Section 8. Laboratory and Specimen Management Procedures

8.1 Overview of Section 8

This section contains information on the laboratory procedures performed in HPTN 091. Laboratory procedures will be performed in a variety of settings, including:

1. Clinics
2. Local laboratories
3. The HPTN Laboratory Center ("LC", Baltimore, MD and Aurora, CO, USA)
4. 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.**

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. References on healthcare worker safety and prevention are available from the US Centers for Disease Control and Prevention at:

https://www.cdc.gov/niosh/topics/healthcare/default.html and
https://www.cdc.gov/niosh/topics/bbp/

Additional reference information can be requested from the HPTN LC. The information provided below is intended to standardize laboratory procedures for HPTN 091 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.

8.2 Specimen Labeling

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

8.2.1 Local Specimen Processing and Storage

For samples that are processed and stored locally, each sample will be entered into the LDMS and labeled with the LDMS generated labels. If needed, any temporary labels (e.g. during plasma processing) for samples will include at least the full PTID, in addition to any other information required by lab SOPs.

8.2.2 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.

8.2.3 Remote Specimen Testing

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

8.2.4 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.ldms.org/resources/manuals/):

Web (Cloud-Based) https://www.ldms.org/resources/ldms/web/

All sites are responsible for ensuring they are using the most recent version of LDMS

and that they have validated the LDMS. All sites must use the *HPTN barcode* label format in order to ensure that both the specimen ID and the global specimen ID assigned to each specimen are printed on LDMS-generated labels.

Examples of two-dimensional LDMS-generated barcode labels are below: PC Windows-based Systems

Row 1:LDMS Specimen ID

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

Row 2: Global Specimen ID

Row 3: Patient Identifier (ID1) and Study/Protocol Identifier (ID2) Row 4: Specimen Date or Harvest Date and Specimen Collection Time

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

Row 6: Volume/Volume Unit and Visit/Visit Unit (VID) Row 7: Other Specimen ID (if applicable)

Web-based Systems:

0500-001RDD00-001
999515640 057.0
07/Jul/2017 08:30
BLD EDT PL2 N/A
1.00 ML 0 Scr

Row 1: Global Specimen ID

Row 2: Patient Identifier (ID1) and Study/Protocol Identifier (ID2) Row 3:Specimen Date or Harvest Date and Specimen Collection Time

Row 4: Primary Type, Additive Type, Derivative Type, and Sub Additive/Derivative Type

Row 5: Volume/Volume Unit and Visit/Visit Unit (VID) Row 6:Other Specimen ID (if applicable)

Questions related to use of LDMS for HPTN 091 should be directed to Vanessa

Cummings (vcummin1@jhmi.edu).

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

LDMS User Support at Frontier Science

LDMS user support is available 24 hours per day by telephone or email to answer your questions about using LDMS, diagnosing problems, and helping your laboratory get the most out of the software. When contacting LDMS User Support, be sure to include your LDMS laboratory number.

Please note LDMS User Support cannot be contacted during the following U.S. Holidays – Thanksgiving Day, Christmas Day, New Year's Day, Memorial Day, and Independence Day.

https://www.ldms.org/contact/

Phone: +1 (716) 834-0900, extension 7311

Email: ldmshelp@fstrf.org

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?

Each site on Windows LDMS must export its LDMS data to Frontier Science (FSTRF) 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.

8.2.5 LDMS Reconciliation

All sites must follow the HPTN LC approved site-specific SOP for regular reconciliation and verification of specimens that are stored; these independent SOPs or detailed Chain of Custody procedures must be followed throughout the study. All sites must also create an HPTN 091 Specimen Log report upon LC request. 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 LOC, HPTN SDMC, and the LC. The HPTN LOC, 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. QCs in the SDQC (Specimen Data Quality Control) Tool must be addressed within two weeks.

8.3 Protocol related testing and sample collection

Samples will be collected and processed at the Screening, Enrollment, and follow-up visits at local processing labs and as indicated in sections 8.4, 8.5 and 8.8 as applicable.

Participants will have specimens collected and tested at local laboratories as listed in section 10.1 in the protocol. Approximately 310 HIV-uninfected TGW will be enrolled in the study and assigned to two different intervention cohorts as outlined in the study protocol. Participants will need to have HIV negative/ non-reactive results at Screening and have at least one negative/non-reactive result at Enrollment in order to be included. Sites will follow the HIV testing algorithm for Screening included in section 8.3.1 HIV Testing of this SSP. If a reactive/positive result is obtained for any HIV test at the Screening or Enrollment visits, the participant is not eligible for the study, even if HIV

infection is not confirmed. Additional testing to confirm suspected HIV infection during Screening and Enrollment will be performed in accordance with local guidelines. If HIV infection is confirmed, participants will receive counseling and be referred for appropriate care, as necessary.

HIV test results from Screening must be available and reviewed prior to Enrollment. If a participant has a reactive test at Enrollment, HIV infection should be confirmed on a second sample collected on a date different from the Enrollment visit as per the HIV testing algorithm.

Participants must have fasted for at least 8 hours, preferably 12 hours, prior to lipid profile sample collection. Sites should verify that a participant has fasted prior to sample collection. If the patient has not fasted, the specimen should not be collected for lipid profile testing, and the participant should be scheduled to return to the site for sample collection. Fasting samples for lipid profile testing will be collected at Screening, Week 26 (Month 6) and Week 78 (Month 18). For the lipid profile testing scheduled at Screening, if the participant has not fasted, sample collection should be rescheduled to occur at the Enrollment visit. For participants initiating GAHT, testing for estradiol and total testosterone must be performed prior to hormonal therapy initiation. A GAHT initiation visit will be scheduled up to 10 days following the collection of samples for estradiol and total testosterone testing for initiation/re- initiation of GAHT. This timeframe will allow for sufficient time for sites to receive laboratory results and for participants taking part in the drug-hormone interaction (DHI) sub-study to complete the DOT phase. Please refer to Section 8.8 for additional details on the DHI sub-study.

Participants who decline PrEP can begin (or reinitiate) PrEP at any time up to and including their Week 65 visit unless contraindicated. At the visit when PrEP is to be initiated or reinitiated, the following procedures should be conducted, and results should be available prior to PrEP dispensation:

- Creatinine clearance
- HIV testing
- Fasting lipid profile (for participants reinitiating PrEP, lipid profile is not required if done within six months of product re-initiation)

Participants may begin co-localized GAHT any time after Enrollment (Immediate Intervention Arm) or after the 6-month visit (Deferred Intervention Arm) up to and including the Week 65 visit unless contraindicated. At the visit when co-localized GAHT is to be initiated or reinitiated, the following procedures should be conducted, and results should be available prior to GAHT dispensation:

- Estradiol and total testosterone testing
- Collect specimens and label tubes according to local regulations and the Blood Collection and Urine Collection SOPs. Blood collection tubes must be filled to the appropriate fill level as indicated by the tube manufacturer. After collection:
- EDTA tubes should be gently inverted at least 8 times (or as specified by manufacturer) after specimen collection to prevent clotting.
- For plasma storage, approximately 20 mL of whole blood should be collected into spray

dried EDTA tubes, e.g. BD 366643 or other, to yield 5 x 1.8 mL plasma aliquots.

Note: Biological samples must be transported in a sturdy, hard-shelled closeable container with a Bio-Hazard sticker/label per local safety regulation.

Table I : Schedule of Study Visits and Specimen Collection –Screening, Enrollment, GAHT Initiation Visit and Follow-up Visits (Adapted from Appendix IA)

Laboratory Procedures	SCR	ENR	GAHT Initiation Visit[4]	Week 13 (Month 3)	Week 26 (Month6)	Week 39 (Month 9)	Week 52 (Month 12)	Week 65 (Month 15)	Week 78 (Month 18)
Dipstick urinalysis (protein and glucose)	X								
GC/CT for NAAT (rectal, urine, pharyngeal)		X		X	X	X	X	X	X
CBC w/differential		X			X		X		X
LFTs (AST, ALT, TBili, alkaline phosphatase)	X			X	X	X	X	X	X
Fasting lipid profile[1]	X[2]	X[2]			X[3]				X[3]
Chemistry testing (BUN or urea, albumin and potassium)	X			X	X	X	X	X	X
Creatinine Clearance	X			X	X	X	X	X	X
Estradiol and total testosterone testing		X		X	X	X	X	X	X

Syphilis testing		**X**		**X**	**X**	**X**	**X**	**X**	**X**
HIV testing	**X**	**X**		**X**	**X**	**X**	**X**	**X**	**X**
HBV testing (HBsAg, HBsAb, HBcAb-Total)	**X**								**X**
HCV Testing	**X**								**X**
Plasma Storage	**X**	**X**		**X**	**X**	**X**	**X**	**X**	**X**
DBS Storage		**X**		**X**	**X**	**X**	**X**	**X**	**X**

[1] Total cholesterol, HDL, triglycerides, and LDL (either calculated or measured). Participants should have fasted for at least 8 hours, preferably 12 hours, prior to sample collection. If participants are not fasting, do not order the lipid testing and reschedule the participant to return to the same for lipid sample collection.

[2] A fasting lipid sample may be collected at either screening ***or*** enrollment. If the fasting sample is collected at the screening visit, it is not required to be collected at the enrollment visit. If a fasting sample is not collected at either the screening or enrollment visits, participants should be scheduled to come to the study site for samples collection with 72 hours of the enrollment visit.

[3] If a participant is not fasting at the Week 26 or 78 visits, the samples should be collected at a split visit within 72 hours of the visit if possible.

[4] For participants randomized to the Immediate Intervention Arm who accept GAHT, GAHT will start at the Enrollment Visit. For participants randomized to the Deferred Intervention Arm who accept GAHT, GAHT will start up to 10 days following the Week 26 (Month 6) study visit.

8.3.1 HIV Testing

All potential participants must have all nonreactive/negative HIV tests at Screening and Enrollment in order to be eligible for the study. If a potential participant has any reactive HIV test at Screening or Enrollment, then are not eligible to enroll in the study, regardless of subsequent HIV test results; these persons will be referred to local care.

HIV testing will be performed using blood collected by phlebotomy at participant visits in accordance with the testing algorithms described in Figures 8-1 through 8-5. Finger-stick and oral fluid testing are not permitted.

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.
HIV testing will be performed at all scheduled study visits (with the exception of the GAHT Initiation Visit). In addition, if a participant has signs or symptoms consistent with acute HIV infection or expresses a concern about recent HIV acquisition, HIV testing will be performed using a ribonucleic acid (RNA) test that is able to detect early HIV infection.

Regardless of whether HIV RNA testing is used for diagnostic testing, HIV acquisition after study Enrollment must be confirmed in all cases using two independent samples collected on different days, as per the HIV testing algorithm.

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

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 HIV tests, so that all tests are performed, read, and confirmed within the specified time frame of testing. 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 confirmation and verification times (second trained staff member confirms initial reading). 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 Figure 8-4 and Table II.

