the HIV rapid test from this visit, and all HIV results from prior study visits are negative or non-reactive.

*If acute HIV infection is suspected, do not administer any further study product. Immediately consult the Protocol Chair, Site PI, PSRT and HPTN LC. In addition to following the algorithm above, the site should send sample for an RNA test that, in the opinion of the site investigator, is able to detect early HIV infection. If possible, the site should select an assay that is FDA-cleared for early HIV diagnosis, such as the APTIMA HIV-1 RNA Qualitative Assay. The site should contact the HPTN LC, Site PI, and Protocol Chair for additional guidance once all of the test results from this visit (including the HIV RNA test) are available.

Table 11.3. Additional Procedures for Participants who have a Reactive or Positive HIV test at any Time after Enrollment.

	HIV confirmation visit following a reactive or positive HIV result.
HIV testing[1]	X
CD4 cell count	X
HIV resistance testing[2]	X
Additional plasma storage[3]	X
HIV Viral Load	X

1. Following approved HIV testing algorithm shown in figure 11.3. HIV rapid testing may be performed in the clinic or the laboratory.

2. Sites may collect specimens for resistance testing at a local laboratory to assist with clinical management; results from resistance testing performed at local laboratories will not be reported to the SDMC. Stored plasma may not be used real-time/local resistance testing.

3. Obtained from 20mL of EDTA whole blood that has been drawn at the same time and date as the sample for HIV testing. See SSP Section 11.4.1 for specimen processing details.

11.3.2. Hepatitis Testing

Testing for HBV and HCV will be performed at Screening. Sites will follow local testing arrangements for the collection and testing of samples, this will be described in the site SOPs.

Test results are required for the enrollment visit.

11.3.3. Safety Testing

CBC and Chemistry testing will be performed at various time points throughout the study. Additional potassium and magnesium testing will be performed at the screening visit. Sites will follow local testing arrangements for the collection and testing of samples, this will be described in the site SOPs.

Test results from the screening visit are required prior to enrollment.

Same day test results are not required prior to the issue of study product.

11.3.3.1. Creatinine Clearance

Calculated creatinine clearance will be performed using the Cockcroft-Gault formula for females:

$$\text{eCcr (female) in mL/min} = \frac{[(140 - \text{age in years}) \times (\text{actual body weight in kg}) \times .85]}{(72 \times \text{serum creatinine in mg/dL})}$$

11.3.4. Coagulation Testing (US sites only)

Coagulation testing (PT/INR and aPTT) will be performed at the week 6 visit only for participants in the Tissue Subset. This testing can also be done at later visits, but results must be available for enrollment into the Tissue Subset. Sites will follow local testing arrangements for the collection and testing of samples, this will be described in the site SOPs.

11.3.5. PapTest (US sites only)

This will be offered at week 8 to participants who elect to enroll into the tissue subset if a satisfactory Pap smear is not documented within the last 12 calendar months. See protocol section 3.1.1 for details. This can also be done at later visits, but results must be available for enrollment into the Tissue Subset. Sites will follow local testing arrangements for the collection and testing of Pap smears.

11.3.6. Pregnancy Testing

All women of reproductive potential will have a βHCG test for pregnancy (sensitivity of ≤ 25 mIU/mL) at each visit listed in table 11.1. Pregnancy testing is not required at subsequent visits if a woman had a positive pregnancy test at a previous visit and is still pregnant.

Sites will follow local testing arrangements for the collection and testing of samples, this will be described in the site SOPs.

The test result from the screening visit is required prior to enrollment.

Same day test results must be available and confirmed to be negative PRIOR to the provision or continuation of study product. This is a requirement at all visits at which study product is to be administered or continued (enrollment, weeks 4, 12, 20, 28, 36 and 44).

Study product must NOT be given if the pregnancy test obtained at this or the prior visit is positive.

Study product must NOT be administered if the participant is pregnant. This must be based on pregnancy test results from previous visits and on the result of the pregnancy test performed at the current study visit.

11.3.7. Urinalysis Testing

Sites will follow local testing arrangements for the collection and testing of urine for urinalysis (protein and glucose). This will be described in the site SOPs.