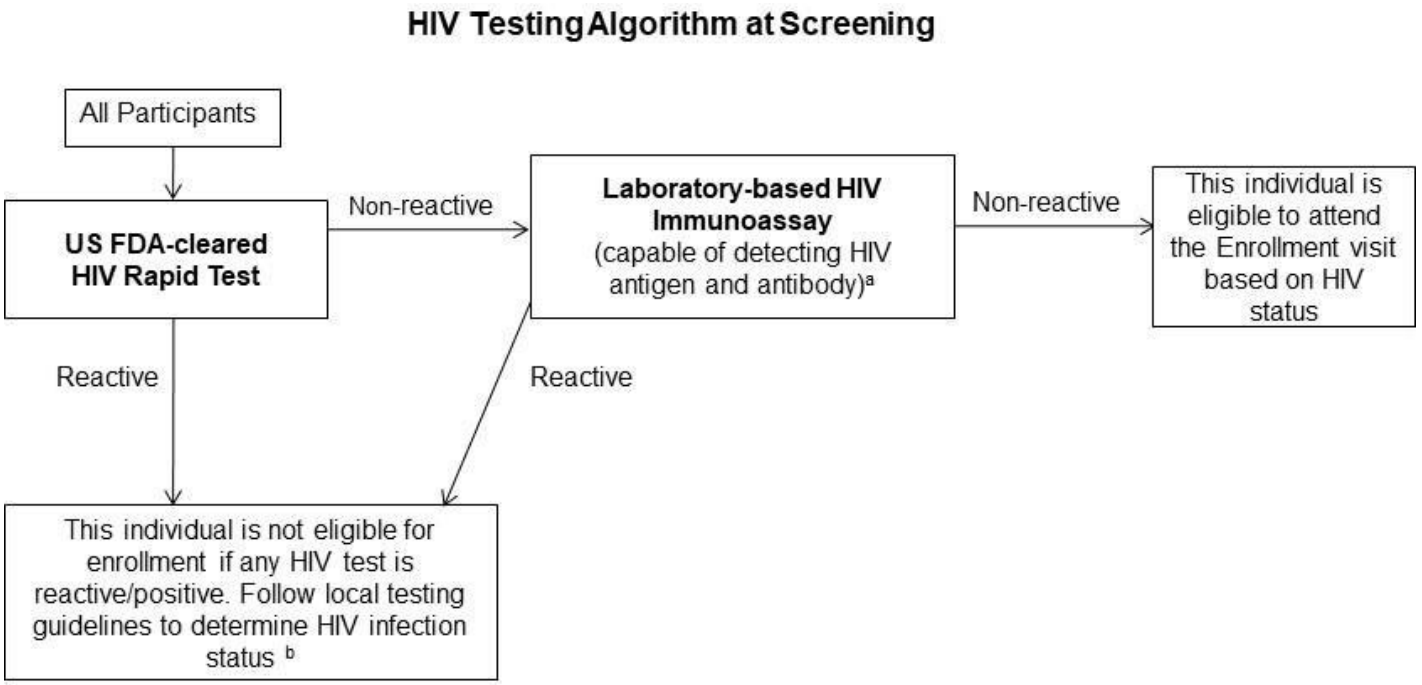
HIV infection must be confirmed using two independent samples collected on different days. Plasma storage is required at every visit at which HIV testing is performed. Every time a blood specimen is drawn for HIV testing, additional blood must be drawn for plasma storage if it does not exceed the visit blood draw limits stated in local consent forms. This includes interim visits, all visits for repeat HIV testing and confirmatory HIV testing. The amount of blood drawn (if not limited by consent forms) should be sufficient to yield 5 plasma aliquots, each containing approximately 1.8 mL of plasma.

Split visits (same visit code but different date), will occur, if for example lipid testing cannot occur at enrollment or weeks 26 or 78, due to a participant not fasting and needing to return for blood collection for fasting lipid profile. Additional details for split visits are provided below:

- For split visits, the laboratory-based HIV instrumented Ag/Ab assay *does not need to be repeated if the split visit occurs less than seven days from the initial visit (i.e., visit X.0) and if HIV testing and PrEP provision was completed at the initial visit.* The exception is HIV confirmation visits, which are interim visits and conducted specifically to perform additional HIV testing.
- If the split visit is seven or more days from the initial visit and the participant *did not receive PrEP*, the laboratory-based HIV instrumented Ag/Ab assay must be repeated Additional information is included in Section 4.3 of the SSP manual. In addition, the collection for DBS should be performed only if DBS was not collected at the initial day of the split visit. Plasma should be stored at any visit where HIV testing is performed.

Store all samples from all visits unless specifically directed to handle stored samples differently by the HPTN LC.

Figure 8-1 HIV Testing Algorithm at the Screening Visit:



NOTES:

[a] This testing must be performed using a laboratory based, non-rapid HIV immunoassay that detects both HIV antigen and HIV antibody (EIA, CIA or CMIA)

[b] Participants with reactive HIV rapid tests will not be able to rescreen

Figure 8-2 HIV Testing Algorithm at the Enrollment Visit:

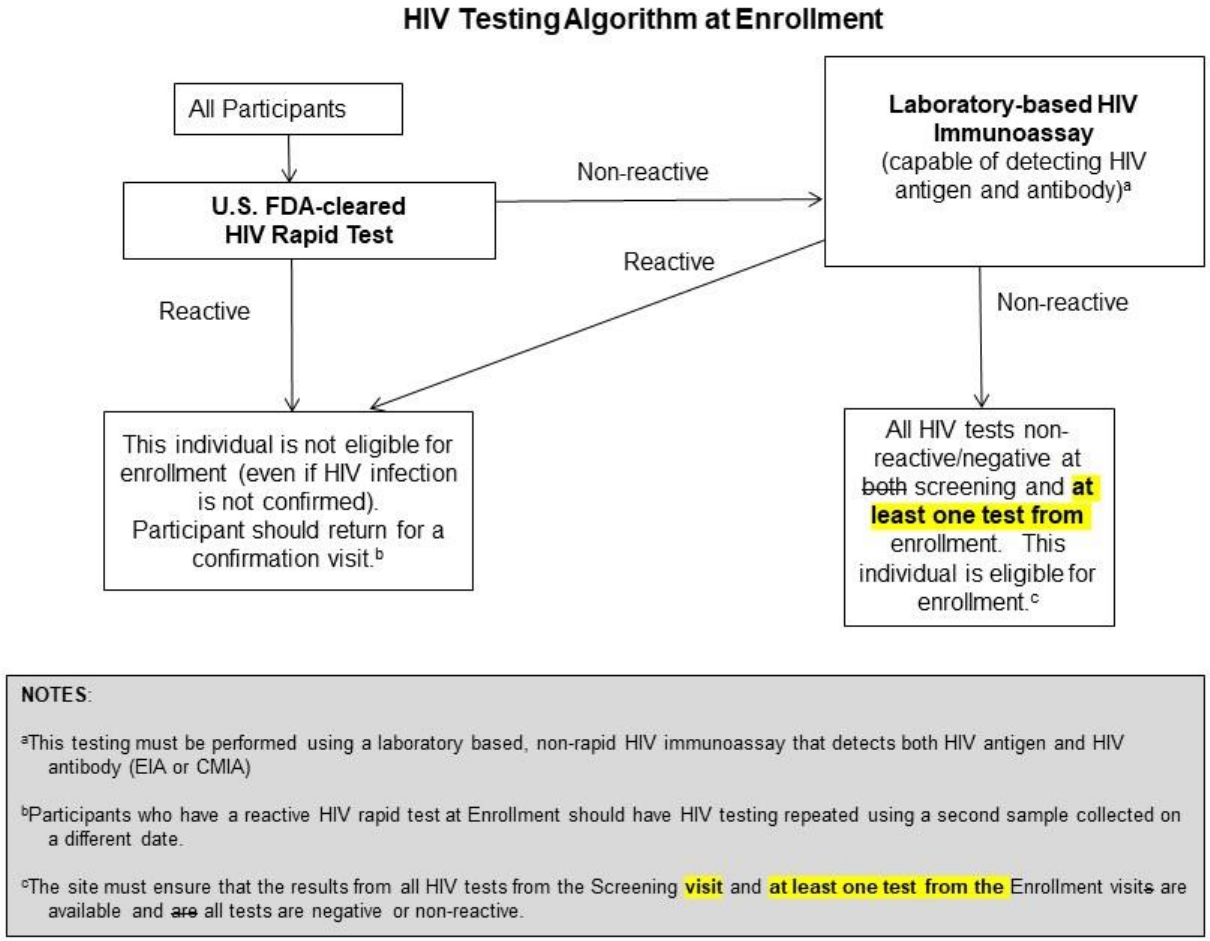


Figure 8-3 HIV Testing Algorithm at Follow-up Visit:

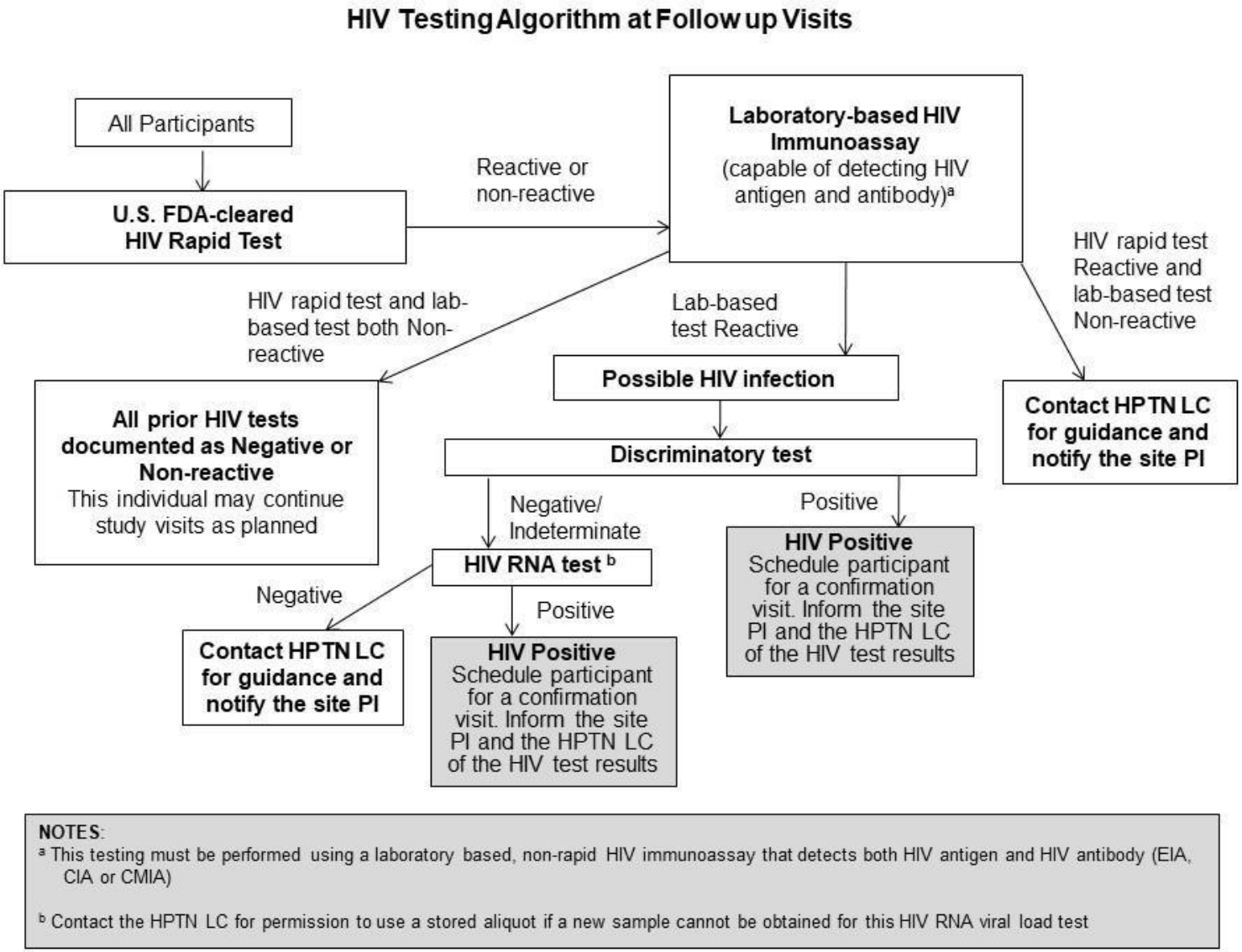
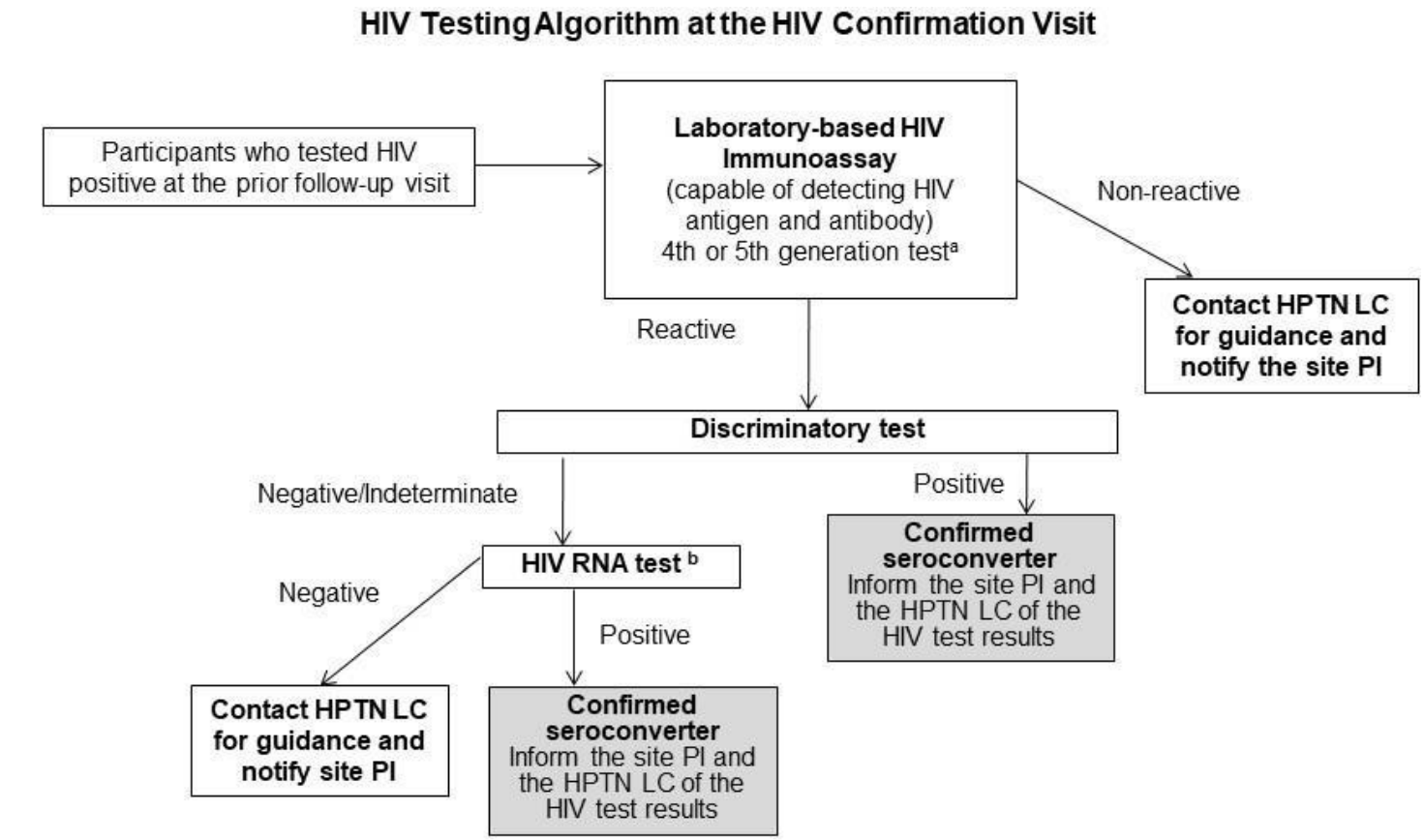


Figure 8-4 HIV Testing Algorithm at the HIV Confirmation Visit:

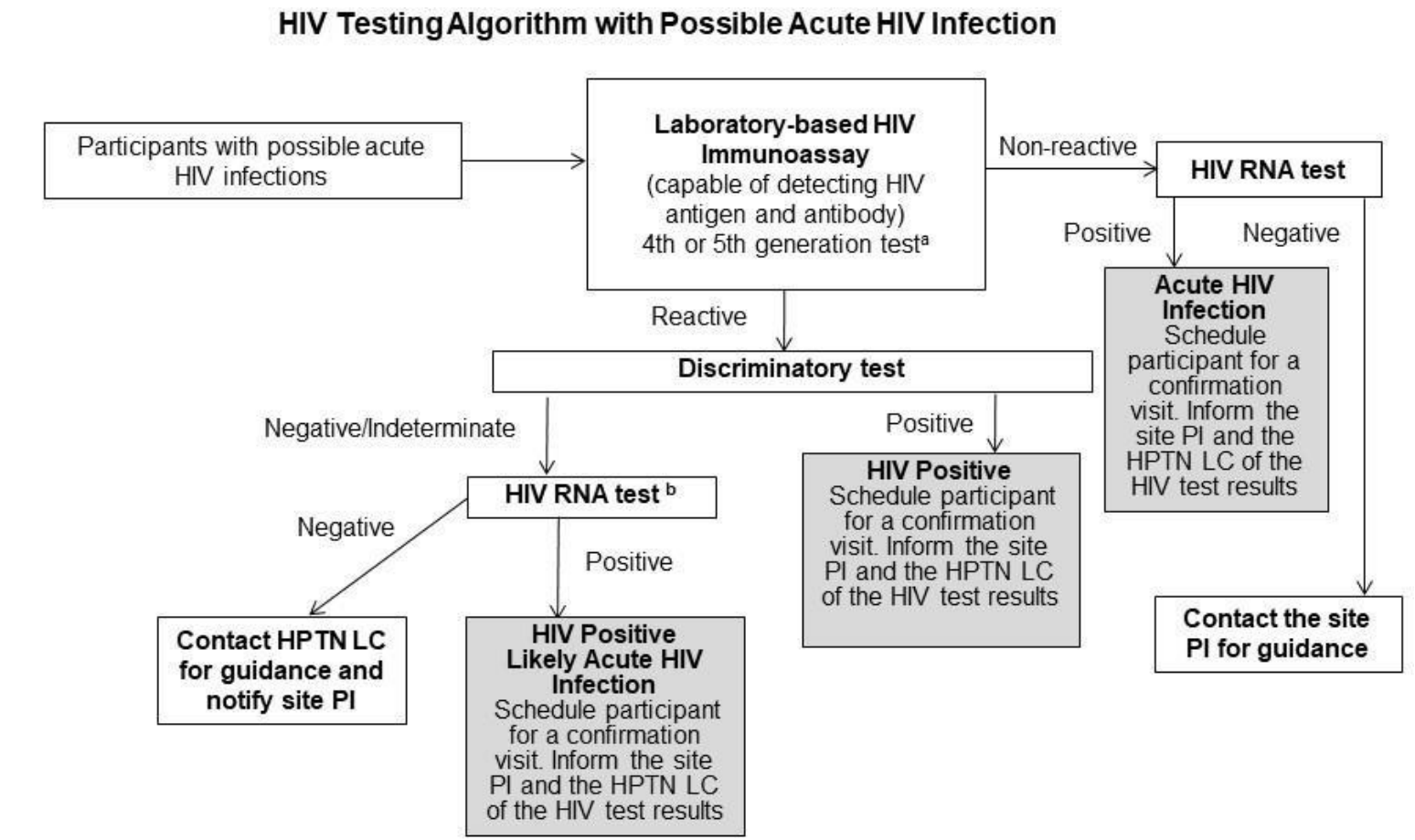


NOTES:

[a] This testing must be performed using a laboratory based, non-rapid HIV immunoassay that detects both HIV antigen and HIV antibody (EIA or CMIA)

[b] Contact the HPTN LC for permission to use a stored aliquot if a new sample cannot be obtained for this HIV RNA viral load test

Figure 8-5 HIV Testing Algorithm with Possible Acute HIV Infection:



NOTES:

[a] This testing must be performed using a laboratory based, non-rapid HIV immunoassay that detects both HIV antigen and HIV antibody (EIA or CMIA)

[b] Contact the HPTN LC for permission to use a stored aliquot if a new sample cannot be obtained for this HIV RNA viral load test

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

Participants who have any reactive or positive HIV test result during follow-up visits will have further testing to confirm infection. Samples from participants who are confirmed to be HIV-infected should be sent to a local laboratory for resistance testing to assist with clinical management (results from resistance testing performed in local laboratories should not be reported to the HPTN Statistical Data Management Center [SDMC] or the HPTN Laboratory Center [LC]). The site IoR or designee should consult the HPTN LC if confirmatory testing does not confirm that the participant is HIV-infected.

Participants who are confirmed to be HIV-infected will be linked for local HIV care and will have an additional visit to follow-up on linkage to care three months after the HIV confirmation visit. At this visit, participants will be terminated from the study. Additionally, participants will be offered linkage to GAHT services.

The confirmatory visit for HIV testing and plasma storage should be performed on a different date than the blood draw that gave the initial reactive or positive HIV test. The HIV confirmation visit should occur within two weeks of the study visit in which the participant had a reactive HIV test result. This HIV confirmatory visit will be an interim visit (for example using visit code X.01).