Results from the enrollment visit are not required prior to enrollment.

11.3.8. Urine or Vaginal Swab for GC/CT Testing and Syphilis Testing.

Sites will follow local testing arrangements for the collection and testing of Urine or Vaginal swabs for GC/CT, and plasma for syphilis testing. This will be described in the site SOPs.

Results from the screening visit are required prior to enrollment.

11.4. Specimen Processing for Sample Storage

11.4.1. Plasma Processing for Storage

Five aliquots of plasma will be prepared from the 20 ml blood tube at each study visit, as indicated below:

20 mL of EDTA whole blood will be drawn for plasma storage at each time point at which HIV testing is performed as indicated in Table 11.1. Sites are requested to store 5 x 1.8 mL aliquots of plasma if possible. HPTN LC should be informed at any time that three or less aliquots of 1.8mL are stored. This plasma will also be used for PK testing at the appropriate visits.

An additional 20 mL of EDTA whole blood will be drawn for plasma storage for participants with a reactive or positive HIV test at any time after enrollment as indicated in Table 11.3. This additional plasma will be stored in the same way.

Sites will follow site specific SOPs for plasma processing which will include the following:

- Collect blood into lavender top blood collection tubes (EDTA) labeled with a SCHARP-provided PTID label. Size and number of collection tubes may vary depending on local lab requirements.

- Deliver this to the local LDMS laboratory along with the LDMS Specimen Tracking Sheet or site specific requisition.

- Using the LDMS Specimen Tracking Sheet, log the sample into LDMS (specimen type = BLD) and generate the appropriate number of LDMS cryovial labels. The lab should store plasma in labeled cryovials. Cryovial size may vary, but 2.0 mL is recommended. Reminder these vials hold 1.8 mL of liquid. Do not add more than 1.8 mL due to expansion issues when freezing.

- Blood processing and plasma storage should be performed within 6 hours of sample collection.

- Centrifuge tube at 800 - 1000 x g for 10 minutes to separate cells and plasma.

- Carefully remove plasma and avoid disturbing the cell layer. Transfer the plasma to an appropriately labelled sterile centrifuge tube.

- Centrifuge plasma again at 800 - 1000 x g for 10 minutes to remove any contaminating debris, cells, or platelets.

- Log samples into LDMS and generate LDMS labels. (PL2) Each aliquot will have its own individual identification number (Global Specimen ID).

- Store plasma in aliquot number order. For example if there is only 3 mL of plasma for archive, store 1.8 mL in aliquot 1. Store the remaining 1.2 mL in aliquot 2 and adjust the aliquot volume in LDMS to indicate 1.2 mL.

- Store the aliquots in the freezer locations assigned in LDMS in a minus 70° to minus 90° freezer.

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

Study sites should plan to store specimens until all of the protocol specified testing (including assessments at the HPTN LC) has been completed and the primary research paper has been published.

LDMS Entry:

LDMS Specimen Code for Plasma Storage

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

Codes used in table:

BLD **Blood**

EDT **EDTA**

PL2 **Plasma, Double Spun**

N/A **Not Applicable**

Other Spec ID: **Not Applicable**

- All plasma vials are stored in the LDMS and in a -70°C to -90°C freezer. Specimens will be shipped to HPTN Laboratory Center (LC) when requested.

All enrolled study participants must consent to collection and storage of their plasma for the duration of their study participation and until all protocol-specified testing has been completed. Participants are asked to consent separately to indefinite storage and possible future research testing of their plasma after the study is completed. Participants may refuse to consent to indefinite storage and possible future research testing and still enroll in the study. After all protocol-specified testing has been completed; the stored plasma of participants who do not consent to indefinite storage and possible future research testing must be destroyed. After all protocol-specified testing has been completed, the HPTN SDMC will provide each site with a list of participants who did not consent to indefinite storage and possible future research testing. Sites should follow local regulations for sample destruction and follow the information provided in the LC section of the HPTN Manual of Operations. Documentation of the destruction should be provided to the HPTN LC.

11.4.2. Whole Blood Storage for Pharmacogenomic Testing

Specimen Type: Whole blood collected in EDTA anticoagulant ("purple top") tubes.