Table II: Schedule of Events for Participants who have a Reactive or Positive HIV Test Result during Study Follow-up (Adapted from Appendix IB)

Laboratory	**HIV Confirmation Visit**
HIV confirmatory testing	X
CD4 Cell Count	X
HIV Viral Load	X
Chemistry testing (BUN or urea, and potassium)	X
LFT (AST, ALT, total bilirubin, alkaline phosphatase)	X
Estradiol and total testosterone testing	X
Serum storage* (DHI Substudy only)	X
Plasma Storage	X
DBS Storage	X

*If indicated.

A separate blood sample (plasma) will be collected for real-time local Resistance Testing for participants who have confirmed HIV infection.

8.3.2 Hepatitis Testing

Testing for HBV (HBsAb, HBsAg, HBcAb Total) and HCV will be performed at Screening and termination visits as indicated in Table I. 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.

8.3.3 Safety Testing

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

Test results from those tests performed at the Screening visit are required prior to Enrollment.

8.3.4 Creatinine Clearance

Calculated creatinine clearance (eCrCl) will be performed at all visits where chemistry testing is performed, using the Cockcroft-Gault formula.

eCrCl (male) in mL/min = [(140 - age in years) x (actual body weight in kg)] / (72 x serum creatinine in mg/dL).

8.3.5 Fasting Lipid Profile

A fasting lipid profile (total cholesterol, HDL, triglycerides, LDL – calculated or measured) will be collected at the Screening or Enrollment, Week 26, and Week 78 visits. Participants should be fasting for at least 8 (preferably 12) hours prior to sample collection. If participants are not fasting, do not order the lipid testing. Note that a fasting lipid profile may be collected at either the Screening or the Enrollment visit. If the participant has not fasted at screening, please collect at the Enrollment visit. If the fasting sample is collected at the screening visit, it is not required to be collected at the Enrollment visit. If a fasting sample is not collected either at the Screening or Enrollment visits, participants should be rescheduled to return for sample collection within 72 hours of the Enrollment visit. If the participant has not fasted at the Week 26 or 78 visits, a fasting lipid profile should not be collected and participants are recommended to return for sample collection and testing within 72-hours of the visit.

Sites will follow local testing arrangements for the collection and testing of the lipid profile. This will be described in the site SOPs.

8.3.6 Urinalysis Testing

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

8.3.7 Syphilis Testing

Sites will follow local testing arrangements for the collection and testing of serum or plasma for syphilis testing. This will be described in the site SOPs.
Syphilis results from the Enrollment visit are not required prior to Enrollment.

8.3.8 Urine Sample for GC/CT Testing.

Sites will follow local testing arrangements for the collection and testing of urine samples for GC/CT nucleic acid testing. This will be described in the site SOPs.
Urine GC/CT results from the Enrollment visit are not required prior to Enrollment.

8.3.9 Rectal, Pharyngeal Swab Collection for GC/CT

Sites will follow local testing arrangements for the collection and testing of rectal and pharyngeal swabs for GC/CT nucleic acid testing. It is preferred that all rectal swabs be provider-performed. Self-collected swabs are acceptable as a second choice if provider-performed collection is not possible.

Rectal GC/CT results from the Enrollment visit are not required prior to Enrollment.

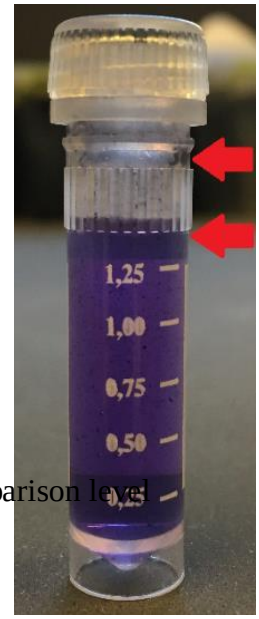
If testing cannot be performed at the local laboratory, testing at another laboratory may be considered following consultation with the HPTN LC.

8.3.10 Plasma Processing for Storage

Approximately 20 mL of EDTA whole blood should be drawn into spray-dried EDTA tubes for plasma storage at each time point at which HIV testing is performed as indicated in Tables I and II. Sites should store 5 x 1.8 mL aliquots of plasma if possible (if extra plasma is obtained, additional aliquots can be stored). The HPTN LC should be informed any time that three or fewer aliquots with 1.8 mL or less are stored.

Note: The 1.8 mL plasma volume stated in this SSP is an approximate volume. This can be estimated (e.g., using the volume markings on the cryovials). The use of a precision pipette is not required for this purpose.

The manufacturer of this example tube only includes gradations up to 1.25 mL for the 2 mL cryovial. The 1.8 mL needed is an approximate volume. For these cryovials, the top of the vertical striped area is an estimated maximum fill 'line' for a limit fill volume to prevent cracking of the container during freezing, and will provide an acceptable 1.8 mL estimate. See photo to the right for reference (Figure 8-6) The plasma level needs to be between the two arrows for 1.8 mL to be delivered and stored. The optimal level is at the indicated top arrow, near the cryovial 'ring' below the cap. Various methods for achieving the desired volume are possible. Example list, other methods are not prohibited or excluded:



- Use of a pipette with a precise measurement (not required)
- Use of a graduated disposable transfer pipette
- A marked-up a cryovial, or filled cryovial with a liquid for a comparison level (properly labeled as a blank for lab safety requirements)

Figure 8-6

An additional approximate 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, whenever blood for HIV testing is drawn. This additional plasma will be stored in the same way.

Sites will follow the instructions below or may follow site specific SOPs for plasma processing which will include the following:

1. Collect blood into lavender top blood collection tubes (K2EDTA) labeled with a SCHARP-provided PTID label. An alternate, site-specific labeling process may be used if an SOP is in place and approved by the HPTN LC, but still must use the PTID with other identifiers. The size and number of collection tubes may vary depending on local lab requirements.
2. Deliver the samples to the local LDMS laboratory along with the LDMS Specimen Tracking Sheet or site-specific requisition that contains the required information.
3. Using the LDMS Specimen Tracking Sheet or site-specific requisition, log the sample into LDMS (specimen type = BLD) and generate the appropriate number of LDMS cryovial labels. The lab should store plasma in labeled cryovials.
    3.1. Cryovial size must be 2.0 mL. We request the use of Sarstedt (cat# 72.694.006) 2 mL Cryovials, or cryovials of the same dimensions. Reminder: Do not add more than 1.8 mL due to expansion of plasma during freezing.
    3.2. Other cryovials types may only be used if specifically approved by the HPTN LC.
4. Blood processing and plasma storage should be performed **within 2 hours** of sample collection (due to Descovy's whole blood stability).
5. Centrifuge tube at 800 - 1000 x *g* for 10 minutes to separate cells and plasma.
6. Carefully remove plasma and avoid disturbing the cell layer. Transfer the plasma to an appropriately labelled sterile centrifuge tube.

7. Centrifuge plasma again at 800 - 1000 x *g* for 10 minutes to remove any contaminating debris, cells, or platelets.
8. Log samples into LDMS and generate LDMS labels (PL2). Each aliquot will have its own individual identification number (Global Specimen ID).
    8.1. Store plasma in aliquot number order. For example, if there is only 3 mL of plasma for storage: store 1.8 mL in aliquot 1, then store the remaining 1.2 mL of plasma in aliquot 2 and adjust the aliquot volume in LDMS to indicate 1.2 mL. The remaining aliquots (3, 4, and 5) should be entered as QNS (Quantity Not Sufficient). Note if there is extra plasma more than 5 aliquots can be stored, ensure the volume of the other aliquot(s) are entered in LDMS.
    8.2. Additional sample condition codes besides QNS, to be used as directed by the HPTN LC, include "SNP" (Specimen Not Processed) and "SNR" (Sample Not Received, CRF received).
9. Store the aliquots in the freezer locations assigned in LDMS in a minus 70º C to minus 90ºC 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 at least for one year after the primary research paper has been published. The sites will be notified by the HPTN LC when they can destroy samples from participants that did not consent to long term storage and when the remaining samples can be destroyed The HPTN LC will seek permission from protocol leadership and network leadership prior to this destruction process. Any samples that are collected in error should not be destroyed without the permission of the HPTN LC.

LDMS Entry:

PL2 aliquots from the 20 mL EDTA draw as follows:

- Several possible tube combinations equaling at least 20 mL (per individual site chain of custody)
- A single primary container of EDTA whole blood is created
- 5 (or more if necessary) PL2 aliquots of 1.8 mL are created (adjusted to approximate aliquot volume as needed during storage)
    - Primary container and aliquot entries should have the "SHV" (short volume) condition code used as needed when incomplete volumes are **obtained**

Note: For Web LDMS, when samples are logged for local testing such as HIV Viral load or CT/NG, please use the condition code of "LLT" (Local Lab Testing) for individual specimens in the Aliquot level of Specimen management. The "Never Store" feature is no longer supported in Web version of LDMS.

- Please remember to contact the HPTN LC when using condition codes

not listed in the SSP.

- No other aliquots are created from this primary container

LDMS Specimen Code for Plasma Storage

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

Codes used in table:

BLD Blood

DPE Spray Dried EDTA

PL2 Plasma, Double Spun

N/A Not Applicable

9.1. All plasma vials are stored electronically in the LDMS and physically in a minus 70ºC to minus 90ºC freezer. Selected aliquots 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 and the HPTN LC will provide detailed instructions for specimen destruction and documentation thereof.

8.4 Dried Blood Spots (DBS)

8.4.1 DBS Supplies:

Possible vendors for DBS supplies: Thermo Fisher Scientific, VWR, Sigma Aldrich, and Market Lab. Some Whatman items may be listed as GE Healthcare Life Sciences. The following supplies may be used. Contact HPTN LC if alternate supplies are to be used.

- EDTA spray dried Blood Collection Tubes
- Whatman Protein Saver Card #903 (Whatman 10534612 or Fisher Scientific # 05- 715-

121). Please handle with gloves and do not touch spot areas.

- Whatman Plastic Sample Bags (Whatman 10548232 or Fisher Scientific # 09-800-16) or Whatman Foil-Barrier Sample Bags (Whatman 10534321 or Sigma Aldrich # WHA10534321).
- Desiccant pack (GE Healthcare Life Sciences (Whatman) 10548234 or Fisher Scientific # 09-800-17).
- Humidity indicator Cards (Manufacturer # MS200032 or MS200033; ADCOA # MS20003-2 or MS20003-3; Fisher Scientific # NC9511648). Or similar products with similar indicator levels, suitable for storage bag size.
- Whatman card drying rack (VWR # 89015-592 or Sigma Aldrich # WHA10539521) or other suitable drying rack.
- Gloves, preferably powder free.
- Water proof marker (Fisher Scientific# 50853571 or VWR # 95042-566)
- LDMS labels.
- A fixed 25 µL, variable 10-100 µL, or 20-200 µL micropipette with appropriate filtered pipette tips. Sites should check with local suppliers for appropriate tips for their micropipettes.

8.4.2 DBS Preparation and Storage

The use of a negative airflow biosafety cabinet is not required for this specimen processing and storage. Sites will follow the instructions below or may follow site specific SOPs for DBS processing and storage.

DBS should be prepared from an EDTA blood tube received in the laboratory. For HPTN 091, it is acceptable to use one of the tubes received for plasma storage before it is processed for plasma storage or a sample received for HIV rapid testing after testing has been performed.

The EDTA tube should be well mixed before preparing the DBS. Pipette 25 µL of whole blood directly onto the center of each spot on the filter paper so that it is contained within the circle (Figure 11.13).

- There will be a total of 5 blood spots created
- Whole blood for DBS should be stored at room temperature (15°C to 25°C) until spots have been created.
- Samples should be processed (spotted) within 6 hours of the time of collection; the actual time of collection should be recorded on the Case Report Form, as well as DBS creation time.
- Ensure that both hands are gloved before handling the Protein Saver (DBS) card; Do not touch the areas where the blood spots will be placed (the filter paper portion).
- Label each Protein Saver Card with study protocol number, PID#, Study date and time of sample collection. Use a waterproof pen or a non-removable label.

- Create an LDMS label and enter specimen information into LDMS. See Figures 11.4 to 11.12.
    - Additional sample condition codes besides QNS, to be used as directed by the HPTN LC, include "SNP" and "SNR".
    - Primary container and aliquot entries should have the "SHV" (short volume) condition code used as needed when incomplete volumes are obtained
    - Please remember to contact the HPTN LC when using condition codes not listed in the SSP.
- Ensure the blood tube has been inverted 8 times and is well mixed. Remove the cap from the EDTA tube and spot 25 µL of blood, using a pipette, onto the center of the designated circles on the Protein Saver Cards (see Figures 11.13 to 11.15 below). Return the cap to the tube and process for other lab tests (i.e. plasma processing) as needed.
    a. The pipette tip should be held approximately 3mm above the spot location and the blood dispensed onto the card with one single dispensing motion from the micropipette. Do not touch, press, or smear the spots.
- Air dry the cards in a card holder or other drying rack (Figure 11.16). Ideally drying time should be between 2 and 16 hours. If storage cannot take place within 16 hours for example over a weekend, an appropriate comment must be made in LDMS to indicate the drying time.
- Keep the DBS cards away from direct sunlight. DBS cards should be dried at the designated lab room temperature which should be between 15 and 40^{O}C. DBS cards should not be dried in excess of 40^{O}C. Do not dry the DBS cards with a fan or any heat source in an attempt to decrease drying time. Air dry only. The use of a biosafety cabinet is not required for the drying of dried blood spots for HPTN
    91. If a cabinet is used there is no requirement for the airflow to be operational or documented for DBS purposes.
- After DBS cards have dried, place DBS card in low gas-permeability plastic bags with humidity indicator and desiccant pack to reduce humidity. See figures 11.17 and 11.18. Indicator cards and desiccant packs should be kept in their manufacturer stock containers (airtight) until the DBS card is dried and ready for freezer storage.
- Store bag in an appropriately labeled box at -70 to -90°C.
    a. If the indicator indicates too much humidity exposure (color change from blue to pink- 40% to 50% level or higher), replace the old desiccant pack and indicator card with a new one and comment the change in LDMS.
    b. There is no need to check the humidity indicators unless DBS are handled for another purpose (i.e. shipping), and action is needed if a problem is noticed.

LDMS Entry:

DBS from EDTA whole blood (example 4 mL draw) as follows:

1. A single primary container of 4 mL EDTA whole blood is created
2. 5 aliquots of 25 µL each are created (1 for each spot on the DBS card)

3. See figures 8-7 to 8-14

LDMS Specimen Code for DBS Storage

Test	Primary LDMS Code	Additive	Derivative	Sub Add/Deriv
Dried Blood Spots	**BLD**	**DPE**	**DBS**	**N/A**

Codes used in table:

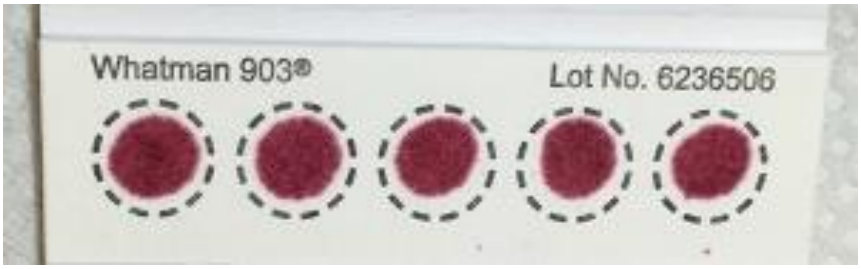
BLD	**Blood**
DPE	**Spray Dried EDTA**
DBS	**Died Blood Spot**
N/A	**Not Applicable**

- All DBS are stored electronically in the LDMS and physically in a minus 70°C to minus 90°C freezer. Selected cards will be shipped to HPTN Laboratory Center (LC) when requested.
- In addition to the illustrations, include the date and time of specimen receipt, date and time of DBS processing (spot time), and date and time of DBS completion and storage for each aliquot. Note the primary aliquot is BLD with 5 aliquots created from the primary specimen. Each aliquot will be 25 µL having its own Global Specimen ID. DBS need to be entered into LDMS and stored in appropriate location so they can be easily retrieved when necessary.

Figure 8-7 Example DBS LDMS Labels for each aliquot

Figure 8-8 Suggested labeling of DBS cards

Figure 8-9 Example of correctly spotted DBS card (25 µL spot volume)



Note: 25 µL spot volume may not completely fill target circle on DBS card.

Figure 8-10 Example of *incorrectly* spotted DBS card

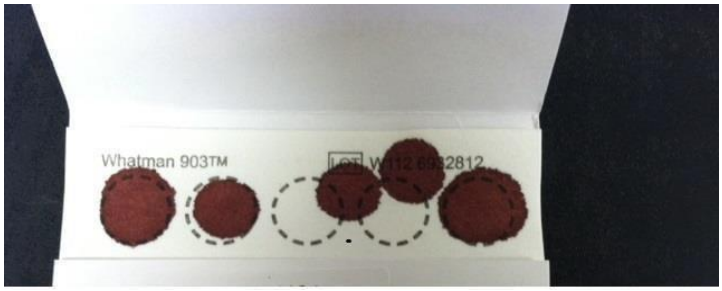


Figure 8-11 Example of incorrectly spotted DBS card (continued)

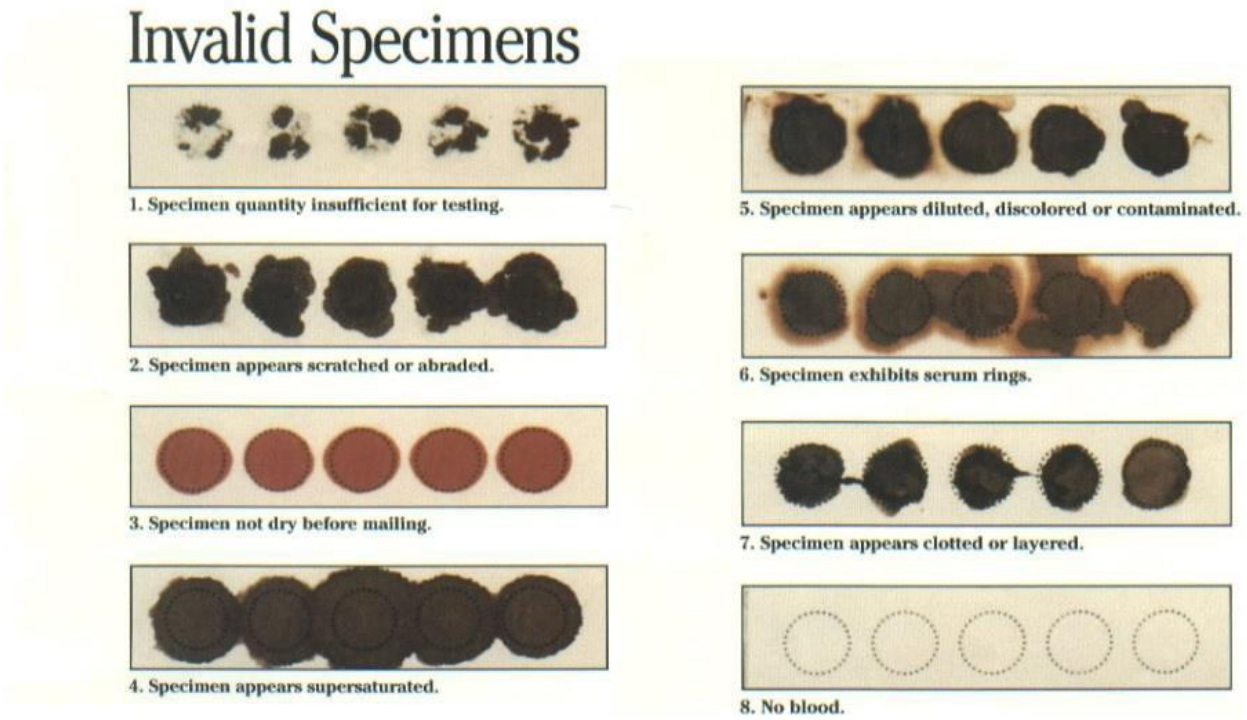


Figure 8-12 Whatman card drying rack (VWR catalogue # 89015-592)

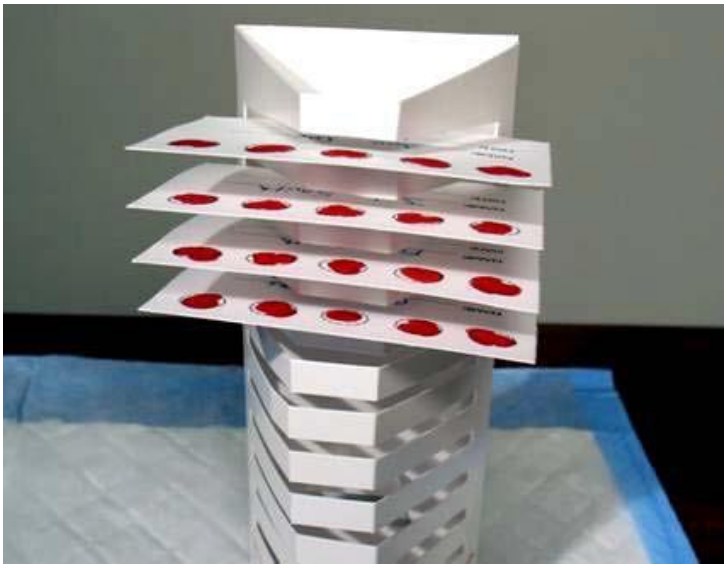


Figure 8-13 Properly labeled and packaged DBS card for storage

Figure 8-14 Properly labeled and packaged DBS card for storage (2)