Specimen volume: Minimum 4 mL whole blood

Handling Instructions: Whole blood is transferred to 5 mL cryovials and frozen at minus 70°C or colder.

Materials

Materials: Thermo Scientific Nalgene Cryovials, 5 mL (Nalgene, Cat. #5000 0050)
Different cryovials may be used but the HPTN LC must be consulted before use**.**

Disposables: Standard Disposable Transfer Pipets (example Fisher, Cat. #137117M)

Procedure –Stepwise

1. An appropriately labeled and filled EDTA whole blood tube will be received.

2. Log specimens into LDMS upon receipt using the following LDMS codes
    a. PRI: BLD
    b. ADD: EDT
    c. DER: BLD
    d. Sub Add/Der N/A
    e. Other Spec ID: PGEN

3. Transfer the whole blood to a labeled Nalgene cryovial using a transfer pipet

4. Do not fill cryovials to more than ¾ capacity

5. Use Parafilm to seal caps of the cryovials to prevent leakage during shipping

6. Ensure PTID, date, visit number and laboratory identifier are on the LDMS label

7. Store whole blood in a freezer at minus 70°C or colder until requested for shipment

8. Ship when requested on dry ice for arrival on Monday through Friday only, site must follow appropriate shipping regulations.

9. Batch shipment to:

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

11.4.3. Cervicovaginal and Rectal Fluid Processing and Storage.

Collection and Storage of Fluid Using a Dacron Swab.

This procedure outlines the preparation of Dacron swabs to be used for the collection of cervicovaginal fluid and rectal fluid.

Note:
It is important that the rectal fluid be collected before an enema is performed (if performed).

It is important that cervicovaginal fluid be collected before the collection of vaginal tissue (select participants at US sites only). The following order of collection is recommended – rectal fluid, cervicovaginal fluid, vaginal tissue.

Required Materials and Equipment

- Two Dacron Swabs (Fisher Scientific Cat. No. 22-029-574)

- Two 15 ml Conical tubes with caps (Fisher Scientific Cat. No. 14-959-49B or equivalent)
- Storage box (Fisher Scientific Cat. No. 03-395-454) and box inserts (Fisher Scientific Cat. No 03-395-462)
- Calibrated analytical balance capable of measuring to 0.0001g – the same balance must be used throughout the procedure.
- Leak-proof transport container (if required)

Preparation of the Dacron swabs

1. Prepare prior to the procedure in a clean environment.
2. Tare the weighing balance and ensure balance has been calibrated.
3. Remove Dacron swabs from the box, wear gloves at all times when handling swabs.
4. The swabs are 6 inches long. Using scissors cut off the top of the shafts to reduce the length of the swabs to approximately 4.5 inches so that each will fit inside the 15mL conical tubes.
5. Place each Dacron swab into its own appropriately labeled 15mL conical tube, labeled with a unique patient identifier.
6. Label conical tubes so that they are identifiable for PTID and also for sample type eg. label one conical tube "**Cervicovaginal**", label the other tube "**Rectal**".
7. Weigh the labelled conical tubes containing swabs and document the weight. An example worksheet is shown in Figure 11.4
8. Record the weighing time (24hour clock)
9. The Dacron swabs are now ready to be used for collection of fluid.

See SSP section 9 for fluid collection instructions.

Storage of specimens

Transport the conical tubes at ambient temperature to the location where post weight will be documented. Following the documentation of the post collection sample weight, samples must be placed in a -80^{o}C freezer or placed in dry ice within an hour of collection. Samples placed in dry ice should be transported for final storage in a -80^{o}C freezer as soon as is possible.

Weigh the Dacron swabs and capped conical tubes again using the same analytical balance used for the pre-weigh.

10. Create an entry for each participant in LDMS, print aliquot labels and label conical tubes.

The following LDMS codes must be used for cervicovaginal fluid:

Primary Code:	VAG
Additive:	NON
Derivative:	FLD
Sub Add/Deriv:	NON

Enter the appropriate weight of the fluid in the Volume field and time of collection.

If a negative value for weight is obtained please add this information as a comment.