Figure 8-15 Example LDMS Entry Visit 2.0 (PC –Windows- based)

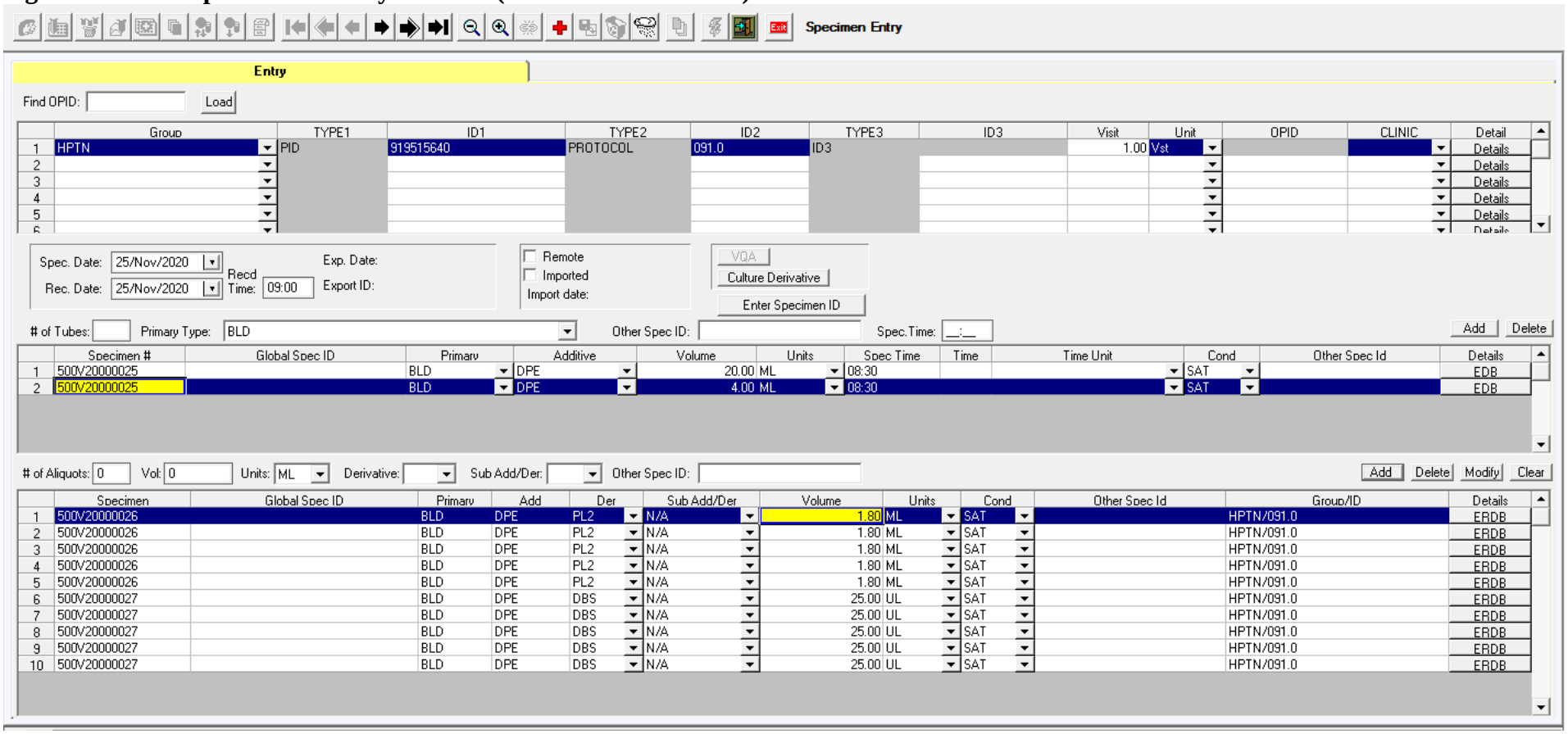
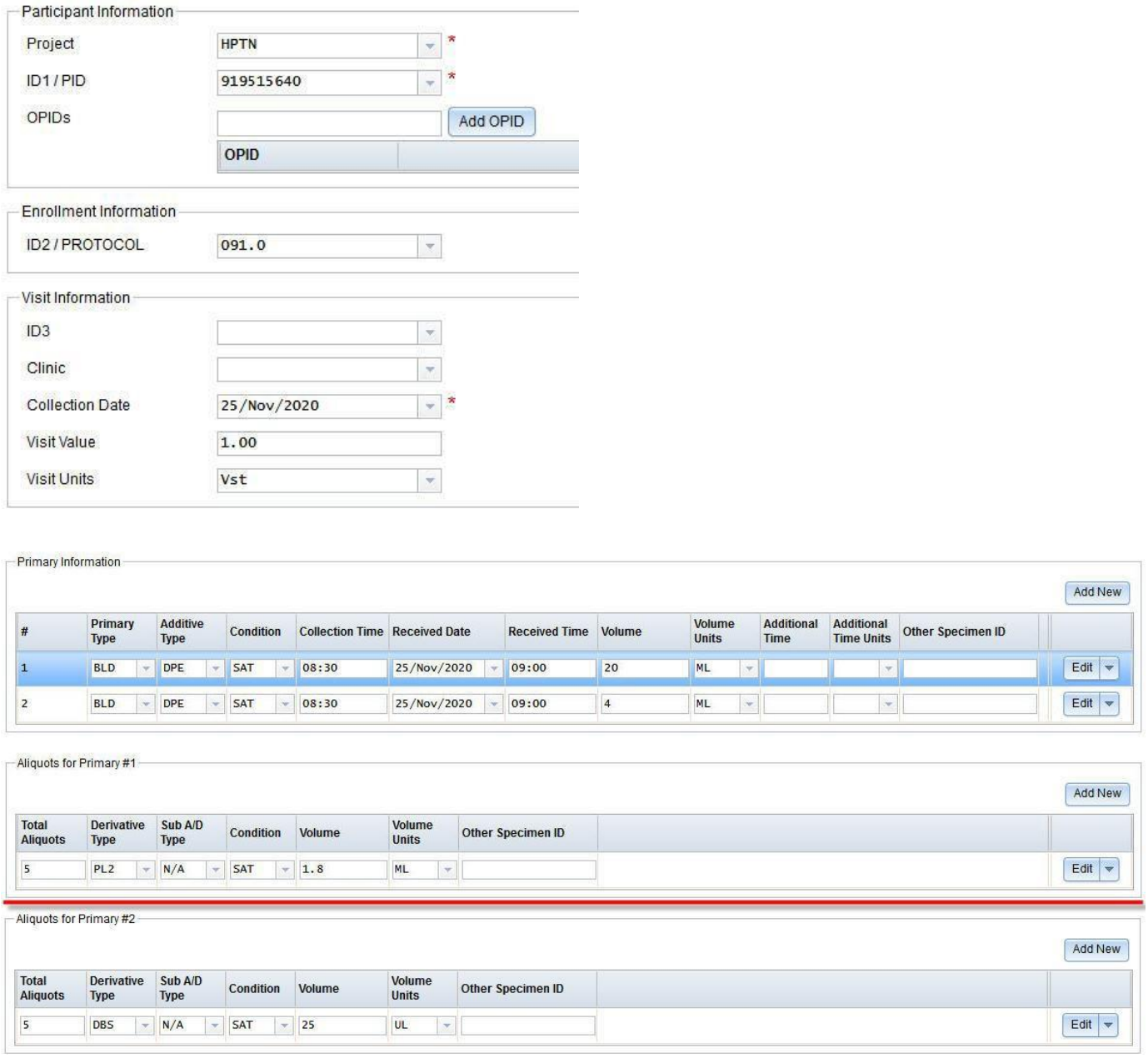


Figure 8-16 Example LDMS Screening Visit 1.0 (Web-based)



8.5 Shipping of Samples to the HPTN Laboratory Center

Each site will ship plasma, whole blood, or DBS samples to the LC or designated laboratory upon request or following a shipping schedule as determined by the LC. The site will batch the shipment, export the LDMS data and notify the SDMC and LC. Additional samples may be specifically requested by the HPTN LC (e.g., archive/back-up samples); in this case, the SDMC will provide the site(s) with specific shipping lists.

Contact the HPTN LC at Johns Hopkins University (Estelle Piwowar-Manning: epiwowa@jhmi.edu, +410-614-6736, Vanessa Cummings: vcummin1@jhmi.edu, +410-614-6737 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 the LDMS Lab number as the ship to lab ID number. The site should export the data to FSTRF after a batch has been made if using windows LDMS and notify HPTN LC with the batch number.

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

Include a copy of the shipping manifest and box map with the shipment. For dry ice shipments, also use UN 1845 along with diagnostics packing Instructions 650, Biological Substance Category B UN 3373, and address the shipment as indicated in the following pages, for Johns Hopkins Hospital (plasma and whole blood) or the University of Colorado (DBS). For some shipments, an alternate address may be provided at the time of request.

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
- Expected date of arrival

Plasma Shipping:

Each site is requested to keep “To Be Shipped” sample storage box(es) in their freezers.

a. Starting at Visit 2 (Enrollment visit), and until the end of the study, all plasma aliquots with an LDMS global ID ending “-01” should be stored in these boxes.

b. The remaining plasma aliquots should be stored as per normal site standards.

All aliquots in these "To Be Shipped" boxes should be shipped, as allowed/requested by the HPTN LC for the remainder of the study.

Samples will be shipped to:

Estelle Piwowar-Manning
Johns Hopkins University Hospital Department of Pathology
Pathology Building, Room 313 600 North Wolfe Street
Baltimore, MD 21287 USA
Phone: 410-502-0752
LDMS Number 300

Other samples, such as those from seroconverters, will also be requested on an ad-hoc basis and may be included in a HPTN LC shipment. Separate shipping instructions will be provided by the HPTN LC team members.

Separate LDMS batches are required for the shipments, of any QA requested samples, and Seroconverter samples if they are sent in the same shipment.

DBS Shipping

DBS sample lists for shipment will be posted on the SCHARP Atlas website for each site. An Email will be sent out by SCHARP to notify each site that the up-to- date shipping list is posted. . DBS cards should be shipped within 2 to 4 weeks of the posting .

Storing DBS by individual participant will simplify the shipment process.

Sites should ship the DBS cards to labs indicated by the HPTN LC. For samples directed to the University of Colorado, sites will ship directly to:

Lane Bushman C/O Pete Anderson
University of Colorado at Denver
Skaggs School of Pharmacy and Pharmaceutical Sciences CAVP Laboratory
C-238-V20, Rm V20-4410
12850 East Montview Blvd Aurora, CO 80045
USA
Phone: 303-724-6132
LDMS Number 533

When shipping DBS, make sure specimens are shipped on dry ice. Check the desiccant packs and humidity indicators before shipping, and replace if needed. Boxes should be placed in watertight secondary containers (Tyvek bags) to protect from humidity while in transit. Make sure to generate an LDMS shipping manifest with each shipment, including all requested information.

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

8.5.1 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 in selected cases (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.

8.5.2 Pharmacology Testing

Samples (plasma and DBS) for drug concentrations will be collected throughout the study. These samples will be collected from all participants, although pharmacology testing may be limited to a subset of the samples. At each visit, a blood sample will be collected. The actual date and time of each blood sample collection will be recorded. This information should be captured on the relevant eCRF.

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

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 externally reviewed and approved. 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.

8.5.3 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.

Results from specialized resistance testing (e.g., minority variants analysis, if performed) will not be returned to study sites.

8.6 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.

8.7 SUBSTUDY

Blood for Non-Viable PBMC Samples for Drug Analysis

NOTE: The substudy will be conducted at selected domestic US Sites ONLY

8.7.1 Required Materials and Equipment

- Two 8 mL Na-Citrate (Blue Top) containing Cell Preparation Tubes (CPT; BD Vacutainer, Cat. # 362761), OR four 4 mL Na-Citrate containing CPTs (BD Vacutainer, Cat. # 362760). NOTE: Sodium Citrate (Na-Citrate) CPT tubes MUST be used; if unable to acquire or any deviation from this product, contact HPTN Lab Center.
- Centrifuge – refrigerated, swing bucket rotor
- Disposable transfer pipettes
- 15 mL conical tubes (Sarstedt No. 62.554.002 PP)
- 1X PBS (Gibco cat. no. 10010 or equivalent)
- Micro-pipets (Rainin P20, P200, P1000 or equivalent)
- Serological Pipettes – 5 mL, 10 mL, 25 mL
- Pipette Tips – 20 µL, 200 µL, 1000 µL
- 2.0 mL Cryovials (VWR 16466-060 or equivalent)
- 0.4 % Trypan Blue (Corning Cellgro 25-900-CI or equivalent)
- 70% Methanol (made with HPLC-grade or Molecular-grade water), ice-cold
- Vortex
- Microscope and Hemocytometer or calibrated and well-controlled cell counter.

Procedure

- Invert the CPT tubes gently to mix the anticoagulant thoroughly. Keep the CPT tubes upright at room temperature (20-25°C) until centrifugation. Document the appropriate sample/study identification information and the blood draw date/time on the appropriate sample/study form.

  NOTE: The time from blood draw to freezing the cell lysate should not exceed 8 h.

- Centrifuge the CPT tubes at room temperature (20-25°C) in a swing bucket rotor centrifuge at 1800 x *g* for 20 minutes. Document the centrifugation start time on the appropriate sample/study form, if applicable.

- Gently invert the CPT tubes 4 – 6 times, without disturbing the underlying gel, to suspend the PBMC in the plasma. Transfer the cells, using a disposable transfer pipette or a serological pipette, from the CPT tubes to an appropriately labeled 15 mL conical

tube. Use one 15 mL conical tube per 8 mL CPT. If using 4 mL CPTs, pool cells from two CPTs into one 15 mL conical tube.

- Add PBS, using a serological pipette, to bring the total volume of each conical tube containing the cells up to the 12 mL mark on the tube. Cap the 15 mL conical tubes and mix by gently inverting.
- Centrifuge the 15 mL conical tubes at 800 x *g* for 5 minutes at 4 °C to pellet the cells.
- Remove as much of the supernatant as possible using a transfer pipet or a serological pipet without disturbing the cell pellet.
- Add 3 mL PBS to each 15 mL conical tube. Suspend the pellet by vortexing.
- Pool all cell suspensions into one 15 mL conical tube.
- Using a serological pipette, and the markings on the conical tube as a guide, make up suspension volume to 10 mL using PBS.
- Mix well by inverting the conical tube 2 – 4 times.
- Take a 0.2 mL aliquot to count cells. **Put aliquot aside and proceed immediately to cell centrifugation and pellet lysis.**
- Centrifuge the 15 mL conical tube at 800 x *g* for 5 minutes at 4 °C to pellet the cells.
- Carefully remove as much of the PBS supernatant as possible without disturbing cell pellet.
- Add 1 mL of well mixed, cold (2-8°C) 70% methanol to the 15 mL conical tube containing the PBMC pellet using a pipette. Suspend the pellet completely using a P1000 (or equivalent) and vortex thoroughly to ensure complete cell lysis.

  NOTE: Prepare 70% methanol lysing solution in a conical tube on the morning of sampling and chill in a -20 °C freezer. Upon sample delivery to the laboratory, and prior to initiating processing, remove the lysing solution from the freezer and keep on ice. Before use, ensure that the solution is completely thawed and mix well by inverting the tube 8 – 10 times to ensure homogeneity.
- Prepare two 2 mL cryovials and transfer 0.5 mL of the lysate into each, using a P1000 or equivalent.
- Cryovials must be labeled appropriately with the appropriate LDMS generated label.
- Store the PBMC lysate in a ≤ -70°C freezer. Document the time each 2.0 mL cryovial was frozen on the appropriate sample/study form.
- Count cells using the hemocytometer-Trypan Blue assay or alternate protocol validated

at local site. Record cell counts on the appropriate data sheet and record **total cell yield** in LDMS. Total cell yield = Dilution adjusted cell concentration from machine/hemocytometer (/mL) x 10 (mL) cells.

NOTE: Time from CPT centrifugation to freezing of lysate should be less than 2 hrs.

LDMS Codes PRI: BLD ADD: CPS DER: CIO
SUB DER: MET

Other Spec ID: PBMC PK

8.7.2 Cell Counting

The standard method for cell counting involves using a hemocytometer and the trypan blue exclusion assay (final dye concentration 0.04%). If sites utilize automated/semiautomated methods for cell counting (*e.g.* Invitrogen Countess Cell Counter, Guava Easycyte Flow Cytometer, Beckman ViCell or equivalent), the alternate method must be validated against the hemocytometer method prior to use. Count at least two independent PBMC preparations using the hemocytometer as well as the preferred local method. Results using the alternate method must be within 20% of hemocytometer results. This validation must be stored on file for future reference if needed.

SITES ARE ENCOURAGED TO USE AUTOMATED/SEMI-AUTOMATED METHODS FOR CELL COUNTING TO REDUCE TECHNICAL VARIABILITY.

Prior to starting the study, site staff must demonstrate proficiency in hemocytometer cell counting. This should be documented by site staff and provided to the HPTN LC for their records.

8.7.3 Required Materials and Equipment for Hemocytometer Counts

- Regular microscope with 10X eyepiece and 10X and 40X objective
- Hemocytometer (FISHER Cat. # 02-671-54 or equivalent) and hemocytometer coverslip (FISHER Cat. # 12-519-10 or equivalent)
- 0.4% Trypan Blue (Corning Cellgro Cat. # 25-900-CI or equivalent)
- Micropipette and tips capable of dispensing 10 µL and 100 µL volumes
- 0.5 mL microcentrifuge tubes

Procedure

- In a 0.5 mL microcentrifuge tube, pipette 50 µL of cell suspension.
- Add 50 µL 0.4% Trypan Blue. Mix well via vortexing. Sample can be analysed 2-5 min after staining. Analysis must be completed preferably within 30 min of cell staining.

- Place hemocytometer cover slip on the hemocytometer as shown in Figure 8.17

- Using a micropipette, load ~10 µL trypan blue-cell suspension into the top chamber of the hemocytometer. Loading is performed by placing the micropipette tip at the junction of the cover slip and the slide and gently expelling the sample. The sample will fill the chamber *via* capillary action. Stop loading as soon as the sample covers the counting grid. Do not overfill the chamber.

  NOTE: This procedure is not intuitive. Loading procedure will be demonstrated to personnel the first time.

- Load 10 µL sample into the bottom chamber of the hemocytometer.

- Place the loaded hemocytometer on the microscope stage and focus using the 10X objective (and 10X eyepiece, total magnification-100X). Focus on the counting grid in the top chamber.

- Once the counting grid is centered, change to 40X objective (total magnification 400X) and focus on the central square. The central square - #5 in Figure 8-17 – comprises 25 sub-squares. One vision field covers one sub-square.

- Moving one sub-square at a time, count live/total cells (as required) in all 25 sub-squares of Central Square (#5, Figure 8-17 1.

  NOTE: Trypan blue is a vital dye. Living cells exclude the dye and will appear bright with a light blue tinge. Dead cells and debris will appear dull dark blue. Cell size and shape will vary depending on sample. PBMCs will appear small and spherical.

- Record total and viable cell number.

- Move the microscope stage to focus on the counting grid in the bottom chamber of the hemocytometer. Repeat steps ~~9.8 and 9~~.9 described above to obtain a cell count for the bottom chamber.

- Determine the average total and viable cell count of both chambers.
  Average cell count = ([Cell count$_{\text{top chamber}}$ + Cell count$_{\text{bottom chamber}}$]/2)

  NOTE: For a preparation with >95% viability, it may be difficult to find a non-viable cell on the grid. In that case total count will equal viable count.

- The cell density of trypan blue cell suspension = **Average cell count X 10^4 cells/mL**.

- The cell density in the original cell suspension = **cell density of trypan blue cell suspension X**
  2. The unit for this number is also ‘cells/mL’.

- **The total cell count in the sample = cell density of original cell suspension (from**

above) X 10. The unit for this number is 'cells'.

- Record and report total cell count in appropriate study document and LDMS field.
- If viable cell counts are recorded, and are < 80% of total cell counts, make a note in the comments field of the LDMS entry for the sample.

LDMS instructions

1. Primary specimen: **BLD**

   Additive: **CPS**

Specimen time: **Draw time** Sample volume: **16** mL Time: Scheduled time point
Time unit: As specified in study procedure Other Spec ID: **PBMC PK**

2. For aliquot information, fields should be filled as follows # of aliquots: **2**

   Aliquot derivative: **CIO**

   Aliquot sub-additive/derivative: **MET**

   Volume: **0.5 mL**

   Other Spec ID: **PBMC PK**

LDMS Specimen Code for Storage

Test	Primary LDMS Code	Additive	Derivative	Sub Add/Deriv
Storage	**BLD**	**CPS**	**CIO**	**MET**

Codes used in table:

BLD Blood

CPS Cell Preparation Tube SCI

CIO Cells in Other (Solution), Non-Viable

MET Methanol

3. Cell count and lysate volume information must be entered in the **aliquot details comments field**.

   Click on the details tab for the **primary sample**. Enter numerical information about lysate volume and total cell count into the comments field. Enter only numbers.