The following LDMS codes must be used for rectal fluid:

Primary Code:	REC
Additive:	NON
Derivative:	FLD
Sub Add/Deriv:	NON

Enter the appropriate weight of the fluid in the Volume field and time of collection.

If a negative value for weight is obtained please add this information as a comment.

11. Place the conical tubes in a -80ºC freezer for storage until shipment is requested by the LC.
12. Record the time that the sample is introduced to the freezer.

Time	Interval between Collection and Freezing
Time unit	hours

Figure 11.4 Example of a Dacron Swab Collection and Storage Worksheet

Fluid Type	LDMS Aliquot Number	Pre Collection Weight in mg.	Post Collection Weight in mg.	Time of post collection weight.	Net weight in mg.	Freezing Time	Time interval between collection and	Performed by
		Tube + Label + Swab	*Tube + Label + Swab*	*24 Hr Format*	*Post weight – Pre weight.*	*24 Hr Format*	*Hours*	
Cervicovaginal								
Rectal								

Cevicovaginal Fluid collection time(24 Hr Format.)

Rectal Fluid collection time(24 Hr Format.)

Time Specimens were received in the processing laboratory (24 Hr Format

Serial Number of balance used for pre and post weight _____.

Comments: (eg presence of blood/stool if seen)

11.4.4. Vaginal Tissue for Processing and Storage – Tissue Subset Participants Only

Note: It is important that cervicovaginal fluid be collected before the collection of vaginal tissue.

The following samples will be collected from participants in the tissue subset (Select participants US sites only) and will be processed and stored using LDMS. Specimens should be collected as described in section 9 of the SSP.

Vaginal Biopsies

Based on a 15 mg biopsy, the following numbers of biopsies are suggested for each testing procedure:

- Vaginal tissue for PK – ideally 2 biopsies (minimum 1)
- Required Materials and Equipment
    - 2 mL Cryovial (Corning #430659 or equivalent)
        - Medium Tischler forceps (Gynex. Order #: 1008-W) or equivalent.
        - Storage box (Fisher Scientific Cat. No. 03-395-464; supplied with insert)
        - Clean, pointed forceps
- Petridish (Falcon # 35-3803 or equivalent)
- Analytical Balance
- Liquid Nitrogen or Dry Ice-Alcohol bath

  Transport medium: See below

Vaginal biopsies should be collected using medium Tischler forceps (3 mm x 5 mm bite size, Gynex. Order #: 1008-W).

Vaginal biopsies should be placed into a suitably labeled 50mL conical tube specimen pot that contains 30mL of the following Transport Medium:

Transport Medium	Volume per 100mL
RPMI Medium 1640 w/L-glutamine, w/HEPES (Invitrogen, Cat # 22400-105)	90.5mL
Fetal Bovine Serum (Invitrogen, Cat # 10082-147; final 7.5%)	7.5mL
Antibiotic/Antimycotic (Invitrogen, 100X, Cat # 15240-104; final 1%)	1mL
50mg/mL Zosyn® (Wyeth, NDC # 0206-8852-16)	1mL

Prepare transport medium in a biological cabinet using sterile conditions and place in a suitable sterile container. Store at 4°C for up to 14 days. Discard any unused portion.

Transport the samples at ambient temperature from the biopsy suite to the location where the sample will be weighed. Transport to the LDMS/storage laboratory should be as soon as possible so that biopsies can be placed in the freezer within one hour of collection**.**

Specimens should be accompanied by a completed LDMS tracking sheet.

Procedure

- Label a cryovial with an LDMS-generated label containing the appropriate sample/study identification information.
- Weigh the labeled cryovial using an analytical balance – use the same analytical balance throughout the procedure. Document the weight of the labeled cryovial on the appropriate sample/study form. See Figure 11.5 for an example of study form.
- Receive biopsy in transport medium. Biopsy should be delivered to the lab to allow freezing within one hour of collection.
- Transfer biopsy to petri dish. Work under sterile conditions.
- Using pointed forceps pick up the biopsy and drain off excess medium by touching biopsy to side of petri dish.
- Transfer biopsy to a pre-weighed cryovial. Ensure biopsy sits at bottom of cryovial.
- Weigh the cryovial containing the biopsy. Document the weight of the cryovial containing the vaginal biopsy on the appropriate sample/study form

- Freeze the cryovial containing the biopsy in Liquid Nitrogen or a dry ice-alcohol bath.