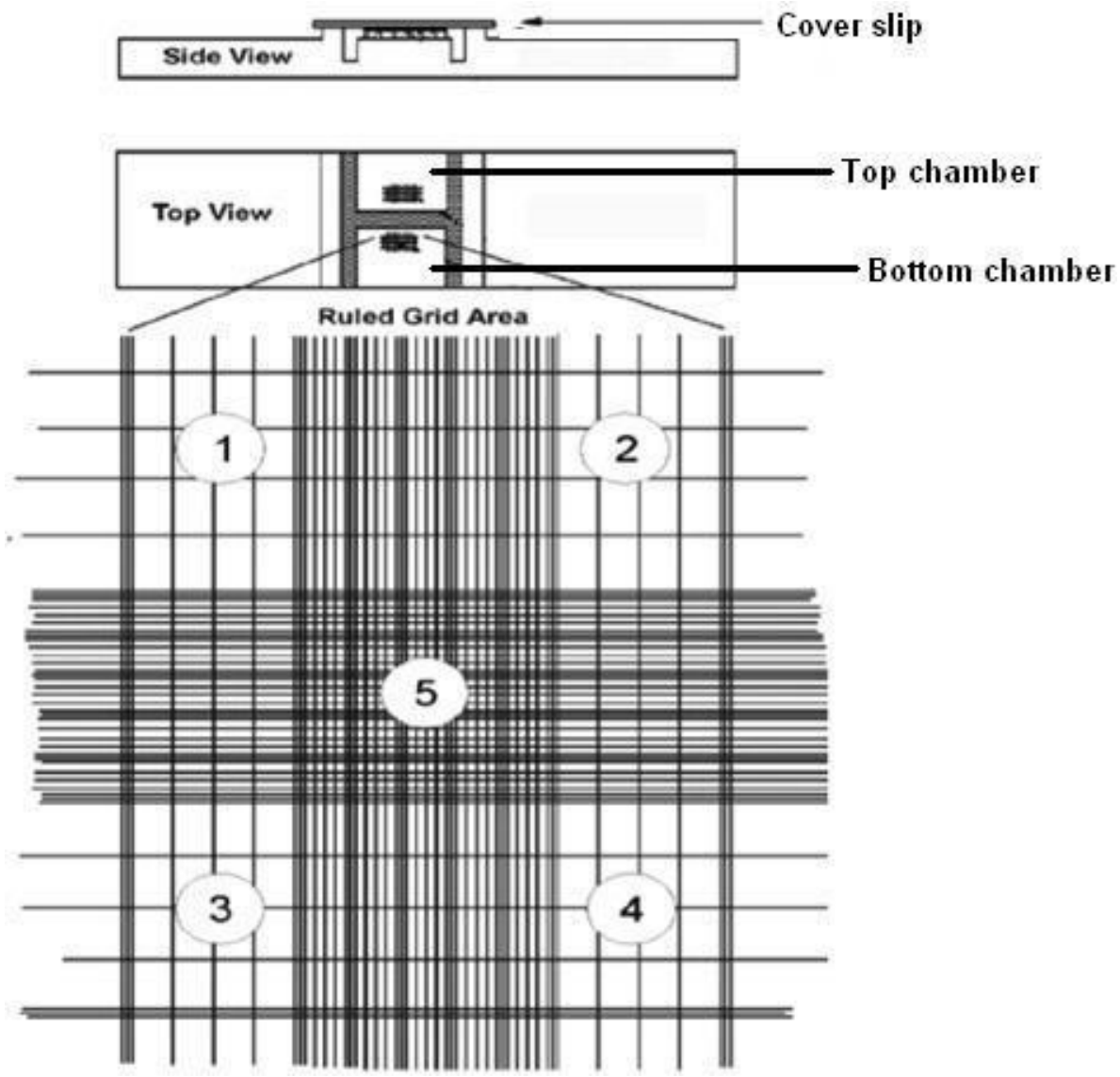
For example, if 3.1x10^7 total cells were lysed in 1 mL 70% MeOH, information should be entered as: 1, 31x10^6. **Please note there must be a space after '1,' and before cell count.**

Upon saving, LDMS will ask if information is to be cascaded to all aliquots. Say **Yes**. **Please adhere precisely to this syntax to enable automated analysis.**

8.7.4 Documentation of Competency

Competency in cell counting using the hemocytometer and trypan blue assay should be assessed by comparing counts performed by site staff and counts determined by analyzing the same sample with the local automated method. Cell counts generated by the trainee must be within 20% of those reported by the automated method. Competency documentation should be maintained at the site.

Figure 8-17



8.8 Serum Processing for Storage for DHI Substudy

Approximately 5 mL of whole blood should be drawn into a Serum Separator tube for serum storage. Sites are requested to store 2 x 1.25 mL aliquot.
Note. The remaining volume can be estimated using the volume markings on the cryovials (for example). The use of a precision pipette is not required for this purpose. The volume should be recorded in LDMS.

The manufacturer of this example tube stops gradations at 1.25 mL for the 2 mL cryovial. For these cryovials, the top of the vertical striped area is an estimated maximum fill 'line' for a limit fill volume to prevent cracking of the container during freezing, and will provide an acceptable 1.8 mL estimate. See photo to the right for reference (Figure 11.4). Various methods for achieving the volume to record in LDMS are possible. Example list, other methods are not prohibited or excluded:

- Use of a pipette with a precise measurement (not required)
- Use of a graduated disposable transfer pipette
- A marked-up a cryovial, or filled cryovial with a liquid for a comparison level (properly labeled as a blank for lab safety requirements)

Sites will follow the instructions below or may follow site specific SOPs for serum processing which will include the following:

10. Collect blood into SST blood collection tubes labeled with a SCHARP- provided PTID label. An alternate, site-specific labeling process may be used if an SOP is in place, and HPTN LC approved, but still must use the PTID with other identifiers. Size and number of collection tubes may vary depending on local lab requirements.
11. Deliver the samples to the local LDMS laboratory along with the LDMS Specimen Tracking Sheet or site-specific requisition that contains the required information.
12. Using the LDMS Specimen Tracking Sheet or site-specific requisition, log the sample into LDMS (specimen type = BLD) and generate the appropriate number of LDMS cryoviallabels. The lab should store serum in labeled cryovials.
    - Cryovial size must be 2.0 mL. We request the use of Sarstedt (cat# 72.694.006) 2 mL Cryovials, or cryovials of the same dimensions. Reminder: Do not add more than 1.8 mL due to expansion of plasma during freezing.
    - Other cryovials types may only be used if specifically approved by the HPTN LC
13. Blood processing should be performed within 6 hours of sample collection, typically within2 hours. Allow the blood to clot for at least 30 minutes before centrifugation
14. Centrifuge tube at room temperature (20˚C to 25˚C) at 1100 to 1300 x *g* for 10 minutes in swinging bucket rotor units or centrifuge at 1100 to 1300 x *g* for 15 minutes in fixed angle units.
15. Carefully remove serum and transfer to an appropriately labelled cryovial.
16. Log samples into LDMS and generate LDMS labels (SER). Each aliquot will have its own individual identification number (Global Specimen ID).
    - Store serum in aliquot number order.
17. Store the aliquots in the freezer locations assigned in LDMS in a minus 70ºC to minus 90ºC freezer.

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

LDMS Entry:

SER aliquots from the SST draw as follows:

- Use SST tube equaling at least 5 mL (per individual site chain of custody)
- A single primary container of whole blood is created

- 2 SER aliquots of 1.25 mL are created (adjusted to approximate aliquot volume as needed during storage)
    - Primary container and aliquot entries should have the "SHV" (short volume) condition code used as needed when incomplete volumes are obtained
    - Please remember to contact the LC when using condition codes not listed in the SSP.
- No other aliquots are created from this primary container

LDMS Specimen Code for Serum Storage

Test	Primary LDMS Code	Additive	Derivative	Sub Add/Deriv
Serum Storage	**BLD**	**SST**	**SER**	**N/A**

Codes used in table:

BLD Blood

SST Serum Separator

SER Serum

N/A Not Applicable

17.1. All serum vials are stored electronically in the LDMS and physically in a minus 70ºC to minus 90ºC freezer. Aliquots will be shipped to LC at Johns Hopkins University; serum and lysed PBMCs should be shipped annually to the HPTN LC (which will be a pass through to LDMS lab 194).

8.9 Plasma for Storage for DHI Substudy

- Collect one 6mL EDTA tube or equivalent for each time point (pre-PrEP dose, 1 hour post dose – plasma storage for PK and 4 hour post dose – plasma storage for PK).
- Process within 1 hour of collection or within a 2-hour window.
- Prepare 1 mL aliquots, at least two, preferable three.
- Notify HPTN LC if less than two aliquots are stored

LDMS Specimen Code for Plasma Storage

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

Codes used in table:

BLD Blood

DPE Spray Dried EDTA

PL2 Plasma, Double Spun

N/A Not Applicable

NOTE: Additional LDMS codes for the DHI sub-study are listed below Table III – Schedule of Events for Participants in Drug- Hormone Interaction Substudy

Table III: Schedule of Events for Participants in Drug-Hormone Interaction Substudy[1] (Adapted from Appendix IC)

Laboratory	Day 1-7 Before GAHT Initiation Visit	GAHT Initiation Visit[2]	Week 13 (or 39) Study Visit	Day 1-7 After Week 13	Day 8 After Week 13 (or 39) (Clinic Visit)
Pre-DOT[1]					
Plasma Storage		X	X		X
Serum Storage[3]		X			X
PBMC storage for PK		X			X
DBS Storage		X	X		X
Post-DOT					
1 hour - Plasma storage for PK		X			X
1 hour - PBMC storage for PK		X			X
4 hour - Plasma storage for PK		X			X
4 hour - PBMC storage for PK		X			X

[1]Please ensure collection of all pre-DOT samples prior to administration of PrEP.

[2]Ensure that all PK collection events occur prior to dispensing GAHT at the GAHT initiation visit. GAHT initiation visit will occur at Week 26 in the deferred intervention arm. For the 1 and 4 hour collections, +/- 15 minutes is allowable.

[3]Samples will be tested for estradiol, free and total testosterone, LH, and FSH. Results will not be returned to participants.

Specific LDMS Codes (Other Spec ID, Time and Time Unit) for samples listed in Table III above:

Pre-dose Plasma Storage – Other Spec ID- PLA PK, Time- 0, Time Unit- Hrs

Pre-dose Serum Storage – Other Spec ID – SER PK, Time- 0, Time Unit- Hrs

Pre-dose PBMC Storage- Other Spec ID – PBMC PK, Time- 0, Time Unit – Hrs

Pre-dose DBS storage- Other Spec ID – DBS PK, Time- 0, Time Unit- Hrs

1hour post-dose Plasma storage – Other Spec ID- PLA PK- Time- 1, Time Unit – Hrs

1hour post-dose PBMC storage – Other Spec ID- PBMC PK- Time – 1, Time Unit- Hrs

4 hour post-dose Plasma storage – Other Spec ID- PLA PK- Time – 4, Time Unit - Hrs

4 hour post-dose PBMC storage – Other Spec ID – PBMC PK, Time- 4, Time Unit- Hrs