- Store the labeled cryovial containing the biopsy in a -80°C freezer. Document the date/time the cryovial containing the biopsy was placed in the freezer.

- Biopsy should be frozen within one hour of collection.

Biopsy should be shipped upon request to the HPTN Laboratory Center at JHU. Copies of the completed study forms should also be sent upon request.

LDMS

Specimen details must be entered into LDMS. Cryovials must be stored using labels produced from the LDMS.

The following LDMS codes must be used.

LDMS FIELD	Code/Information to be entered
Primary code	VAG
Additive	BTM
Derivative	VAG
Subderivative	N/A
Volume	Weight of biopsy in mg
Units	mg
Time	Interval between Collection and Freezing
Time unit	hours
Other Spec ID	PK
Comments	Note any contamination or other details.

Figure 11.5 Example of Study Form for Vaginal Biopsy Storage.

Storage of Vaginal Biopsy for PK

Participant ID Number (PTID) ……..

Date of Biopsy Collection ……..

Visit Number ……..

BIOPSY ID	Weight (Milligrams)			Time (actual, 24 h format)		Time interval
(LDMS aliquot ID)	Cryovial	Cryovial + Biopsy	Biopsy	Collection hh:mm	Freezing hh:mm	(collection to freezing) Hours
Biopsy 1.						
Biopsy 2.						

Serial Number of Balance Used.

Weighing Performed By.

11.5. Shipping of Samples to the HPTN LC

Each site will ship plasma samples to the LC upon receiving shipping request lists. The site will batch the shipment, export the LDMS data and notify the LC about the shipment. Any additional samples may be specifically requested by the HPTN LC (e.g., archive/back-up samples).

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

Sites will ship samples to the LC using the LDMS following the LC approved Shipping SOP indicating Lab 300 as the ship to lab ID number.

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

Include a copy of the shipping manifest and box map. For dry ice shipments, use diagnostics packing code 650, UN 3373, and address the shipment to:

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

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

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

11.5.1. HIV QA testing

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

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

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

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

11.5.2. Pharmacology Testing

Plasma samples for drug levels will be collected beginning at the enrollment visit (Day 0) and at weeks 4 through week 76. These samples will be collected from all participants, although PK testing may be limited to a subset of the samples. At these visits the blood will be collected PRIOR to the administration of study product. The actual date and time of each blood sample collection will be recorded, as well as the time of each injection. This information should be captured on the relevant CRF.

Specimens for pharmacology testing will be stored on site for shipment to the HPTN LC upon request.

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

Stored plasma may also be tested for the presence of other ARV drugs or other substances.

11.5.3. Pharmacogenomic Testing

A whole blood specimen for pharmacogenomic analysis will be collected at the enrollment visit. Samples will be stored on site for shipment to the HPTN LC upon request. Assays will be performed at the HPTN LC. Results will not be returned to the sites or study participants.

11.5.4. Other testing

The HPTN LC will perform QA testing, including testing to determine HIV infection status in selected cases. Additional assays may be performed at the HPTN LC or a laboratory designated by the HPTN LC. This testing may include the following tests for participants who acquire HIV infection: HIV viral load, HIV resistance testing, HIV subtyping, and other tests to characterize HIV viruses and/or the host response to HIV infection. Results will not be returned to the sites or study participants, with the exception of HIV testing (if results obtained at the HPTN LC do not agree with site results) and the exception for resistance test results, noted below.

Resistance testing will be performed at the HPTN LC or a laboratory designated by the HPTN LC. This testing will be performed retrospectively at the end of the study. If real-time resistance testing is needed for clinical management, that testing should be arranged by the site outside of the study; separate specimens should be collected for that testing. For sites that do not have the capacity for local resistance testing for clinical care, results from resistance testing may be provided at the end of the study at the request of the site IoR, with approval of the HPTN LC and Protocol Chair. Results from specialized resistance testing (e.g., minority variants analysis, if performed) will not be returned to study sites.

11.6. Laboratory Monitoring

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